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OP-01

INFLAMMATION MARKERS AND EFFECTORS IN LONG TIME INFECTED AND NEWLY DIAGNOSED HIV PATIENTS

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Introduction: The advances made in HIV treatment caused a significant reduction of death rate; however despite viral suppression, inflammation and immune cell activation persist, being associated with poor outcome. Based on these, we aimed to evaluate inflammation and T cell profile in long time infected and newly diagnosed HIV patients.
Methods: The CD4 and CD8 T cell subsets and activated cells were evaluated by flow cytometry. The levels of IL-6, C-reactive protein (CRP), cyclophilin A (CypA) were evaluated by ELISA.
Results: HIV infected patients showed increased level of inflammatory markers (CRP, IL-6 and CypA), especially those newly diagnosed, being associated with high HIV viral load. Despite viral suppression, in long time infected HIV patients these markers continue to be high. CypA level correlated positively with viral loads and negatively with CD4/CD8 ratios. Newly diagnosed HIV patients presented high percentages of terminally differentiated CD8T cells and of memory phenotype CD4T cells. High CD8T cell activation was associated with a more advance HIV infection stage.
Conclusion: HIV infection induce an inflammatory status and altered T cells profiles, defects that persists despite viral suppression.
Acknowledgment: supported by UEFISCDI, Grant HIV-ID, 260/2015.

MARKERI SI EFECTORI INFLAMATORI LA PACIENTII NOU DIAGNOSTICATI SI CEI CU DURATA LUNGA A INFECTIE HIV

Introducere: Progresele realizate in tratamentul infectiei HIV a condus la scaderea ratei mortalitatii; totusi, inflamatie si activarea celulelor imune persista in pofida supresiei virale, fiind asociat cu evolutie nefavorabila. Ne-am propus sa evaluam inflamatia si profilul celulelor T la pacientii nou diagnosticati cu HIV si la cei cu infectie de lunga durata
Metode: Subseturile de celule T CD4 si CD8 si celulele activate au fost evaluate prin citometrie in flux. Nivelurile de IL-6, proteina C-reactiva, ciclofilinaA (CypA) s-a evaluat prin ELISA.
Rezultate: Pacientii cu infectie HIV au prezentat nivel crescut al markerilor inflamatori (CRP, IL-6, CypA), in special cei nou diagnosticati, fiind asociat cu incarcaturi virale ridicate. Acesti markeri continua sa fie crescuti la pacientii cu infectie de lunga durata, in pofida supresiei virale. Nivelul de CypA s-a corelat direct cu incarcatura virala si indirect cu raportul CD4/CD8. Pacientii nou diagnosticati cu HIV au prezentat procente ridicate de celule T CD8 terminal diferentiate si celule T CD4 cu fenotip de memorie. Nivelul crescut de celule T CD8 activate s-a asociat cu stadiu avansat al infectie HIV.
Concluzii: Infectia HIV induce inflamatie si alterarea profilului celulelor T, defecte ce persista in pofida supresiei virale.
Acknowledgment: finantat UEFISCDI, Grant HIV-ID, 260/2015.

OP-02

POSSIBLE EPIGENETICS BIOMARKERS IN PANCREATIC ONCOGENESIS

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Pancreatic cancer is the fourth leading cause of cancer deaths, being responsible for 7% of all cancer-related deaths in both men and women, it remains a major challenge and early detection could decrease the mortality caused by this disease. An important role in initiation and progression of this disease is played by gradual accumulation of various genetic and epigenetic alterations. Along with GNMT gene promoter methylation and beta-catenin epigenetic modification our attention was focused onlong non-coding RNAs (lncRNAs) and the relation with neoplastic transformation. Little is known about the role of lncRNAs in pancreatic duct adenocarcinoma (PDAC). The aim of this study was to analyze the expression profiles of lncRNAs in pancreatic cancer. For this purpose, differentially expressed lncRNAs between paired samples (normal/pancreatic adenocarcinoma) were evaluated using Human LncProfiler qPCR Array Kit (System Biosciences). Selected lncRNAs were further validated in 30 paired samples. Using Human LncProfiler Array, 42 lncRNAs presented significant differential expression levels between PDAC tissues and paired normal tissues (> 2-fold change; p< 0.05. 27 lncRNAs being significantly upregulated (among them: Alpha 280, E2F4 antisense, Evf1 Evf2, Hoxa11as, Tsix, Meg3, Malat1) while 15 were significantly downregulated (EgoA, SRA, Lust, PCGEM1, Gomafu, and Tmevpg1). In order to validate the results two selected lncRNAs (Malat1 and Meg3) were quantified in 30 sample pairs (qRT-PCR). Results indicated that both lncRNAs were highly expressed in pancreatic cancer compared with adjacent normal tissues (p<0.001) especially in advanced stages of disease (III-IV). These data show different patterns of expression of lncRNA in pancreatic cancer and suggest a potential role for these epigenetic factors in this pathology.
Acknowledgment: This study was supported by PCCA 90/2012 and POSDRU/186/3.2/S/155295

POSIBILI BIOMARKERI EPIGENTICI IN ONCOGENZA PANCREATICA

Cancerul pancreatic este a patra cauza principala a deceselor provocate de cancer, fiind responsabil de 7% din totalul deceselor legate de cancer la barbati si femei, aceasta rămâne o provocare majoră și depistarea precoce ar putea reduce mortalitatea cauzată de această boală. Un rol important in initierea si progresia acestei boli este jucat de acumularea treptata a diferitelor modificari genetice si epigenetice. Impreuna cu metilarea promotorului genei GNMT si modificarea epigenetică a geneibeta-catenina, atenția noastră s-a concentrat pe ARNs long non-coding (lncRNAs) și relația acestuia cu transformarea neoplazică. Se cunosc puține despre rolul lncRNAs în adenocarcinom pancreatic ductal (PDAC). Scopul acestui studiu a fost acela de a analiza profilurile de expresie ale lncRNAs in cancerul pancreatic. În acest scop, lncRNAs exprimate diferentiat între probe pereche (tesut normal/ adenocarcinom pancreatic) au fost evaluate cu ajutorul Kit Array Human LncProfiler qPCR (Sistem Biosciences). lncRNAs selectate au fost validate în continuare în 30 de probe pereche.Cu ajutorul LncProfiler Umane Array, 42 lncRNAs au prezentat niveluri de expresie semnificativ diferite între țesuturile PDAC și țesuturile normale asociate (fold change>2 ori; p <0,05)/ 27 lncRNAs fiind semnificativ supraexprimate (printre ele:. Alfa 280, E2F4 antisens, Evf1 Evf2, Hoxa11as, Tsix, Meg3, Malat1), în timp ce la 15 expresia a fost semnificativ scazuta (EgoA, SRA, Lust, PCGEM1, Gomafu și Tmevpg1). Pentru a valida rezultatele a două lncRNAs selectate (Malat1 și Meg3), acestea au fost cuantificate în 30 de eșantioane perechi (qRT-PCR). Rezultatele au indicat că ambele lncRNAs au fost puternic exprimate in cancerul pancreatic, comparativ cu țesuturile normale adiacente (p <0,001), în special în stadiile avansate ale bolii (III-IV). Aceste date arata diferite modele de exprimare a lncRNA in cancerul de pancreas și sugerează un rol potențial pentru acești factori epigenetice în această patologie.
Acknowledgment: Acest studiu a fost sustinut de PCCA 90/2012 și POSDRU / 186 / 3.2 / S / 155295

OP-03

CROSS-TALK BETWEEN VASCULAR AND IMMUNE CELLS ACCELERATES THE EVOLUTION OF ATHEROSCLEROTIC PLAQUE: THE MECHANISMS INVOLVED

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Introduction: Monocytes, macrophages (MAC), smooth muscle cells (SMC), and their interactions have key roles in the pathogenesis of vascular diseases. In atherosclerosis, SMC often reside in close proximity to macrophage clusters. We hypothesized that their interaction, either by direct contact or indirectly by the different factors released from SMC and/or MAC influence the evolution of atherosclerotic plaque. In this context, we investigated the effect of the cross-talk between SMC and monocytes/MAC on key molecules of atherosclerosis evolution (inflammatory molecules, metalloproteases, matrix proteins) and angiogenesis, as well as the molecular mechanisms involved.
Materials and Methods: Human aortic SMC isolated from the media of fetal thoracic aorta, human endothelial cells line EAhy926, THP monocytes and MAC (obtained by differentiation of monocytes with PMA for 3 days) were cultivated using standard cell culture methods. The cell-cell cross talk was investigated either by cell to cell direct interaction or using trans-well chambers, where SMC were grown on the filter and at confluence were placed in the wells above differentiated MAC. Inflammatory and matrix molecules, metalloproteases, vascular endothelial growth factor (VEGF) were determined by quantitative PCR, Western Blot, ELISA assay and zymography. The capacity of co-culture conditioned media to induce endothelial tube formation was determined using Matrigel. The signaling mechanisms involved was investigated using specific inhibitors for transcription factors and signaling molecules, and siRNA technology.
Results: The experiments indicated that the cross-talk between monocytes or macrophages and SMC affects the behavior of the cells, resulting in: (i) increased expression of inflammatory molecules (TNFα, IL-1β, IL-6, resistin) in SMC and monocytes; (ii) decreased matrix proteins synthesis (collagen I, III and elastin) in SMC; (iii) increased expression and activity of gelatinase MMP-9 and collagenase MMP-1 in both MAC and SMC; (iv) increasedlevel of VEGF released in the conditioned media of cell co-culture. Moreover, the conditioned media collected from MAC-SMC co-culture had anotable angiogenic effect on endothelial cells, promoting tube formation. The JAK/STAT and TLR2 signaling pathway along with NF-κB, AP-1 and STAT3 transcription factors were found responsible for these modifications.
Conclusions: The macrophages-SMC cross-talk results in an increased expression of pro-atherogenic and pro-angiogenic molecules that are known to participate to the progression and aggravation of atherosclerosis. Future studies to understand the complex interplay between these key cells of the atherosclerotic plaque, may offer new therapeutic strategies in vascular medicine.
Acknowledgements: This work was supported by grants of the Romanian National Authority for Scientific Research and Innovation, CNCS – UEFISCDI, project number PN-II-RU-TE-2014-4-0965, project number PN-II-ID-PCE-2011-3-0928 and by the Romanian Academy.

COMUNICAREA CELULELOR VASCULARE CU CELULELE SISTEMULUI IMUN ACCELEREAZAEVOLUTIA PLACII ATEROSCLEROTICE: MECANISME IMPLICATE

Introducere: Monocitele, macrofagele (MAC), celulele musculare netede (CMN) si interactiunile dintre ele au roluri cheie in patogeneza bolilor vasculare aterosclerotice. In ateroscleroza, CMN se situeaza deseori in imediata apropiere a grupurilor de macrofage. Noi am presupus ca interactia dintre aceste celule, atat prin contact direct cat si prin diferiti factori eliberati de CMN si/sau MAC influenteaza evolutia placii aterosclerotice. In acest context, am investigat efectul comunicarii dintre CMN si monocite sau MAC asupra unor molecule-cheie ale evolutiei aterosclerozei (molecule inflamatorii, metaloproteaze, proteine matriceale) si asupra angiogenezei, precum si mecanismele moleculare implicate.
Materiale și metode: CMN aortice umane izolate din media aortei toracice fetale, linia de celule endoteliale umane EaHy926, monocitele THP si MAC (obtinute prin diferentierea monocitelor cu PMA timp de 3 zile) au fost

cultivate folosind metode standard de culturi celulare. Comunicarea dintre celule s-a realizat prin interactia directa dintre monocite si CMN sau folosind camere duble, in care CMN au fost cultivate pe filtru si, la confluenta au fost plasate in godeurile unde se aflau MAC diferentiate. Expresia moleculelor inflamatorii si matriceale, a metaloproteazelor si factorului de crestere endothelial (VEGF) au fost determinate prin PCR cantitativ, Western Blot, teste ELISA si zimografie. Capacitatea mediului conditionat de a induce formarea de tubi endoteliali de angiogeneza a fost determinata folosind Matrigel. Mecanismele de semnalizare implicate au fost investigate cu ajutorul unor inhibitori specifici pentru factorii de transcriere si pentru moleculele de semnalizare si cu ajutorul tehnologiei siRNA.

Rezultate: Datele noastre arata cainteractia dintre monocite or MAC si CMN afecteaza comportamentul ambelor tipuri de celule, rezultand in: (i) cresterea expresiei moleculelor inflamatorii (TNFa, IL-1β, IL-6, rezistina) in CMN și monocite; (ii) scaderea sintezei proteinelor matricei extracelulare (colagen I, III și elastina) in CMN; (iii) expresie si activitate crescuta a gelatinazei MMP-9 si a colagenazei MMP-1 in MAC si CMN; (iii) nivel crescut al VEGF eliberat in mediul conditionat al co-culturii MAC-CMN. Mai mult decat atat, mediul conditionat colectat de la MAC si SMCaflate inco-culturaa avut un efect angiogenic notabil asupra celulelor endoteliale, promovand formarea de tubi de angiogeneza. Caile de semnalizare JAK/STAT si TLR2, impreuna cu factorii de transcriptie NF-kB, AP-1 și STAT3 sunt responsabili pentru modificarile observate.

Concluzii: Comunicarea dintre monocite sau MAC cu CMN duce la crestera expresiei moleculelor pro-aterogene si pro-angiogene si scaderea expresiei proteinelor matriceale, modificari care mediaza progresia si agravarea aterosclerozei. Cercetarile viitoare pentru a intelege interactiunea complexa dintre aceste celule cheie ale placii aterosclerotice, pot oferi noistrategii terapeutice in medicina vasculara.

Acknowledgements: This work was supported by grants of the Romanian National Authority for Scientific Research and Innovation, CNCS – UEFISCDI, project number PN-II-RU-TE-2014-4-0965, project number PN-II-ID-PCE-2011-3-0928 and by the Romanian Academy.

OP-04

EXPERIMENTAL MODELS FOR INFLAMMATORY BOWEL DISEASE: WHICH IS BETTER?

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Background: Ideally, the inflammatory bowel disease (IBD) experimental models should be characterized by a predictable clinical course, histopathology as well as inflammation similar to human ulcerative colitis (UC) and Crohn' disease (CD). The aim of this study was develop two inexpensive models of IBD in rats using as chemical agents dextran sodium sulfate (DSS) and trinitrobenzene sulfonic acid (TNBS).

Materials and methods: Experiments were conducted on 15 male Wistar rats, 14 weeks old, randomized into three groups, of 5 rats each: group A as control group received tap water; group B received 5% DSS in their drinking water, during 7 days and group C received intrarectally a single dose of 100 mg/kg TNBS. The effect of acute administration of DSS and TNBS was evaluated by the assay of the activity of whole blood glutathione peroxydase (GPx), a marker of the oxidative stress, using a commercially available Randox kit. Experimental colitis was estimated by the clinical course of the disease. After euthanasia, the entire colon was removed, perfused and immersed in 10% formalin. Colonic sections underwent morphological examination after standard hematoxilin eosin stain. The histological scoring system previously described by Rachmilewitz for grading the severity of DSS and TNBS induced colitis in rodent models was employed. In

order to evaluate the differences in the 3 groups the Kruskal-Wallis test was used. The post-hoc analysis using Mann-Whitney test was used to identify the source of differences. Because of multiple comparisons the significance level was adjusted after a Bonferroni correction and it was equal to 0.02.
Results: After DSS/TNBS inoculation, the rats showed decrease in the body weight, diarrhea, bloody stools as well as histological lesions of colitis compared to the control rats. DSS administration increased the GPx activity compared to the control and TNBS-inoculated groups but not statistically significant. We also observed that the mean level of hemoglobin (Hb) concentration was significantly higher in DSS-treated group compared to the control group (14.32 g/dL vs 12.58 g/dL). In addition, the mean level of Hb concentration was significantly higher in DSS-treated group compared to TNBS-treated group (14.32 g/dL vs 11.89 g/dL) (p= 0.001). Mean Hb levels was lower, but not statistically significant, in TNBS-treated group compared with controls (11.89 g/dL vs 12.58 g/dL). Mean of colon weight was significantly different (p = 0.001) in control rats (2.38g) compared to the TNBS group (3.87g). Mean colon weight values were also significantly different (p= 0.002) in TNBS-treated group (3.87g) compared to the DSS-treated group (2.52g). The statistically significant differences between the colon length (cm) values in the control and the TNBS group were confirmed by a post-test analysis (Mann Whitney test; p = 0.019). Other statistically significant differences between the colon length (cm) values in the TNBS-treated group compared to the DSS-treated group (mean rank = 3 versus 8) were identified and validated by the Mann Whitney test (p = 0.007). Histological examination of the colonic mucosa following the administration of DSS showed multifocal erosions with discrete to mild inflammatory infiltrate represented by polymorphonucleates (PMN), lymphocytes and plasma cells. For the TNBS-induced colitis the histological changes were represented by multifocal areas of ulcerative colitis with mild to severe inflammatory infiltrate. Whole blood GPx values displayed a direct dependence on the chemical agent used. Our results indicate the existence of a correlation between the clinical findings, histopathology, proinflammatory state and oxidative stress.
Conclusions: The experimental DSS/TNBS-induced bowel inflammation used in this study corresponds to the human IBD and is reproducible with characteristics indicative of acute inflammation in the case of the mentioned protocols.

MODELE EXPERIMENTALE DE BOALĂ INFLAMATORIE INTESTINALĂ: CARE ESTE MAI BUN?

Introducere: În mod ideal, modelele experimentale de boală inflamatorie intestinală (IBD) trebuie să se caracterizeze printr-o evoluție clinică previzibilă, histopatologie şi inflamatie similare colitei ulceroase (UC) si bolii Crohn (CD) umane. Scopul acestui studiu a fost să dezvolte două modele ieftine de IBD la sobolani utilizând ca agenti chimici dextran sulfatul de sodiu (DSS) si acidul trinitrobenzen sulfonic (TNBS).
Materiale şi metode: Experimentele au fost realizate pe 15 sobolani masculi, rasa Wistar, în vârstă de 14 săptămâni, distribuiți aleator in trei grupuri a câte 5 sobolani fiecare: grupul A considerat grup de control a primit apă; grupul B a primit timp de 7 zile o solutie de DSS 5% in apa de băut, iar sobolanilor din grupul C li s-a administrat intrarectal o doza unică TNBS de 100 mg/kg. Efectul administrării acute de DSS si TNBS a fost evaluat prin determinarea activității glutation peroxidazei (GPx) din sângele integral, un marker al stresului oxidativ, folosind un kit commercial Randox. Colita experimentală a fost estimată prin úrmărirea evolutiei clinice a bolii. După eutanasiere, intregul colon a fost indepărtat, perfuzat si imersat intr-o solutie de formalină 10%. Sectiunile colonice au fost supuse examenului morfologic după colorarea standard cu hematoxilină eozină. Modul de calculare a scorului histologic a fost descris anterior de Rachmilewitz in vederea stabilirii severității colitei induse de DSS si TNBS in modele de colită murine. Pentru evaluarea diferentelor dintre cele trei grupuri a fost utilizat testul Kruskal-Wallis. Analiza ulterioară ce apelează la testul Mann-Whitney a fost folosită pentru identificarea sursei acestor diferente. Din cauza comparatiilor multiple, pragul de semnificatie a fost ajustat consecutiv unei corectii Bonferroni la 0.02.
Rezultate: Ca urmare a administrării de DSS/TNBS, sobolanii au scăzut în greutate, prezentând diaree, melenă precum şi leziuni histologice specifice colitei spre deosebire de lotul de control. Administrarea DSS a crescut activitatea GPx față de lotul de control şi grupul cu TNBS, dar fără semnificație statistică. S-a observat deasemenea că media concentrației de hemoglobină (Hb) a fost semnificativ mai mare în grupul tratat cu DSS-în comparație cu grupul de control (14.32 g/dL vs 12.58 g/dL). În plus, media concentrației de Hb a fost semnificativ mai mare în grupul cu DSS în comparație cu grupul cu TNBS (14.32 g/dL vs 11.89 g/dL) (p= 0.001). Media concentrației de Hb a fost mai mică, dar fără semnificație statistică, în grupul tratat cu

TNBS față de cel de control (11.89 g/dL vs 12.58 g/dL). Media greutății colonului a fost semnificativ diferită (p = 0.001) în lotul de control (2.38g) în comparație cu lotul tratat cu TNBS (3.87g). Media greutății colonului a fost deasemenea diferită semnificativ (p= 0.002) în grupul cu TNBS (3.87g) în comparație cu grupul tratat cu DSS (2.52g). Diferențele semnificative statistic dintre valorile lungimii colonului (cm) în lotul de control şi grupul cu TNBS au fost confirmate printr-o analiză post-test (testul Mann Whitney; p = 0.019). Alte diferente semnificative statistic între valorile lungimii colonului (cm) din grupul tratat cu TNBS față de cel tratat cu DSS (mean rank = 3 versus 8) au fost identificate şi validate cu testul Mann Whitney test (p = 0.007). Examenul histologic al mucoasei colonice consecutive administrării DSS a evidențiat prezența eroziunilor multifocale cu infiltrat inflamator discret până la mediu reprezentat de polimorfonucleare (PMN), limfocite şi plasmocite. În cazul colitei induse de TNBS, modificările histologice au fost reprezentate de arii multifocale de colită ulcerativă cu infiltrat inflamator de nivel mediu până la sever. Valorile activității GPx din sângele integral au fost dependente în mod direct de agentul chimic utilizat. Rezultatele indică existența unei corelații între tabloul clinic, histopatologic, statusul proinflamator şi stresul oxidativ.

Concluzii: Inflamația intestinală experimentală indusă de DSS/TNBS în acest studiu corespunde bolii inflamatorii umane şi este reproductibilă având, în cazul protocoalelor menționate, caracteristicile unei inflamații acute.

OP-05

MODERN IMMUNOGLOBULINS (IGY) (4). PREVENTION AND TREATMENT OF INFECTIONS WITH ANTIBIOTIC-RESISTANT SPECIFIC PATHOGENIC GERMS IN HUMANS

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Since 1893 when Klemperer discovered immunoglobulins in the chicken egg yolk, many studies have been carried out on the production of specific antibodies and their capacity to protect the organism against infections. During 2000-2006, experts throughout the world talked for the first time about the egg as a pharmaceutical product and about the yolk as an important source of immunoglobulins which can be used for diagnosis, prophylaxis and treatment in humans and animals. The benefits of using chicken antibodies are outstanding compared to those of mammalian antibodies. Among these, the capacity of chicken antibodies to recognize various epitopes than the mammalian antibodies. In the last 25 years, there has been great interestin poultry (Y) immunolgobulins (Ig) as anti-infectious product. The initial studies were independently conducted in Europe, USA, Canada and Japan. Encouraging results were obtained regarding the efficacy of oral IgY against infectious agents and efficacy for the immunesystem recovery of immunosuppressed people. Based on the studies conducted on IgY specific to many pathogenic germs, toxins as well as to some drug-antibody conjugates, science has made a remarkable progress marking the beginning of IgY era. To this extent, ROMVAC S.A. specialists have developed and manufactured since 2012, biological products PC2 ofIMUNOINSTANT product range which gave good results innosocomial infection treatment, paradontosis treatment and other Staphilococus aureus MRSA or Psoeudomonasae ruginosa infections treatment.

IMUNOGLOBULINELE MODERNE (IGY) (4). PREVENIREA ŞI COMBATEREA INFECȚIILOR CU GERMENI PATOGENI SPECIFICI REZISTENȚI LA ANTIBIOTICE LA OM

Incepand cu anul 1893 cand Klemperer a descoperit în gălbenușul ouălor de găină imunoglobulinele, au fost derulate numeroase studii privind productia de anticorpi specifici si capacitatea acestora de a proteja organismul impotriva infectiilor. În perioada 2000-2006 experți din toată lumea au discutat pentru prima dată oul ca produs farmaceutic si despre gălbenuş ca o sursă importantă de imunoglobuline care pot fi folosite în diagnostic, profilaxie şi tratament la animale şi om. Avantajele folosirii anticorpilor produsi de gaina sunt de necontestat

comparativ cu beneficiile folosirii anticorpilor produsi de mamifere. Printre acestea se numara capacitatea anticorpilor produsi de gaina de a recunoaste mai multi epitopi decat anticorpii de mamifere. In ultimi 25 de ani a aparut un interes deosebit pentru imunolgobulinele (Ig) de pasare (Y) ca produs antiinfecțios. Studiile de început au fost realizate independent in Europa, USA, Canada și Japonia. Au fost obtinute rezultate promițătoare cu privire la eficiența administrării orale a IgY contra agenților infecțioși si eficienta in redresarea statusului imunitar al persoanelor imunosupresate. Pe baza studiilor făcute folosind IgY specific pentru numeroși germeni patogeni, toxine ca și a unor conjugare medicamente-anticorpi, știinta a realizat un progres considerabil care marchează începutul erei IgY. In acest context, specialistii de la ROMVAC S.A.au dezvoltat si realizeaza din 2012 produse biologice PC2 din grupul IMUNOINSTANT care se folosesc cu rezultate bune in tratamentul infectiilor nosocomiale tratamentul paradontozelor, dar și al altor infecții cu Staphilococus aureus MRSA sau Psoeudomonas aeruginosa.

OP-06

3D DIFFUSE TENSOR IMAGING IMPORTANT ACQUISITION IN EXPANSIVE TUMORAL AND NON TUMORAL INTRACRANIAL LESIONS

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Introduction: Diffusion tensor imaging (DTI) is a MRI technique that enables the measurement of the diffusion of water in tissue in order to produce neural tract images. The idea of using diffusion data to produce images of neural tracts was first proposed by Aaron Filler & colleagues (1991). Conventional magnetic resonance (MR) imaging has been the standard clinical tool to characterizing and localizing brain tumors. Topical, MR imaging is used to determine the appropriate therapy and for neurosurgical planning if lesion resection is possible. However, even the most anatomically detailed MR imaging does not allow an assessment of specific white matter (WM) tracts. DTI data can be used to visualize the major WM tracts of the brain. DTI is an MR technique that can indirectly evaluate the integrity of WM by measuring water diffusion and its directionality in three dimensions. DTI has been applied to differentiate edema from tumor, in patients with brain tumor for tumor characterization and to assess structural properties of the adjacent tracts. Knowledge of the anatomical relationship between tumor and WM tracts could improve preoperative risk analysis and decrease the risk of WM tract injury during surgery. To minimize injury to the WM tracts adjacent to the tumor, knowledge of their structural integrity would be important.
Material and Method: In the surgery of patients with brain tumors, preservation of vital cerebral function is as important as maximizing tumor resection. The associated morbidity of aggressive resections can be significantly reduced by carefully preservation of vital cerebral function, and the quality of life of these patients will be largely improved. Simultaneously maximizing tumor resection can reduce the chance of recurrence of tumors and improve longer patient survival and long-term functional status. For realizing these two goals, many imaging modalities were used to assess brain tumors, which include conventional MRI, positron emission tomography, magnetoencephalography, and functional MRI. These tools were used to determine the relationship of tumors with surrounding cortical function areas but provide no information concerning the status of the eloquent white matter tracts. Knowledge of the structural integrity and location of eloquent white matter tracts relevant to cerebral tumors is crucial in neurosurgical planning, because damage to these clinically eloquent pathways can result in postoperatively neurological deficits as damage of functional cortical areas. It is very important for designing appropriate neurosurgical plan that determining the exact location of tumors relevant to eloquent white matter tracts. DTI is an important progress in the field of MR imaging. It is the only imaging method that can visualize the 3D structures of white matter tracts in the brain in vivo. Recently some researchers have reported that DTI can be used to illustrate the relationship of clinical eloquent white matter tracts with brain tumors, and they were all restricted to preoperative studies. The localization of tumors in relation to the white matter (WM) tracts (infiltration, deflection), has been one the

most important initial applications. In surgical planning for some types of brain tumors, surgery is aided by knowing the proximity and relative position of tumor. The use of DTI for the assessment of white matter in development, pathology and degeneration has been the focus of over 50, 000 research publications since 2005. In tissue with an ordered microstructure, like cerebral white matter, orientation can be quantified by measuring its anisotropic diffusion. Diffusion-tensor calculations permit the characterization of diffusion in heterogeneously oriented tissue. The spatial orientation of myelinated fiber tracts can then be represented as distinct white matter maps in easily read, color-coded directional maps. Recently, various investigators have used directional diffusion information to create maps of white matter connectivity. These techniques may be valuable for tract identification when the white matter tracts are displaced by tumor. DTI is a useful new preoperative diagnostic tool for evaluating lesions close to vital cortical and subcortical structures. The authors obtain extremly eloquent results using the DTI procedure in delimited intracranial tumors such as meningiomas – fractional anizotropy can be used to diferentiate malignant mengiomas from beningn one -, metastases, pineal tumors and low grade gliomas. The DTI procedure is extremly useful in all intracranial expansive tumoral and it is, at the present, indispensable in determining the strategy and pre-surgery planning. Also, in post surgery period we can evaluate by the help of MRI and DTI the neurosurgery intervention and the effects of the nervous tracts. DTI has a great value also in intracranial non tumoral expansive lesions such as MS.

Conclusions: The recent development of DTI allows direct examination, in vivo, of some aspects of brain microstructure. DTI has already shown to be of value in studies of neuroanatomy, fiber connectivity, and brain development. It has become interesting for investigation of different brain pathology, such as expansive tumoral lesion, cerebral ischemia, trauma, MS, epilepsy and metabolic disorders. However, further improvement in technique and stable postprocessing analyses is needed to increase the utility of DTI in both research and clinical applications. The actual data reveals an ascension of using DTI prcedures in all expansive or non expansive intracerebral lesions.

OP-07

MOLECULAR PROGNOSTIC MARKERS IN PANCREATIC ADENOCARCINOMA

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Introduction: Vascular endothelial growth factor (VEGF) and its receptors (VEGFR1 and VEGFR2) are the most important angiogenesis stimulating factors in pancreatic cancer.

Material and method: This was a prospective study of 108 consecutive patients, with clinical and imaging suspicion of pancreatic neoplasms, based on samples obtained through endoscopic ultrasound-guided fine needle aspiration (EUS-FNA).

Aim of the study: To evaluate VEGFR1 and VEGFR2 gene expression in EUS-FNA samples and to identify prognostic markers in pancreatic adenocarcinoma.

Results: EUS procedures. EUS-guided FNA had an accuracy of 93% for the diagnosis of pancreatic adenocarcinoma. Molecular analysis. Based on real-time qPCR analysis, VEGFR1 and VEGFR2 expressions were present in 90% and 65% of the analysed samples obtained through EUS-guided FNA, respectively. Survival rate. Almost 90% of patients died during the study, with a median survival rate of only 9 months. The survival was directly influenced by the initial stage and by the presence of VEGFR1 and VEGFR2 gene expression. Prognostic factors. We further used a Cox model regression analysis. Thus, VEGFR1 was found to be a negative predictive factor for tumor resectability and for the survival of less than 6 months from diagnosis. VEGFR2 was identified as a predictor for survival of less than 12 months from diagnosis. The VEGFR1 /

VEGFR2 coexpression was the factor with the largest negative prognostic implications for the survival at 6 months and for the mortality in pancreatic adenocarcinoma.
Conclusion: EUS is a very effective technique for the diagnosis and staging of pancreatic adenocarcinoma, with an accuracy that can reach up to 93%. Furthermore, the role of molecular analysis of EUS-guided FNA samples was established by the assessment of VEGFR1, VEGFR2 and coexpression VEGFR1 / VEGFR2, which were prognostic markers in pancreatic cancer.

MARKERI MOLECULARI PROGNOSTICI IN ADENOCARCINOMUL PANCREATIC

Introducere: Factorul de creștere a endoteliului vascular (VEGF) și receptorii săi (VEGF-R1 and VEGF-R2) sunt cei mai importanți factori de stimulare a angiogenezei in cancerul pancreatic.
Material și metodă: Au fost studiați din punct de vedere epidemiologic, clinic, endosonografic, citologic si genetic, 108 pacienți cu suspiciune clinică și imagistică de neoplasm pancreatic.
Obiective: Evaluarea expresiei genetice a VEGF-R1 și VEGF-R2 din probele de puncție fină aspirativă ghidată echoendoscopic (EUS-FNA) și identificarea unor markeri prognostici în adenocarcinomul pancreatic.
Rezultate: Examinarea endosonografică. Ecoendoscopia are o acuratețe de aproximativ 93% in diagnosticarea cancerului pancreatic. Analiza moleculară. Expresiile VEGF-R1 și VEGF-R2 au fost prezente în 90%, respectiv 65% din probele analizate. Supraviețuire. Aproximativ 90% din pacienți au decedat, cu o spuraviețuire medie de 9 luni. Supraviețuirea a fost direct influențată de stadializarea avansată și de prezența expresiilor VEGF-R1 și VEGF-R2. Factori prognostici. Utilizând un model de regresie Cox, pentru identificarea factorilor cu implicație prognostică, s-a constatat că VEGF-R1 este marker prognostic pentru statusul de inoperabilitate și pentru supraviețuirea sub 6 luni de la diagnostic. VEGF-R2 este factor predictiv pentru supraviețuirea sub 1 an de la diagnostic, iar co-expresia VEGF-R1 / VEGF-R2 reprezintă factorul cu cea mai mare implicație prognostică în ceea ce privește supraviețuriea sub 6 luni și decesul pacienților cu adenocarcinom pancreatic.
Concluzii: EUS reprezintă o metodă eficientă de diagnostic și stadializare în adenocarcinomul pancreatic, cu o acuratețe de 93%. Mai mult, analiza moleculară a probelor citologice recoltate prin EUS-FNA a demonstrat că VEGF-R1, VEGF-R2 și coexpresia VEGF-R1/VEGF-R2, pot fi considerați markeri prognostici în cancerul pancreatic.

OP-08

MOLECULAR MARKERS IN ARTERIAL HYPERTENSION

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Hypertension is a complex disease determined by the interconnection between multiple molecular, patho-physiological and environmental factors. This paper reviews the potential role of various molecular markers in the mechanisms associated with the development and consequences of arterial hypertension. Molecular markers are effective tools that have been used to identify small changes (particular DNA sequences) within the mapping population allowing for segregation of traits and identity. The importance of genetic determination in hypertension is best represented by the fact that family history increases the risk of developing hypertension by approximately 4 times. Genomic technologies make it possible today to genotype millions of genetic variants across the human genome. So far the most powerful approaches for finding genetic variants influencing susceptibility to hypertension have been genome wide association studies (GWAS) between single nucleotide polymorphisms (SNPs) and disease phenotypes. Connecting SNPs to causal pathways proved to be challenging. Only 2 GWAS analyzed hypertension as a dichotomous trait and resulted in identification of tractable signals in pathways of blood pressure regulation. One SNP was located in UMDO (uromodulin gene) and the second one was found near the endothelial NO synthase (eNOS) gene. Not all features of gene regulation are encoded in genes or DNA sequences. DNA methylation, histone modification, and alteration of microRNA expression - referred to as epigenetics - may also contribute to blood pressure gene regulation. MicroRNAs, as essential gene

expression regulators, modulate cardiovascular development and disease and are emerging as potential biomarkers and therapeutic targets in cardiovascular disease. Several studies analyzed the association between various biomarkers and hypertension. Recent studies have shown that tissue expression and plasma concentrations of several inflammatory markers and mediators are associated with increased risk of hypertension. Also it has been proved that subjects with resistant hypertension have higher levels of inflammatory cytokines as well as an increased arterial stiffness indicating that inflammatory mechanisms may play an important role in the pathogenesis and progression of hypertension. Unfortunately, despite considerable research, the molecular mechanisms of arterial hypertension remain incompletely understood. The quest to solving the genetic origins and molecular pathways of hypertension has not yet come to an end, and further research in this field will help improve the understanding of blood pressure pathophysiology and may eventually lead to the development of novel treatment approaches for hypertension.

MARKERI MOLECULARI IN HIPERTENSIUNEA ARTERIALA

Hipertensiunea arteriala este o patologie complexa determinata de interconexiunea dintre numerosi factori moleculari, fiziopatologici si de mediu. Lucrarea de fata isi propune sa treaca in revista rolul diferitilor markeri moleculari in mecanismele legate de aparitia hipertensiunii si a complicatiilor acesteia. Markerii moleculari sunt un mijloc eficient de a identifica mici modificari (in special secvente de ADN) in populatia studiata permitand segregarea identitatii si a trasaturilor. Importanta determinarii genetice in hipertensiune este cel mai bine evidentiata de faptul ca prezenta antecedentelor heredocolaterale de hipertensiune creste de 4 ori riscul aparitiei acesteia. Tehnologiile genomice actuale fac posibila genotiparea milioanelor de variante genetice din genomul uman. Studiile de asociere genomica (GWAS) dintre polimorfismul uninucleotidic (SNP) si diversele fenotipuri reprezinta cea mai buna abordare pentru gasirea variantelor genetice ce influenteaza susceptibilitatea aparitei hipertensiunii. Conexiunea dintre SNP-uri si caile fiziopatologie s-a dovedit a fi dificila. Doar 2 studii de asociere genomica au analizat hipertensiunea ca o entitate dihotomica si au identificat o conexiune clara cu 2 cai de semnalizare implicate in hipertensiune: una la nivelul genei uromodulinei (UMOD) si cealalta alaturata genei sintetazei oxidului nitric (eNOS). Nu toate caracteristicile reglarii genelor sunt codificate in secvente ADN. Metilarea ADN-ului, modificarea histonelor si alterarea expresiei micro ARN (ce fac studiul epigeneticii), ar putea contribui la reglarea genelor implicate in controlul tensiunii arteriale. MicroARN urile, ca reglatori esentiali ai expresiei genelor, moduleaza evolutia bolilor cardiovasculare, avand potentialul de a deveni biomarkeri si chiar tinte terapeutice. Mai multe studii au analizat asocierea dintre diversi biomarkeri si hipertensiune. S-a demonstrat ca expresia tisulara sau concentratia plamatica a diferitilor markeri si mediatori ai inflamatiei este asociata cu cresterea riscului de hipertensiune. De asemena s-a descoperit ca pacientii cu hipertensiune rezistenta la tratament au nivele ridicate de citokine inflamatorii si rigiditate crecuta a peretelui arterial indicand un posibil rol al inflamatiei in patogeneza si progresia hipertensiunii. In ciuda studiilor facute pana acum, mecanismele moleculare ale hipertensiunii raman incomplet elucidate. Incercarea de a gasi originea genetica exacta si mecanismele aparitiei hipertensiunii arteriale nu a luat sfarsit, impunandu-se continuarea cercetarilor in domeniu, ce vor duce la aprofundarea intelegerii mecanismelor fiziopatologice si in cele din urma la noi abordari terapeutice in hipertensiune.

OP-09

FROM MOLECULAR MARKERS TO PERSONALIZED BRAIN TUMOURS THERAPY

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The most commonly used chemotherapy in brain cancer is the combination of procarbazine, lomustine and vincristine (PCV). The alkylating agent TMZ is another therapeutic approach used for the treatment of both low

and high grade astrocytic tumors, largely replacing the PCV treatment, as a result of its oral administration and minor side effects. Addition of TMZ to surgery and radiotherapy acts as the standard treatment of high grade glioma. Intrinsic or acquired resistance to chemotherapy is critical points in treatment efficacy. Several growth factor receptor (GFR) family members are overexpressed or overactivated in glioma, playing an important role in treatment resistance. Aberrant activity of several GFRs and their intracellular signaling has been reported in the majority of high grade gliomas. Here, we investigated the effect of several approaches that inactivate GFRs activity and signaling on brain tumour cells viability in vitro. GFRs inactivation induced cytotoxicity in brain tumour cell lines and induced apoptosis by activating Caspase 3, 8 and 9.

DE LA MARKERI MOLECULARI LA TERAPII PERSONALIZATE IN TUMORILE CEREBRALE

Modalitatea chimioterapică cea mai frecventă în tratamentul tumorilor cerebrale este combinația de procarbazină, lomustină și vincristină (PCV). Agentul de alchilare temozolomidă (TMZ) este o altă abordare terapeutică utilizată pentru tratamentul tumorilor astrocitare de grad înalt şi de grad scăzut, înlocuind în mare măsură tratamentul PCV, ca urmare a administrării sale orale și a efectelor secundare minore. Tratamentul multimodal cuprinzând TMZ, chirurgie şi radioterapie este de asemenea utilizat ca terapie standard în glioamele de grad înalt. Rezistența intrinsecă sau dobândită la chimioterapie este una dintre problemele majore în eficacitatea tratamentului tumorilor cerebrale. Numeroși receptori ai factorilor de creștere (GFR) sunt supraexprimați sau supractivați în gliom, jucând un rol important în rezistența la tratament. Activitatea aberantă a acestor receptori membranari și a semnalului intracelular transdus de aceștia a fost raportată în majoritatea glioamelor de grad înalt. În studiile noastre am investigat efectul inactivării GFRs și a moleculelor din cascada de semnalizare intracelulara asupra viabilității celulelor tumorale cerebrale in vitro. Rezultatele noastre au arătat că inactivarea GFRs a produs citotoxicitate în liniile celulare tumorale cerebrale si a indus apoptoză prin activarea caspazelor 3, 8 și 9.

OP-10

UPPER GASTROINTESTINAL CANCERS

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Upper gastrointestinal cancers — such as hepatocellular carcinoma, cholangiocarcinoma or pancreatic ductal adenocarcinoma—are notoriously resistant to anti-cancer systemic therapies, and often recur even after aggressive local therapies leading to dismal survival rates. Recent developments in gastrointestinal oncology have offered renewed hoped for the development of more efficacious therapies. For example, our understanding of the oncogenic drivers in carcinogenesis has increased exponentially, and may potentially allow personalization of therapy.In addition, a great interest has been recently to target immune checkpoints, and unleash the potential of the immune system against cancers. However, optimal translation of these studies into new therapeutic approaches will require a concerted effort in clinical trials and in preclinical studies.I will present results from clinical correlative studies and preclinical models of these diseases performed at our institution and in collaboration with the Fundeni Clinical Institute. The insights gained from this “bench-to-the-bedside and back” approach raisethe hope for a more efficient development of targeted agents in liver and pancreatic cancers, with the goal of increasing survival in patients afflicted with these aggressive diseases.

OP-11

LACTOFERRIN-DERIVED PEPTIDES WITH ANTI- HEPATITIS B VIRUS ACTIVITY- IN VITRO STUDIES

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Lactoferrin (Lf) is an iron binding glycoprotein which was shown to exhibit antiviral activity at an early phase of infection. It was suggested that Lf interaction with host cell surface molecules such as glycosaminoglycans (GAGs) could be part of the mechanism of action. Two GAGs binding sites located in the N-terminal (N-t) region of Lf could be involved in its antiviral activity, one of them being a cationic cluster (GRRRR). We have investigated seven human Lf (HLf)-derived peptides (HLP), corresponding to the N-t domain of the native protein (1-47 amino acids sequence) for their capacity to prevent hepatitis B virus (HBV) infection and replication using HepaRG and HepG2.2.2.15 cell lines. Of the series tested, four peptides demonstrated inhibition of HBV infection in HepaRG cells between 40 to 80%. The most potent inhibitor was HLP1-23, a peptide containing the GRRRR cluster which prevented HBV infection at 250 µM by neutralizing the viral particles. In an effort to improve the antiviral activity of HLP1-23 we further used computer modeling followed by chemical synthesis. A new mutant peptide with increased overall positive charge and aromaticity, supposedly displaying improved affinity through additional GAGs binding site and increased stability through supplementary aromatic stacking interactions was designed. The new peptide HLP1-33 was assayed for potential cytotoxicity in HepaRG cells and anti-HBV activity on HepaRG cell system. The results revealed that HLP1-33 peptide has good solubility in aqueous solution and is not toxic for concentrations up to 100 µM. Preliminary results showed that HLP1-33 is able to inhibit HBV infection on HepaRG cells by 50% at 100 µM. Inhibition of HBV replication in HepG2.2.2.15 cellular systems is currently under investigation. Lf-derived peptides may constitute a non-toxic approach for potential clinical therapy in inhibiting early steps of HBV infection or protection of regenerated hepatocytes from de novo infection.

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OP-12

BONE MARROW NICHE, A DRUG FREE SANCTUARY FOR CANCER STEM CELLS

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Survival of patients with hematological malignancies and cancer in general depends on our ability to eliminate the last bastion of malignant cells, the so called “minimal residual disease”. Inspired by Sir Paget’s “soil and seed” hypothesis, it is now clear that there is a constant feedback between the cancer cells and their surrounding microenvironment. This “molecular conversation” reinforces a protective niche that can support cancer cells and needs to be overcome in order to eliminate minimal residual disease and improve cure rates in our patients. Studying the role of bone marrow mesenchymal microenvironment, we have discovered that normal hematopoietic stem cells are intrinsically programmed to undergo differentiation and form blood with subsequent exhaustion. It is the bone marrow niche that controls stem cell behavior to match physiological needs and maintain stem cells for the life span of the organism. During these studies, we have uncovered that bone marrow mesenchymal cells express drug detoxifying enzyme at levels comparable to hepatocytes. More so,

these mesenchymal cells are able to metabolize chemotherapy and create true “drug-free sanctuaries” in the bone marrow. The biochemical barrier generated by the bone marrow mesenchymal cells is in many ways similar to the better known “blood-brain barrier” and should be by-passed in order to eliminate resting cancer stem cells. Our more recent data bring to light how the malignant cells promote their survival by re-enforcing this barrier. Tools to by-pass this protective mechanism have been developed in our laboratory and are in phase II clinical trials at John Hopkins and soon to be tested at Fundeni Clinical Institute.

OP-13

HEPATOCYTE TELOMERASE EXPRESSION IN CHRONIC LIVER DISEASE AND PRIMARY LIVER TUMORS - ARGUMENTS FOR HEPATOCYTE REPROGAMMING?

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Hepatocellular carcinoma is diagnosed in > 90% of cases in the context of liver cirrhosis. Chronic liver disease is characterized by hepatocyte senescence due to prolonged hepatocyte division to support liver regeneration. Hepatocyte reprogramming to a stem-cell like phenotype is a new concept in chronic liver disease and supports liver regeneration as an adaptative mechanism. Telomerase (TERT) overexpression during chronic liver disease is a potential marker of hepatocyte reprogramming. According to latest studies, TERT gene promotor harbors the most frequently encoutered mutations in hepatocellular carcinoma, suggesting that it is an active gene promotor in liver cirrhosis, as a premalignant condition. TERT expression has been investigated in hepatocellular carcinoma nodules, as well as in chronic liver disease, by quantitative gene expression as well as immunohistochemistry. TERT over-expression has been detected by qRTPCR in 21.9% of HCC nodules and by immunohistochemistry in 77.7% of HCC cases. This difference is explained by the fact that TERT is already expressed in non –tumoral liver cirrhosis so that tumoral over-expression is registered in only a subgroup of HCC cases. There was no correlation between TERT over-expression and clinical parameters such as tumor diameter, Milan criteria, Edmondson Steiner tumor grading, or risk of tumor recurrence, however there was a tendency towards lower overall survival in patients with HCC TERT over-expression. Relative gene expression of reprogramming factors OCT4, SOX2 and NANOG in HCC nodules vs. adjacent non-tumoral cirrhotic tissue has also been investigated. Significant SOX2 over-expression was detected in 59.5%, NANOG over-expression in 26.2% of cases and OCT4 over-expression in 4.9% of HCC cases, respectivelly. No correlations could be identified between TERT over-expression and reprogramming factors over-expression in HCC, suggesting that TERT activation and reprogramming events secondary to reprogramming factors over-expression are distinct processes during the oncogenic process. TERT and reprogramming factors gene expression studies could bring new insights into liver regeneration process during chronic liver disease and liver cirrhosis, as a premalignant condition.

EXPRESIA HEPATOCITARA DE TELOMERAZA IN BOALA HEPATICA CRONICA SI TUMORILE HEPATICE PRIMARE – ARGUMENTE PENTRU PROCESUL DE REPROGRAMARE HEPATOCITARA?

Carcinomul hepatocelular este diagnosticat in >90% din cazuri la pacientii cu ciroza hepatica. Boala hepatica cronica este caracterizata de senescenta hepatocitara in urma episoadelor repetate de diviziune hepatocitara care sustin procesul de regenerare hepatica. Reprogramarea hepatocitara la un fenotip cecular stem-like sustine procesul de regenerare hepatica in cursul bolii hepatice cronice, fiind fenomen adapatativ asociat senescentei hepatocitare. Supraexpresia de telomeraza (TERT) in boala hepatica cronica este un indicator potential al reprogramarii hepatocitare din cursul bolii hepatice cronice. Conform unor studii recente, promotorul telomerazei regrupeaza cele mai multe mutatii in carcinomul hepatocelular, sugerand ca este un promotor extrem de activ inca din stadiul de ciroza hepatica, drept conditie premaligna. Expresia TERT a fost investigata in carcinomul hepatocelular si in tesutul cirotic non-tumoral prin qRT-PCR si imunohistochimic. Supraexpresia TERT la nivelul nodulilor tumorali s-a inregistrat la 21.9% din cazurile studiate, expresia TERT fiind detectabila imunohistochimic in 77.7%

din cazurile de hepatocarcinom. Aceasta diferenta este explicata de faptul ca TERT este deja exprimata in tesutul cirotic, supraexpresia ei fiind decelata astfel doar intr-un subgrup de carcinoame hepatocelulare. Nu s-a inregistrat nici o corelatie intre supraexpresia tumorala TERT si parametrii clinici precum dimensiunea nodulului tumoral, clasificarea Milano, gradingul tumoral Edmondson Steiner sau riscul de recidiva tumorala dupa o procedura potential curativa. S-a inregistrat insa o supravietuire globala mai scazuta la pacientii cu supraexpresie tumorala de telomeraza. Expresia genica relativa a factorilor de reprogramare celulara OCT4, SOX2, si NANOG in nodulii HCC comparativ cu tesutul cirotic non-tumoral a fost de asemenea investigata. Supra expresia SOX2 s-a inregistrat in 59.5% din cazuri, NANOG in 26.2% din cazuri iar OCT4 in 4.9% din cazuri. Nu s-au evidentiat corelatii intre supra-expresia TERT si supraexpresia factorilor de reprogramare celulara, sugerand faptul ca activarea expresiei TERT si reprogramarea secundara supraexpresiei factorilor de reprogramare celulara sunt procese independente, in cursul cascadei oncogenice din carcinomul hepatocelular. Studiul expresiei TERT si a factorilor de reprogramare celulara pot aduce noi informatii cu privire la procesul de regenerare hepatica din boala hepatica cronica si ciroza hepatica, drept conditie premaligna.

OP-14

100% SVR12 WITH INTERFERON-FREE REGIMENS IN BOTH PRE AND POSTTRANSPLANT PATIENTS WITH HEPATITIS C ALTHOUGH ON TREATMENT VIRAL KINETICS DIFFER BETWEEN CIRRHOSIS AND LT RECIPIENTS

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Background: Over recent years, the efficacy of antiviral C therapy has improved dramatically using new direct-acting antiviral (DAA) agents, leading to SVR rates over 90% in non-transplant patients, as well as in transplanted recipients.
Aim: To present our experience with DAA agents in cirrhotics on the waiting list for LT and in LT recipients, regimens obtained by compassionate-use.
Methods: Our EAP cohort consisted of patients 19 patients with liver cirrhosis and 17 patients with recurrent hepatitis C after LT. All patients received associated ribavirin. The 3D regimen was administered 12 weeks for patients with cirrhosis and 24 weeks for LT recipients.
Results: There were analyzed 17 females and 19 males with a mean age of 52.5±6.9 years.In cirrhotic population median MELD score was 11.6 (range 9-16). 3 patients from the LT recipients had F4 METAVIR, 4 patients F3 METAVIR, 6 patients F2 METAVIR and 4 had F1 METAVIR. Median time since LT was 15 months (range 6-87 months). On treatment virologic response was 100% in both cirrhotics and LT recipients. End of therapy and sustained virological response (SVR 12) in this whole cohort of patients was 100%. However, at week 2 after beginning of therapy, 6 out of 19 cirrhotic patients were HCV RNA undetectable and only 2 out of 17 LT recipients (p=0.12). Undetectable HCV RNA at 4 weeks of therapy was present in 17 patients with cirrhosis and in 8 patients with recurrent hepatitis C (p=0.01). At week 6 all patients with cirrhosis had negative viral load while LT recipients became all negative by week 8.
Conclusion: Although there is a significant difference regarding viral load decrease on interferon free therapy between patients with liver cirrhosis and LT recipients, most of them noncirrhotic, SVR12 is 100%.

100% SVR12 CU REGIMURILE INTERFERON-FREE LA PACIENTII PRE SI POST-TRANSPLANT HEPATIC CU HEPATITA CRONICA C DESI CINETICA VIRALA IN TIMPUL TRATAMENTULUI DIFERA INTRE PACIENTII CU CIROZA HEPATICA SI CEI TRANSPLANTATI

Introducere: In ultimiiani, eficacitatea terapiei antivirale impotriva VHC s-a imbunatatit dramatic prin folosirea noilor agenti antivirali cu actiune directa (DAA) conducand la rate de RVS de peste 90% atat la pacientii transplantati cat si la cei netransplantati.

Scop: Sa prezentam experienta noastra cu agentii DAA la ciroticii aflati pe lista de asteptare pentru TH si la pacientii transplantati, regimuri obtinute printr-un program compasional.
Metode: In studiul nostrum au fostinclusi 19pacienti cu ciroza hepatica si 17 pacienti cu hepatita recurenta C posttransplant hepatic. Toti pacientii au primit tratament antiviral in asociere cu ribavirina.Regimul 3D a fost administrat timp de 12 saptamani pentru pacientii cu cirozasi 24 de saptamani pentru pacientii transplantati.
Rezultate: Au fost analizati 19 barbati si 17 femei cu o varsta medie de 52.5±6.9 ani. In populatia cirotica scorul MELD median a fost de 11.6 (limite 9-16).Trei pacienti transplantati au avut scorul F4 METAVIR, 4 pacienti scorul F3 METAVIR, 6 pacienti F2 METAVIR si 4 pacienti F1 METAVIR. Timpul median de la TH a fost de 15 luni (limite 6-87 luni).Raspunsul virusologic in cursul tratamentului a fost 100% atat la cirotici cat si la transplantati. Raspunsul virusologic la sfarsitul tratamentului si raspunsul virusologic sustinut (RVS) a fost 100% in intreaga cohort de pacienti.La 2 saptamani de tratament 6 din 19 pacienticiroticierau ARN VHC nedetectabil si numai 2 din 17 pacienti transplantati (p=0.12). ARN VHC nedetectabil la 4 saptamani de tratament s-ainregistrat la 17 pacienti-ciroticisi 8 pacienti cu hepatitarecurenta C (p=0.01). La saptamana 6 totipacientii cu ciroza aveau incarcatura virala negativa, in timp ce pacientii transplantati au fost negative la 8 saptamani.
Concluzii: Desi a fost o diferenta semnificativa in scaderea viremiei in cursul tratamentului interferon free intrepacientii cu cirozasi transplant hepatic, majoritatea non-cirotici, rata RVS a fost 100%.

OP-15

MARKERS OF HEPATITIS B VIRUS INFECTION IN PEOPLE WHO INJECT DRUGS

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Background: Before the introduction of routine HBV immunization of all newborns in 1995, Romania was an endemic country for HBV infection, mostly acquired perinatally. During the last 5 years an alarming increase in the number of injecting drug users (IDUs) co-infected with HIV and viral hepatitis was reported, fact that may impact the local HBV prevalence.
Objective: The aim of our study was to determine the prevalence of HBV infection among IDUs admitted in a single tertiary facility and to evaluate their epidemiological and virological characteristics.
Methods: A cross sectional study on 123 IDUs tested between 2011 -2015 for HIV infection and viral hepatitis serological markers and active viral replication.
Results: Out of 123 evaluated IDUs, 93 (75.6%) tested positives for anti-HBc IgG antibodies, among them 39.7% (37/93) were HBsAg carriers, 34.4% (32/93) were immune after natural infection and 25.8% (24/93) presented isolated HBcIgG antibodies. The majority of HBsAg carriers were young males (94.5%), with a median age at drug use initiation of 17 years; mostly using both heroin and ethnobotanical drugs (67.5%) for a median time of 8.5 years. The majority of HBsAg carriers, 91.8% (34/37), were also HCV co-infected and 62.1% (23/37) HIV co-infected. Overall, 64.8% (24/37) of the HBV chronic carriers presented detectable HBV-DNA, but active viral replication (ADN-VHB>1000 UI/mL) was found in only 35.1% (13/37), associated with significantly higher hepatic cytolysis (ALT-1396 vs. 265 UI/mL). Among HIV-infected IDUs, HBsAg portage was more frequently associated with higher HIV viral loads (5.00 vs. 4.77 log10 copies/mL, p=0.18), but good immunologic status (median CD4 cell count >500/mm3 in 34.2% vs. 28.5%, p=0.34), and accompanied by active HBV viral replication (38.4% vs. 10%, p<0.001). In patients with active viral replication, the most commonly detected HBV genotype was D (8/13, 61.5 %) followed by genotype A (4/13, 30.7%). Patients with genotype D had lower HBV viral load (154000 vs. 8212500 UI/mL) and were more often HIV co-infected (72.2% vs. 33.3% p<0.001).
Conclusions: A high percentage of IDUs have markers of past or present HBV infection, frequently associated with both HCV and HIV co-infections. Nevertheless, active viral replication is present only in a third of the HBs

chronic carriers, more frequently among those co-infected with HIV. HBV screening and vaccination among individuals in vulnerable groups are mandatory in order to reduce the transmission risk and liver disease progression.

OP-16

EPIGENETIC ALTERATIONS IN CERVICAL CARCINOMA

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Background: High-risk HPV(hr-HPV) genotypes infection associates with cervical dysplasia and carcinogenesis. Cervical cancer is one of the most frequent cancers affecting women in Romania. Infection with hr-HPV is the main cause for cervical neoplasia but there are other cofactors associated with cervical carcinogenesis. Epigenetic regulation of gene expression is an important feature ofcancerogenesis and involves epigenetic changes such as DNA methylation, histone modification, non-coding RNAs activity.
Objective: to investigate the expression of epigenetic factors involved in HPV oncogenic transformation in cervical samples.
Material and methods: Starting from our previous studies based on E6, E7 HPV18 silencing in HeLa cell line, we decided to investigate the expression levels of selected epigenetic factors involved cervical carcinogenesis and for this purpose 159 cervical specimens (20÷77 years old, median: 36.7) from women with hr-HPV-induced dysplastic cervical lesions (CIN1; CIN2+) and squamous cervical carcinoma (SCC) were selected. All samples were tested for hr-HPV DNA presence (Linear Array, Roche). 40 women (20–48 years old, median: 32) with negative cytological smears and HPV DNA negative were included as control group. Total RNA was isolated and targeted genes expression levels were analysed by qRT-PCR. Statistical analysis was performed using GraphPad Prism 6.
Results: The obtained data revealed a different pattern of expression for enzymes involved in DNA methylation (DNMTs) and in chromatin remodelling (KDMs) in precancerous /cancerous lesions as compared with controls. Moreover, our results identified a significantly differential pattern of expression for some long non-coding RNAs in cervical carcinoma vs controls. We found that among all the investigated factors DNMT1 (p=0.0013), KDM1B (p=0.004), KDM6A (p=0.004), KDM6B (p=0.0225) methylases and lncRNAs MALAT1 (p=0,0036), H19 (p=0.007) and MEG3 (p=0.003) display significant levels correlated with histological degree and also with viral infection.
Conclusions: Our results sustain the involvement of epigenetic changes in HPV-induced cervical dysplasia and stress the necessity of epigenetic events evaluation among cervical samples proposing their assessment as a potential diagnostic tool along with other valuable markers.
Acknowledgements: The present study was supported by national research project: PN-II-RU-TE-2014-4-2502 (OncoNuRD).

MODIFICARI EPIGENETICE IN CANCERUL CERVICAL

Introducere: Infecția persistentă cu genotipurile HPV reprezintă un eveniment important în procesul de transformare malignă a epiteliului cervical. Infecția cu anumite genotipuri HPV de risc înalt (hr-HPV) poate conduce la apariția neoplaziei cervicale intraepiteliale și a carcinomului invaziv. Infecția cu hr-HPV reprezintă principala cauză pentru apariația cancerului cervical, dar au fost identificați și alți cofactori asociați cu transformarea neoplazică. O caracteristică importantă a procesul de cancerogeneză este reglarea expresiei genice prin intermediul modificărilor epigenetice (metilarea ADN, modificările histonelor şi activitatea ARN necodificator).
Scopul acestui studiu este de a investiga nivelul de expresie al unor factori epigenetici implicați în transformarea malignă HPV-indusă în probe cervicale.
Materiale şi metode: Pornind de la rezultatele obținute prin silențierea oncogenelor virale E6 şi E7 HPV18 în linia celulară HeLa, am decis investigarea nivelului de expresie al unor factori epigenetici selectați implicați în cancerogeneza cervicală iar în acest sens au fost selectate 159 de paciente cu vârsta medie de

36,7 (între 20-77 ani) cu leziuni displazice de col. De la acestea s-au prelevat chirurgical fragmente biopsice pentru investigații anatomo-patologice şi pentru izolarea acizilor nucleici în vederea testării virale (ADN HPV) şi a determinării prin Real-Time PCR a nivelului de expresie pentru factorii investigați. Analiza statistică s-a realizat utilizând GraphPad Prism 6.
Rezultate: Datele obținute au indicat un pattern diferit de expresie pentru enzimele implicate în metilarea ADN (DNMT) sau remodelarea cromatinei (KDM) n leziunile precursoare şi cancer cervical comparativ cu controalele. Totodată rezultatele au identificat o expresie semnificativă a unor ARNlnc în cancerul cervical vs. controale. S-a observat că dintre factorii studiați metilazele: DNMT1 (p=0,0013), KDM1B (p=0,004), KDM6A (p=0,004), KDM6B (p=0,0225) şi ARNlnc: MALAT1 (p=0,0036), H19 (p=0,007) şi MEG3 (p=0,003) prezintă un profil de expresie diferit corelat cu gradul de severitate al leziunii şi cu infecția virală.
Concluzii: Rezultatele obținute susțin implicarea modificărilor epigenetice în transformarea neoplazică HPV-indusă şi subliniază importanța studierii acestora în cancerogeneza cervicală.
Acknowledgements: Studiul a fost realizat cu ajutorul proiectului PN-II-RU-TE-2014-4-2502 (OncoNuRD).

OP-17

EARLY MOLECULAR MARKERS OF PREHYPERGLYCAEMIC STAGES OF CLINICAL DIABETES

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The prevention of any diabetes phenotype need to be preceeded by a good prediction. The sooner it is predicted, the higher is the chance to prevent the various phenotypes of diabetes. For that, our strategy is to go backward, from the clinical onset to the first identified trigger of autoimmune diabetes (type 1 diabetes - T1D and intermediary diabetes - IDM) or non-autoimmune diabetes (type 2 diabetes - T2D and other phenotypes). We have in view to use various risk factors: clinical, biochemical, immunological, hormonal, chemochemical, adipochemical and genetical. Such markers could be obtained by analyzing skin, sweat, saliva, blood (antibodies, branched or aromatic amino acids, lipids and carbohydrates derivatives, proinsulin, insulin, adiponectin and others) and, occasionally, tissues available during various surgical interventions (adipose tissue, pancreatic tissue, lymph nodes, vessels, skin, liver, etc.). Preliminary results obtained by some new devices and dedicated software will be presented and discussed.

OP-18

CHOLANGIOCARCINOMA: GENETIC LANDSCAPE AND ITS IMPLICATIONS

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Cholangiocarcinoma (CCA) is a malignant tumor of bile duct epithelial cells with with a 5-year survival rate of less than 5%. Notably the incidence rate is increasing worldwide. Chronic inflammation resulting from liver fluke infection, hepatitis and other inflammatory bowel diseases is a major contributing factor to carcinogenesis, likely through accumulation of serial genetic and epigenetic alterations resulting in aberration of oncogenes and tumor suppressors. Recent studies making use of advances in high-throughput genomics have revealed the genetic landscape of CCA, greatly increasing understanding of its underlying biology and improve treatment options. A series of highly recurrent mutations in genessuch as TP53, KRAS, SMAD4, BRAF, MLL3, ARID1A, PBRM1 and BAP1, which are known to be involved in cell cycle control, cell signaling pathways and chromatin dynamics, have led to investigations of their roles, through molecular to mouse modelling studies. On the other

hand, potential therapeutic targets such as FGFR translocation and IDH mutations have been identified leading to ongoing clinical trials. Comparisons between liver fluke-related and non-infection related CCA have revealed significant differences in genetic and epigenetic pattern, indicating that different causative etiologies may induce distinct somatic alterations and frequencies, even within the same tumor type.

OP-19

PATTERNS OF PROTEIN PHOSPHORYLATION IN BCR-ABL1 NEGATIVE MYELOPROLIFERATIVENEOPLASMS

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Introduction: BCR-ABL1 negative myeloproliferative neoplasms (MPN) are clonal disorders of hematopoietic stem cells (HSC) that share a common pathogenic feature - constitutive activation of the JAK2/STAT pathway. JAK2V617F mutation is the main phenotipic driver mutation in MPN, followed by somatic mutation in calreticulin (CALR) gene. According to recent data, CALR mutants activate specifically the thrombopoietin receptor (TpoR) and, subsequently, JAK2. However, the mechanisms of CALR-induced JAK2/STAT pathological signaling need to be better characterized.
Material and methods: The first objective of our study was to establish the prevalence of CALR mutations in a lot of JAK2V617F negative MPN patients by Sanger sequencing of CALR exon 9. Secondly, representative patients with a 52-bp CALR deletion (del52) were selected for analyzing the protein phosphorylation profiles in white blood cell lysates using a human phospho-kinase array. Both matched controls and JAK2 V617 positive patients were included in the experiment.
Results: CALR mutations were detected in 52 of the 90 JAK2V617F negative MPN patients (57.7%),type 1 CALR mutation (del52) being the most prevalent (61.5% of total CALR mutations).Phospho-kinase profiling indicated a similarCALR and JAK2V617F phosphorylation pattern of Tor, Stat2 and Srcand a CALR specific phosphorylation pattern of Gsk3, Msk1/2, Hsp27. These preliminary results suggest new insights in the signaling mechanisms for MPN driver mutations that need to be explored by further studies.
Acknowledgements: PCCA PN II 133/2014 and POS CCE O1.1.2. 433/2012.

PROFILURI DE FOSFORILARE PROTEICĂ ÎN NEOPLASMELE MIELOPROLIFERATIVE BCR-ABL1 NEGATIVE

Introducere: Neoplasmele mieloproliferative BCR-ABL1 negative (MPN) sunt afecțiuni clonale ale celulei stem hematopoietice (HSC), care au ca element patogen comun activarea constitutivă a căii JAK2 / STAT. JAK2V617F este principala mutație driver în MPN, urmată de mutațiile somatice în gena calreticulinei (CALR). Conform datelor recente, mutațiile CALR activează în mod specific receptorul trombopoietinei (TpoR) și, ulterior, JAK2. Cu toate acestea, este necesară o caracterizare mai bună a mecanismelor semnalizării patologice JAK2 / STAT induse de CALR.
Material si metode: Primul obiectiv al studiului nostru a fost de a stabili prevalența mutațiilor CALR într-un lot de pacienți cu MPN JAK2V617F negativi prin secvențiere Sanger a exonului 9CALR. Pentru analiza profilului de fosforilare a proteinelorîn leucocite folosind kitul Human Phospho-Kinase Array au fost selectați pacienți reprezentativi cu deleție de 52-pb în gena CALR, pacienți cu mutație JAK2 V617F și controale.
Rezultate: Mutații în gena CALR au fost identificatela 52 dintre cei 90 de pacienti cu MPN JAK2V617F negativi (57,7%), tipul 1 de mutație CALR (del52) fiind cel mai prevalent (61,5% din totalul mutațiilor CALR). Analiza fosfokinazelor a indicat un profil similar de fosforilare al proteinelor Tor, Stat2 și Src la pacienții JAK2V617F pozitivi cât și la cei cu del52 CALR. De asemenea, s-a constatat un profil de fosforilare al proteinelor GSK3, MSK1/2, Hsp27 specific pentru CALR. Aceste rezultate preliminare sugereaza elemente noi in mecanismele de

semnalizare in MPN ce trebuie să fie explorate prin studii suplimentare.
Mentiune: PCCA PN II 133/2014 and POS CCE O1.1.2. 433/2012.

OP-20

REDOX DANGER SIGNALS IN RHEUMATOID ARTHRITIS

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Background: The sophisticated pathophysiology of rheumatoid arthritis (RA) is under intensive debate. A paradigm shift occurred lately, bringing in the forefront an interconnected web of intrinsic and extrinsic stresses that may underlie chronic inflammation in RA.
Aim: The aim of the study was to investigate the profile of inflammatory and redox-mediated signals in peripheral mononononuclear cells (PBMC) from RA patients, in correlation with disease activity.
Methods: RA patients non-responsive to synthetic DMARDs were monitored during biologic therapy. Systemic inflammatory markers were assessed at molecular level by pathway-focused PCR arrays (Qiagen) related to oxidative stress and antioxidant response.
Results: Molecular data indicated that good clinical outcome in RA was accompanied in particular cases by an increase of inflammatory cells in blood (over-expression of several pro-inflammatory cytokine genes). PBMC from responsive RA patients also exhibited increased intracellular oxidative activity, sustained by over-expression of some Nox enzymes (Nox2-NCF2, CYBB; DUOX). The intrinsic and environmental oxidative stress triggered in this cases a robust antioxidant response, mirrored by over-expression of SOD2, CAT, GPX(5), GST(Z1,P1) and PRDX1-6, hence accounting for inflammation decline.
Conclusion: Accumulation of inflammatory cells in peripheral blood may accompany the therapy-induced decline of disease activity. Besides pro-inflammatory cytokines, the intrinsic oxidative stress in PBMC and an imbalanced antioxidant response can drive systemic manifestations in RA.
Acknowledgments: The study was supported by the PCCA project 124/2014 and by the project PN09_33_02.07/2009, financed by the Romanian Ministry for Education and Scientific Research.

SEMNALE DE ALARMA REDOX IN ARTRITA REUMATOIDA

Introducere: Mecanismele patogene complexe ale artritei reumatoide (RA) sunt in plin proces de reevaluare, avand in vedere schimbarea de paradigma care aduce in prim-plan imunitatea innascuta si o retea de stresori care sustin inflamatia cronica in RA.
Scop: Studiul are ca scop evaluarea profilului molecular al semnalelelor inflamatoare mediate redox la nivelul celulelor mononucleare periferice (PBMC) de la pacienti RA, in corelatie cu activitatea bolii.
Metoda: Au fost luati in studiu pacienti RA neresponsivi la DMARD sintetice si carora li s-a instituit in consecinta terapie biologica. Utilizand metoda PCR array (Qiagen) au fost investigate modificarile de expresie pentru 84 gene asociate stresului oxidativ si raspunsului antioxidant.
Resultate: Screeningul molecular a evidentiat faptul ca evolutia clinica buna a pacientilor RA a fost insotita in unele cazuri de cresterea numarului de celule inflamatorii in sange periferic (supra-exprimarea unor gene care codifica pentru citokine pro-inflamatoare). PBMC de la pacientii RA responsivi la terapie prezinta de asemenea activitate oxidativa intracelulara crescuta, sustinuta de supra-exprimarea unor enzime din familia Nox (NOX2-NCF2 si CYBB; DUOX). In acelasi timp, stresul oxidativ a declansat un raspuns antioxidant robust in cazul pacientilor RA responsivi, evidentiat prin supra-exprimarea genelor SOD2, CAT, GPX(5), GST(Z1,P1) and PRDX(1-6), ceea ce sustine evolutia buna a pacientilor pe parcursul terapiei biologice.
Concluzii: Acumularea de celule inflamatorii in sange periferic poate fi consecinta scaderii activitatii bolii ca urmare a terapiei biologice anti-reumatice in RA. Pe langa citokinele pro-inflamatorii, stresul oxidativ intrinsec la nivelul PBMC, in conjunctie cu raspunsul antioxidant ineficient, ar putea constitui un mecanism care sustine

manifestarile sistemice ale RA.
Studiul a fost finantat de Ministerul Educatiei Nationale si Cercetarii Stiintifice prin proiectele PCCA 124/2014 si PN09_33_02.07/2009.

OP-21

LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 (LPPLA2) – INFLAMMATORY BIOMARKER IN CARDIOVASCULAR DISEASES

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Lipoprotein-associated phospholipase A2 (LpPLA2) is an enzyme produced in cells involved in atherosclerotic process and is mainly bound to the LDL in the circulation, while the remaining is distributed among HDL, VLDL and lipoprotein a (Lp(a)). Its activity is correlated with the intensity of atherosclerotic process. LpPLA2 plays a central role in the pathophysiology of atherosclerosis, from its initiation to the progression of cardiovascular complications. Numerous epidemiological studies have demonstrated that increased circulating levels of LpPLA2 predict an increased risk of acute cardiovascular events (myocardial infarction, stroke) and cardiovascular mortality. LpPLA2 is a marker of vascular inflammation and represents the link between the lipid metabolism and the low-grade inflammation that is characteristic of cardiovascular or metabolic diseases. LpPLA2 has some advantages that make it superior to other inflammatory markers such as high sensitivity C reactive protein: minimal bio-variation, high specificity for vascular inflammation, and plasma level independent of insulin resistance. The studies performed in The National Institute of Pathology „Victor Babes" in collaboration with the specialty clinics of The Faculty of Medicine "Carol Davila" have contribute to the introduction of LpPLA2 into The Cardiovascular Disease Risk Assessment Guidelines in 2008, and in The European Guidelines on cardiovascular disease prevention in clinical practice in 2012. We also have demonstrated that LpPLA2 is consistently correlated with heart failure, regardless of its etiology. Currently a lot of evidence supports the role of LpPLA2 as a risk marker, a prognostic biomarker, and possible therapeutic target.

FOSFOLIPAZA A2 ASOCIATA LDL (LPPLA2) – BIOMARKER AL INFLAMATIEI IN BOLILE CARDIOVASCULARE

Fosfolipaza A2 asociata LDL (LpPLA2) este o enzima produsa de celulele implicate in procesul aterogen si circula legata in principal de LDL si in mica proportie de VLDL si lipoproteina a (Lp(a)). Activitatea ei se coreleaza cu intensitatea procesului aterogen. LpPLA2 ocupa un loc central in fiziopatologia aterosclerozei de la initierea ei si in progresia complicatiilor cardiovasculare. Numeroase studii epidemiologice au demonstrat ca nivelul plasmatic ridicat al LpPLA2 indica un risc crescut pentru evenimente cardiovasculare acute (infarct miocardic, accident cerebral) si mortalitate cardiovasculara. LpPLA2 este un marker al inflamatiei vasculare si reprezinta o legatura intre metabolismul lipidic si inflamatia "low grade" caracteristica bolilor cardiovasculare sau metabolice. Comparativ cu alti biomarkeri inflamatori (exemplu: proteina C reactiva cu specificitate inalta), LpPLA2 prezintă un număr de avantaje: independenta fata de indicele de masă corporala si de rezistenta la insulina, variabilitate biologica mica si specificitate mare pentru inflamatia vasculara. Cercetările efectuate in Institul National „Victor Babes" in colaborare cu clinicile de specialitate ale UMF „Carol Davila" au contribuit la introducerea LpPLA2 in algoritmul investigațional de risc vascular în anul 2008 si ulterior, in anul 2012, in Ghidurile Europene de practica in medicina cardiovasculara. Cercetarile efctuate la pacientii cu insuficienta cardiaca au aratat ca activitatea LpPLA2 este mult crescuta, indiferent de etiologie, comparativ cu subiectii normali. In prezent exista o multitudine de evidente referitoare la rolul LpPLA2 ca factor de risc, biomarker prognostic și posibila tinta terapeutica.

OP-22

REAL TIME PCR METHOD FOR RAPID DIAGNOSIS OF INFECTIOUS MENINGITIS HIV-INFECTED PATIENTS

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Objectives: To assess Real Time PCR technique as a method for rapid diagnosis of infectious meningitis compared to standard diagnostic procedures in HIV-infected patients.
Material and Methods: We investigated 250 cerebrospinal fluide / CSF collected from HIV-infected children and adults, admitted to Hospital for Infectious and Tropical Diseases "Dr. V. Babes "from 2013 to 2015m, with clinical suspicion of meningitis infection and negative classical microbiological diagnosis. Molecular technique was used both for rapid diagnosis, monitoring clinical evolution under treatment, but also to detect viral-bacterial parasitic co-infections in refractory patients to administered medication. CSF were investigated by classical methods: no. nucleated elements / mmc, qualitative proteinorachia and quantitative albuminorachia, glycorrhachia, Lowenstein-Jensen medium cultivation and automated MB / BACT. Bacteriological, mycological and parasitological classic diagnosis was performed,. For Real Time PCR method was used nucleic acid extraction kit directly from CSF, "Master Pure Complete DNA & RNA Purification Kit" / Epicentre Biotechnologies, the commercial primer "Primer Design" UKsi Light Scanner 32 / Idaho Technologies.
Results: There were 250 CSF collected by lumbar puncture in HIV-infected patients with suspected infectious meningoencephalitis in context clinico-epidemiological imaging and biological suggestive. Out of 250 CSF, where: 1 (0,4%) ZN smear (+), 16 (6,4%) cultures LJ and 22 (8,8%) MB/ BACT automatic system cultures. 73 (29.2%) were positive CSF by RT PCR: Mycobacterium tuberculosis: 25 (10%), JCV: 16(6,4%), Toxoplasma gondii: 29 (11,6%), Cryptococcus neoformans: 3 (1,2%), associations: M. tuberculosis + Cryptococcus neoforman /1, M. tuberculosis + JCV / 1.
Conclusions: Tuberculous meningitis, often associated with HIV infection have various characteristics of CSF microbiological and biochemical appearance, are mainly paucibacillary with negative Ziehl-Neelsen smears and cultures. 29,2% CSF negative by classical microbiological diagnosis were positive by Real Time PCR technique for M. tuberculosis, T. gondii, JCV and Cryptococcus neoformans.

REAL TIME PCR - METODA DE DIAGNOSTIC RAPID AL MENINGITELOR INFECTIOASE LA PACIENTII INFECTATI HIV

Obiective: utilizarea tehnicii Real Time PCR ca metoda rapida de diagnostic al meningitelor infectioase, comparativ cu procedurile clasice, standard de diagnostic, in cazul pacientilor infectati HIV.
Material si Metode: S-au investigat 250 lichide cefalorahidiene/ LCR recoltate de la copii si adulti infectati HIV, internati in Spitalul Clinic de Boli Infectioase si Tropicale "Dr. V. Babes", in perioada 2013-2015, cu suspiciune clinica de meningita infectioasa si diagnostic microbiologic clasic, negativ. Tehnica moleculara s-a utilizat atat pentru diagnostic rapid, pentru monitorizarea evolutiei clinice sub tratament, dar si pentru depistarea co-infectiilor bacteriene- virale- parazitare la pacientii refractari la medicatia administrata. LCR s-au investigat prin metode clasice: Nr. elemente nucleate/ mmc, proteinorahie calitativa si albuminorahie cantitativa, glicorahie, clorurorahie; cultivare pe mediu Lowenstein –Jensen si in sistem automat MB/BACT. S-a efectuat diagnostic bacteriologic, micologic si parazitologic clasic. Pentru metoda Real Time PCR s-a utilizat kitul de extractie acizi nucleici direct din LCR, "Master Pure Complete DNA&RNA Purification Kit"/ Epicentre Biotechnologies, primer-ii comerciali "Primer Design" U.K.si Light Scanner 32/ Idaho Technologies.
Rezultate: S-au recoltat prin punctie lombara 250 LCR de la pacienti infectati HIV cu suspiciune de meningoencefalita infectioasa, in context clinico- epidemiologic, imagistic si biologic sugestiv. Pentru cele 250 LCR analizate pentru diagnostic Mycobacterium tuberculosis, am obtinut 1 (0,4%) frotiu ZN (+); 16 (6,4%) culturi LJ si 22 (8,8%) culturi in sistem automat MB/BACT. 73(29,2%) LCR au fost pozitive prin tehnica RT PCR: Mycobacterium tuberculosis: 25(10%); JCV: 16(6,4%); Toxoplasma gondii: 29(11,6%), Cryptococcus neoformans: 3 (1,2%), asocieri M. tuberculosis+ Cryptococcus neoformans- 1; M. tuberculosis+ JCV/ 1.

Concluzii: Meningitele tuberculoase, frecvent asociate infectiei HIV au variate particularitati ale aspectului microbiologic si biochimic al LCR, sunt preponderent paucibacilare, cu frotiuri Ziehl- Neelsen si culturi negative. 29,2% din LCR cu diagnostic microbiologic clasic negativ, au fost pozitive prin tehnica Real Time PCR pentru M. tuberculosis, T. gondii, JCV si Cryptococcus neoformans.

OP-23

MECHANISMS IN SALIVARY GLANDS REGENERATION

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Introduction: Salivary glands secretion may be diminished to dissolution in primary or secondary diseases of glandular parenchyma. The intrinsic recovery and regeneration potential of the glandular tissue allows restoration of salivary secretion level if the causing lesion is below a certain severity degree.
Methods: Study of salivary glands regenerative capacity includes in vitro experiments on primary cell cultures, and also in vivo experiments, by unilateral ligation of the murine submandibular gland followed by deligation after 5-7 days.
Results: Primary cell cultures from main salivary glands lead to the formation of floating cellular aggregates, called salispheres. Selected cells from salispheres, once injected into atrophic salivary glands (post irradiation), stimulate local reparatory reaction, leading to gradual regeneration. After excretory duct deligation, the salivary gland heals spontaneously, without external cellular contribution, provided that vegetative innervation remained intact.
Conclusions: Salivary glands present an internal regeneration potential, which acts until a certain threshold, and which can be reactivated even if that threshold is exceeded, by injecting cells with induction capabilities. Identification of the cellular substrate of these processes may lead to optimisation of treatment for xerostomia and "dry mouth" syndrome-related diseases.
Acknowledgment: This work is partially supported by Project PN 16.22.02.03, "Study of the regenerative capacity of salivary glands"

MECANISME IMPLICATE IN REGENERAREA GLANDELOR SALIVARE

Introducere: Secreția glandelor salivare poate fi diminuată până la dispariție în cazul unor afectări primare sau secundare ale parenchimului glandular. Potențialul intrinsec de reparare și regenerare a țesutului glandular permite refacerea nivelului de secreție salivară în cazul în care leziunea cauzatoare nu a depășit un anume grad de severitate.
Material şi metodă: Studiul capacității regenerative a glandelor salivare include experimente in vitro prin culturi celulare primare, precum și in vivo, prin ligatura unilaterală a ductului excretor al glandei submandibulare murine și eliberarea acestuia după un interval de 5-7 zile.
Rezultate: Culturile celulare primare din glandele salivare mari duc la formarea de agregate celulare non-aderente, numite salisfere. Celule selecționate din salisfere, odată injectate în glande salivare atrofiate (post-iradiere), stimulează răspunsul reparator local și duc către o regenerare treptată.După deligaturarea ductului excretor, glanda salivară își revine spontan, fără aport celular exogen, cu condiția ca inervația vegetativă să nu fi fost afectată.
Concluzii: Există la nivelul glandelor salivare un potențial intern de regenerare, care acționează până la un anume prag și poate fi reactivat chiar și în cazul depășirii acelui prag, prin suplimentarea cu celule cu capacități inductorii. Identificarea substratului celular al acestor procese poate duce la optimizarea tratamentului afecțiunilor care diminuează capacitatea secretorie salivară, de timpul xerostomiei sau sindromului de "gură uscată".
Acest studiu a fost finanțat parțial de proiectul PN 16.22.02.03, "Studiul capacității regenerative a glandelor salivare".

OP-24

INTRATHORACIC TUMORS: FROM MOLECULAR DIAGNOSIS TO TREATMENT

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Intrathoracic tumors are being discovered accidentally, by chest x-ray or CT, or after clinical manifestations and investigations carried out subsequently. The nature of these tumors (malignant or benign) represents a medical emergency, the final diagnosis being established in the outcome of histopathology and / or immunohistochemistry results. In the case of localized tumors, surgery combined with chemotherapy and radiotherapy is the standard current therapy for almost all tumours; in the metastatic stage an important role in prolonging survival is played by molecular targeted therapy.
Objectives: Genetic and epigenetic transformations occur during malignant transformation, accumulates continuously during clonal expansion, influences the processes of invasion, metastasis and resistance to treatment. Identification and characterization of molecular changes are crucial for the treatment of neoplastic diseases. Knowing the characteristics of both the tumor and the individual genetic alterations will lead to significant improvement in prognosis. The purpose of this review is to summarize recent discoveries in the biology of intrathoracic tumors and their involvement in therapeutic strategies.
Methods: Analysis of current therapeutics guidelines and specialized publications in the field of intrahoracic tumors, especially metastatic lung cancer.
Conclusions: The use of molecular targeted therapy in metastatic lung cancer led to an improvement in the patients prognosis by increasing overall survival, and also the quality of life.

TUMORILE INTRATORACICE: DE LA DIAGNOSTICUL MOLECULAR LA TRATAMENT

Fomațiunile tumorale intratoracice sunt descoperite fie întâmplator, prin radiografie pulmonară sau computer tomograf, sau în urma manifestărilor clinice şi a investigațiilor efectuate ulterior. Stabilirea naturii acestor tumori (malignă sau benignă) reprezintă o urgență medicală, diagnosticul final fiind stabilit în urma rezultatului histopatologic si/sau imunohistochimic. Dacă în cazul tumorilor localizate, tratamentul chirurgical combinat cu chimioterapia si radioterapia reprezintă standardul teraputic actual pentru majoritatea localizarilor, în stadiul metastatic un rol foarte important pentru prelungirea supraviețuirii îl joacă terapia țintită molecular.
Obiective: Modificările genetice si epigentice apar în timpul transformărilor maligne, se acumulează continu în timpul expansiunii clonale, influențând procesele de invazie, metastazare si rezistență la tratament. Identificarea și caracterizarea acestor modificări moleculare sunt de o importanță crucială pentru tratamentul bolilor neoplazice. Cunoașterea atât a caracteristicilor tumorale cât şi a alterărilor genetice individuale vor duce la imbunătățirea semnificativă a prognosticul pacienților. Scopul acestei analize este de a rezuma descoperirile recente din biologia tumorilor intratoracice și implicarea acestora în strategiile terapeutice.
Metode: Analiza ghidurilor teraputice actuale şi a publicațiilor de specialitate din domeniul tumorilor intratoracice si implicarea acestora in strategiile terpeutice.
Concluzii: Folosirea terapiei țintite molecular în cancerul metastazat a dus la imbunătățirea prognosticului pacienților, prin creşterea supraviețuirii globale, dar şi a calității vieții.

OP-25

METABOLIC RISK FACTORS EVALUATION IN A YOUNG POPULATION WITH NORMAL BMI

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Background: Inside the non obese population, as defined by body mass index (BMI), metabolic risk factors are relatively prevalent, but there isn't any study in the Romanian young population that had assessed the relationship between insulin resistance and the cardio-vascular risk factors for metabolic syndrome.
Method: We have conducted a cross sectional study of 103 young individuals with BMI <30, without diabetes, cardio-vascular or hepatic conditions to whom fasting glucose, triglycerides, high density lipoprotein cholesterol, insulinemia, waist circumference and arterial pressure (AP) were recorded. Insulin resistance was evaluated using the HOMA-IR index. Statistic data processing data included Pearson relation and multiple regression (backward method), using the SPSS version 21 software.
Results: Waist circumference, diastolic AP and HOMA-IR were highly correlated. Multiple regression showed a strong correlation between HOMA-IR and waist circumference ($p<0.001$) and diastolic blood pressure ($p=0.008$) that was maintained inside the women group ($p=0.016$ and $p=0.032$, respectively). In men, HOMA-IR correlated only with waist circumference ($p=0.031$).
Conclusion: Metabolic risk factors such as waist circumference, diastolic blood pressure and HOMA-IR are strongly correlated inside the non obese young population.

FACTORII DE RISC METABOLIC INTR-O POPULAȚIE DE ADULȚI TINERI NON OBEZI

Introducere: Deși în cadrul populației non obeze, definită prin indicele de masăcorporală (IMC), factorii de risc metabolic au o prevalență relative ridicată, nu există nici un studio în populația tânără din România privind relația din trerezistența la insulin și factorii de risc cardio-vasculari aparținând sindromului metabolic.
Metodă: Am derulat un studiu transversal pe un grup de 103 de adulți tineri cu IMC <30, fără diabet, boli cardio-vasculare sau hepatice, la care am măsurat glicemia a jeun, trigliceridemia, colesterolul HDL, insulinemia, circumferința abdominal și tensiunea arterială (TA). Rezistența la insulină a fost evaluată prin indicele HOMA (HOMA-IR). Analiza statistica incluscorelația Pearson și regresia multiplă, (metoda backward), utilizând programul SPSS versiunea 21.
Resultate: Intrecircumferința abdominală, TA diastolică și HOMA-IR a fost găsită o corelație strânsă. Regresia multiplă a aratat o corelatie strânsă între HOMA-IR și circumferința abdominală ($p<0.001$) și TA diastolică ($p=0.008$) care s-a menținut în subgrupul de femei. ($p=0.016$ și, respectiv, $p=0.032$). In subgrupul de bărbați, HOMA-IR a fost corelat doar cu circumferința abdominală ($p=0.031$).
Concluzii: Factorii de risc metabolic precum circumferința abdominală, TA diastolică și HOMA-IR sunt strâns corelați în cadrul populației tinere, non obeze.

OP-26

AIDS IN ROMANIA FROM 1988 TO 2016. IN MEMORIAM OF ACADEMICIAN PROFESSOR NICOLAE CAJAL

I.V. Patrascu, C. Chiurciu
Romvac Company SA

One of the most important scientific research in human medicine it is the great program for AIDS in ROMANIA. Together with professor academician Nicolae Cajal we developed and conducted programs, beginning from 1987 we initiated studies for AIDS. The illustrious scholar and academician Professor Nicolae Cajal, director of the

Institute of Virology Stefan S. Nicolau was agreed to sign all my letters between me and the Institute in Japan, between me and the American Universities and WHO. Academician Professor Nicolae Cajal agreed to set up a laboratory on the second floor of the Institute, he let me bring colleagues and specialists from ROMVAC. The entire team from ROMVAC made free arrangements, had equipped the laboratory and participated in what was to be done there. In June 1989 we discovered the AIDS to the children in hospitals Fundeni and Pantelimon. To the first 300 children tested in Fundeni Hospital and Pantelimon Hospital (in our days - Victor Gomoiu Hospital) HIV incidence was 13 %. Those results, discreetly hidden, by a presentation of courage were made public at the Institute of Virology S . Nicolau at the festive session for the 23 August 1989. Professor Nicolae Cajal was attentive, concerned about what we do and assist the astounded at what happened in Romania. Side by side we decided that it was not an evolution of HIV diagnosis but a program that allowed us to discover stages of disease outbreaks existing across the country. We suggested Professor Cajal to appeal to WHO, to come urgently in Romania and to control me if I had right or wrong. The WHO correspondence, signed by myself went to the Medicine DU Monde organization in 1990. The WHO Experts were coming in Romania, they were presented to Academician Cajal. Experts WHO have confirmed our results, they brought with them samples of serum from one of the most gorgeous actions of an epidemiological program accomplished in the world. WHO, at our suggestion recommended in February 1990 that the human medical work around the world to reconsider and go to: - Use of disposable materials; - Declare unconditional outbreaks of AIDS; - Reducing the parenteral administration and use of oral drugs administration; This phase in the history of human medicine in Romania shoes us the professional commitment of a great scientist, which was Nicolae Cajal. Today, in our days, side by side with Ing. C. Chiurciu, CEO of ROMVAC COMPANY S.A. I continuing what I did for years with Professor Nicolae Cajal and I am having outstanding achievements in the same scientific direction. We are able to inform you that the ROMVAC who was part of the AIDS program, we have achieved organic products-PC2 generation, which can be use in patients with AIDS/aids IN Romania. WE wish to let you know that ROMVAC is able to initiate a program of research and production of vaccine for AIDS/AIDS prepared adaptive mechanisms on the activity of the immune system developed by Professor N. Jerne from the Pasteur Institute in Paris in 1975, received also the Nobel Prize. We, the specialists from ROMVAC, are standing at your disposal!

SIDA IN ROMANIA 1988-2016. IN MEMORIAM ACADEMICIAN PROFESOR DR. NICOLAE CAJAL

In activitatea mea de cercetare in medicina umana, una dintre realizari a constituit-o cercetarea privind SIDA in Romania. Prin colaborare cu academicianul Nicolae Cajal, alaturi de care am elaborat programe cu deosebita importanta stiintifica si medicala incepand cu anul 1987 am organizat activitatea de cercetare in domeniul SIDA. Nicolae Cajal a fost cel care a semnat intreaga corespondenta purtata de mine cu un institut din Japonia, cu universitati americane si cu OMS. Din iunie 1988, profesorul Cajal a fost de acord sa aduc colegi si specialisti de la ROMVAC. In luna iunie 1989 am descoperit SIDA la copiii din spitalele Fundeni si Pantelimon. La primii 300 de copii, pacienti ai spitalelor Fundeni si Pantelimon incidenta infectiei cu HIV a fost de 13%. Aceste rezultate sunt facute public la sesiunea din 23 august 1989 din cadrul Institului de Virusologie S.Nicolau. Profesorul Nicolae Cajal actioneaza in consecinta, frapat fiind de ceea ce se intampla in Romania si, la sugestia mea, apeleaza la OMS.Corespondenta trimisa la OMS am dus-o la MEDICINE du MONDE. De aici a pornit programul OMS. Prin urmare, specialistii OMS ajung in Romania, unde aduc cu ei probe de ser de la una din cele mai frumoase actiuni dintr-un program epidemiologic din lume. OMS, la sugestia noastra, recomanda in februarie 1990 ca activitatea medicala din intreaga lume sa se reconsidere prin: - utilizare amaterialelor de unica folosinta; - declararea neconditionata a focarelor de SIDA; - reducerea administrarilor parenterale si folosirea administrarii orale a medicamentelor; Aceasta etapa marcanta din istoria medicine umane romanesti surprinde dedicatia si contributia profesionala a unui mare savant, cel care a fost NICOLAE CAJAL. Astazi, alaturi de Ing. Chim. C. Chiurciu, director general al ROMVAC S.A., continui ceea ce am facut alaturi de profesorul Nicolae Cajal. Suntem in masura sa va informam ca, la Romvac am reusit sa realizam produse biologice – PC2, din generatia a-3-a care pot fi folosite la pacientii cu SIDA. Romvac este in masura sa organizeze si sa initieze un program de cercetare si productie de vaccin anti AIDS preparat dupa profesorul N. Jerne de la Institutul Pasteur din Paris in 1975, premiat cu Nobel. Romvac, prin specialistii sai va sta la dispozitie.

OP-27

ONCOGEN - STRATEGY FOR EXCELLENCE IN RESEARCH

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The OncoGen Centre is the first state-of-the-art center for gene therapies in Romania, financed by European Funds dedicated to leading-edge research in areas of: Advanced therapies in cancer - Tumor cell biology; Regenerative medicine - Adult stem cells study; Health and environmental factors; Immunotherapies. OncoGen also oversees the maintenance of a relationship between basic research and clinical research, by a high throughput bench-to-bedside approach, enabling the rapid translation of research results into clinical practice, aiming to improve the outcomes for patients through personalized treatment. The mission of the OncoGen center is to develop advanced methods for the diagnosis and therapy of cancer and chronic degenerative diseases, which have a high morbidity and limited therapeutic options. The research team will accomplish this mission through an interdisciplinary approach of fundamental, translational and clinical research activities, through the creation of educational programs, and through the development of partnerships with the academia and with the industry. The center will enable the development of new therapeutics for human use, through the high safety standards and conditions that it fulfills. The inclusion of the GMP facility of the OncoGen center in the European network of similar centers will facilitate the running of multicentric clinical trials, for the development of innovative therapies in cancer and in chronic degenerative diseases. The aim is to promote research that can be applied in advanced therapies using human stem cells for regenerative medicine, and for the production of immunotherapeutics based on modern technologies of molecular biology.

ONCOGEN - STRATEGIE PENTRU EXCELENTA IN CERCETARE

OncoGen este primul centru de terapii genice de ultima generatie din Romania, finantat din fonduri europene si dedicat cercetarii in domenii precum: Terapii avansate in cancer - Biologia celulelor tumorale; Medicina regenerativa - Studiul celulelor stem adulte; Sanatate si factori de mediu; Imunoterapii. OncoGen supervizeaza si mentinerea legaturii intre cercetarea fundamentala si cea clinica, printr-o abordare bench-to-bedside de inalta eficienta, permitand transferul rapid al rezultatelor cercetarii in practica clinica, cu obiectivul de a ameliora prognosticul pacientilor prin tratament personalizat. Misiunea centrului OncoGen este dezvoltarea de metode avansate de diagnostic si terapie a cancerului si a bolilor cronice degenerative cu morbiditate crescuta si cu optiuni terapeutice limitate. Echipa de cercetatori va îndeplini aceasta misiune atat printr-o abordare interdisciplinara a activitatilor de cercetare fundamentala, translationala si clinica, cat si prin sustinerea de programe educationale si parteneriate cu mediul academic si cel industrial. Prin conditiile si standardele de inalta siguranta pe care le indeplineste, acest centru va permite dezvoltarea de noi produse terapeutice pentru uz uman. Includerea unitatii GMP din Centrul Oncogen in reteaua europeana de centre cu dotari similare va facilita derularea de studii clinice multicentrice pentru dezvoltarea de tratamente inovatoare in cancer si bolile cronice degenerative. Se urmareste promovarea cercetarii cu potential aplicativ in domeniul terapiilor avansate cu celule stem umane in medicina regenerativa si pentru obtinerea de produse imunoterapeutice bazate pe tehnologii moderne de biologie moleculara.

OP-28

MODELING PROTEIN-DNA INTERACTIONS WITH EXPERIMENTAL CONSTRAINTS. A STUDY CASE ON RAG PROTEINS AND PAIRED COMPLEX FORMATION

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Over the past decade computational techniques such as string and structural bioinformatics, molecular modeling and simulation became increasingly useful inimproving our understandingof biological systems at molecular level. Intertwining such techniques with experiment is twofold valuable. On one hand it allows generating more accurate models of the structure, interactions and dynamics of large, complex biomolecular systems. On the other handsuch improved models areinstrumental in elaborating working hypotheses on biological processes and functions that can be further tested in the lab. An interesting example of such strategy in molecular medicine is provided here by the work on the Paired Complex structure comprising RAG1 and RAG2 proteins (1) and their DNA interactors the Recombination Signal Sequences 12RSS (2) and 23RSS (3).

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OP-29

CLASS I RESTRICTED PRESENTATION OF N-GLYCOSYLATED ANTIGENS

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The degradation process of the antigens specific to MHC-I presentation depends mainly on the proteasomal proteases in the cytosol. However, since many antigens are glycoproteins, including tumour antigens or viruses envelope proteins, their glycosylation status could also affect their processing and presentation. Here, we have investigatedthe ability of a tumour antigen to undergo proteasome degradation and generate immunoepitopes in the absence of its multiple N-glycosylation sites. The methods developed include LC/MS and T cells presentation assays to investigate the individual role of N-glycans in proteins with multiple N-glycans.Using human CD8+ T cell clones specific for a glycosylated epitope of tyrosinase, we found that transfectants of single and triple N-glycosylation mutants are recognized by specific T cells. The aglycosylated antigenwas able to trigger higher CD8+ T cell activation. The LC/MS analysis showed significant increase of the amount of the peptideepitope resulted from the antigen processsing. The generation of the peptide by the antigen processing machinery is therefore largely independent of antigenN-glycosylation. However, whilst distal N-glycans had no effect on the epitope generation, the mutants lacking the epitope glycan generated the antigenic peptide more efficiently. We conclude that epitope located N-glycans limit the ability of human tyrosinase to provide HLA-A2 restricted antigen for recognition by specific CD8+ T cells.These findings may help understanding the glycobiology of the antigen presentation pathway and improve the design of optimized peptide-based vaccines including aglycosylated antigens

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OP-30

GOLD NANOPARTICLES ENHANCE THE EFFECT OF TYROSINE KINASE INHIBITORS IN ACUTE MYELOID LEUKEMIA THERAPY

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Background and aims: Every year, in Europe, acute myeloid leukemia (AML) is diagnosed in thousands of adults. For most subtypes of AML, the backbone of treatment was introduced nearly 40 years ago as a combination of cytosine arabinoside with an anthracycline. This therapy is still the worldwide standard of care. Two-thirds of patients achieve complete remission, although most of them ultimately relapse. Since the FLT3 mutation is the most frequent, it serves as a key molecular target for tyrosine kinase inhibitors (TKIs) that inhibit FLT3 kinase. In this study, we report the conjugation of TKIs onto spherical gold nanoparticles.
Materials and methods: The internalization of TKI-nanocarriers was proved by the strongly scattered light from gold nanoparticles and was correlated with the results obtained by trans¬mission electron microscopy and dark-field microscopy. The therapeutic effect of the newly designed drugs was investigated by several methods including cell counting assay as well as the MTT assay.
Results: We report the newly described bioconjugates to be superior when compared with the drug alone, with data confirmed by state-of-the-art analyses of internalization, cell biology, gene analysis for FLT3-IDT gene, and Western blotting to assess degradation of the FLT3 protein.
Conclusion: The effective transmembrane delivery and increased efficacy validate its use as a potential therapeutic.

OP-31

ALTERATION OF BRAIN BARRIERS AND PROGRESSION OF NEURODEGENERATION

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Barriers of the brain, namely blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSF), are unique structures which are able to maintain the homeostatic environment of the cerebral tissue, to control the traffic between blood and brain and to discretely toon the composition of CSF. An important amount of data accumulated already to show that BBB and BCSF are importantly altered in neurodegenerative diseases, such as Alzheimer's disease (AD). My group and others showed that molecular pathogenic operators of AD, such as β-amyloid peptide, are able to change the expression of tight junction proteins and to alter consequently the

barrier properties, at least in experimental models. I will summarize here the data supporting BBB and BSCF alteration in AD and other neurodegenerative diseases and I will propose future experimental and therapeutic approaches in the field.

MODIFICAREA BARIERELOR CEREBRALE ȘI PROGRESIA PROCESELOR NEURODEGENERATIVE

Barierele creierului, și anume bariera hemato-encefalică (BHE) și bariera hemato-lichidiană (BHL, lichid cefalo-rahidian, LCR), sunt structuri unice capabile să mențină mediul homeostatic al țesutului cerebral, să controleze traficul molecular între compartimentele capilar și cerebral și să regleze fin compoziția LCR. S-a acumulat deja o cantitate importante de date publicate în literatura de specialitate care arată că BHE și BHL sunt modificate sever în bolile neurodegenerative, spre exemplu în boala Alzheimer (BA). Grupul meu cât și alte grupuri au arătat că operatori patogenici moleculari ai BA, cum ar fi peptidul beta-amiloid, sunt capabili să altereze expresia proteinlor de joncțiune strânsă și consecutiv propriteățile de barieră, cel puțin în modele experimentale. Voi rezuma aici datele care demonstrează afectarea BHE și BHL în BA și alte boli neurodegenerative și voi propune abordări noi viitoare atât experimentale cât și terapeutice.

OP-32

SEVERE CASES OF INFLUENZA DURING THE 2014/2015 SEASON

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Background: Every year different strains of influenza viruses emerge due to the continuous antigenic drift or sometimes due to gene reassortment causing an antigenic shift. Influenza viruses can be associated with multiple severe complications that can sometimes lead to death. Our aim is to analyze epidemiological, clinical and virological characteristics of the influenza season 2014/2015 in Bucharest.
Methods and Materials: Retrospective study of patients diagnosed with influenza and hospitalized in a tertiary facility during 2014/2015 season. Positive diagnosis was based on RT- PCR and viral isolation in MDCK cell cultures.
Results: 110 patients (median age 43 years, 81,8% women) were hospitalized. Only 3 patients were under 18 years of age. A positive laboratory diagnosis was available for 57 patients: 32 were infected with a B stain, 14with an A/ H3strain and11 with the A/H1pdm09 strain. None of the patients was vaccinated. The peak of the influenza seasonal infection was in February- March, with an uniforme presence of all 3 strains throughout the season. 71.8% (79 patients) received antiviral therapy with Oseltamivir. A severe form of influenza was diagnosed in 30 cases (27,27%) , out of which 21 had respiratory complications and 9 neurological complications. Only 6 of these patients were arrived to receive antiviral treatment in the first 48 hours from the onset of the disease, the others had delayed presentation. The majority of neurological complications 8/9 (89%) were present in patients infected with a B influenza strain. The mortality rate was high (7/ 110) 4 with AH1 and 3 with B strain. Viral isolation identified a strain of influenza virus B (B/Phuket/3073/2013 (B/Yam -lineage) distinct from the WHO recommended strain included in the 2014/2015 vaccine (B/Massachusetts/2/2012-like virus).
Conclusion: Influenza is not just a mild diseases, a significant number of patients can have a severe disease with pulmonary or neurological complications. The continuous variability of the influenza viruses can give rise to virulent strains that escape the immune response, especially when a large part of the population remains unvaccinated.

CAZURILE SEVERE DE GRIPĂ ÎN TIMPUL SEZONULUI 2014/2015

In fiecare an, apar tulpini diferite ale virusurilor gripale din cauza modificarilor antigenice minore (drift antigenic) sau, uneori, din cauza rearanjariiantigenice majore (shift antigenic). Virusurile gripale pot fi asociate cu mai multe complicații severe, care pot duce uneori la deces. Scopul nostru este de a analiza caracteristicile epidemiologice, clinice si virusologice ale gripei in sezonul 2014/2015 din București.
Material si metodă: Studiu retrospectiv al pacienților diagnosticați cu gripă și internați într-o instalație terțiară în timpul sezonului 2014/2015. Diagnosticul pozitiv sa bazat pe RT-PCR și izolarea virală în culturi de celule MDCK. Rezultate 110 pacienți (mediana de 43 de ani, 81,8% femei) au fost spitalizați. Doar 3 pacienți au fost sub 18 ani. Diagnosticul de laborator a fost pozitiv la 57 de pacienți: 32 au fost infectati cu virus gripal tip B, 14 cu o tulpină A/H3 și 11 cu tulpina A/H1pdm09. Nici unul dintre pacienți nu a fost vaccinat. Varful sezonului a fost in lunile februarie-martie, cu o prezență uniforma a tuturor celor 3 tulpini pe tot parcursul sezonului. 71,8% (79 pacienți) au primit tratament antiviral cu oseltamivir. Forme severe de gripă au fost diagnosticate în 30 de cazuri (27,27%), din care 21 au avut complicații respiratorii și 9 complicații neurologice. Doar 6 dintre acești pacienți au primit tratament antiviral în primele 48 de ore de la debutul bolii, restul s-au prezentat tardiv pentru internare. Majoritatea complicatiilor neurologice 8/9 (89%) au fost prezente la pacienții infectați cu o tulpină B de gripa. Rata de mortalitate a fost ridicată (7/110) 4 cu AH1 și 3 cu tulpina B. La izolarea virala s-a identificat o tulpină de virus gripal B (B / Phuket / 3073/2013 (B / Yam -lineage) diferită de tulpina recomandată de OMS si inclusă in vaccinul pentru sezonul gripal 2014/2015 (B/Massachusetts/2/2012-like virus).
Concluzii: Gripa nu este doar o afectiune ușoară, un număr semnificativ de pacienți pot avea o formăseveră deboală cu complicații pulmonare sau neurologice. Variabilitatea continuă a virusurilor gripale poate da naștere unor tulpini virulente care scapă răspunsului imun, în special atunci când o mare parte a populației rămâne nevaccinată.

OP-33

FROM MOLECULAR PATHOLOGY TO INFORMATIONAL PATHOLOGY

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Introduction: The great advances of genetics, biochemistry, immunology and other fundamental sciences, transferred all the human pathology from the organic level at the molecular level. So we know today very well the molecular substrate almost of all the diseases we face. Therefore diagnosis and treatment of these diseases is today based on molecular changes posed. However between molecular changes and their clinical manifestations there is not a linear relationship because they do not always produce the disease. Not always mutations of different genes, and molecular markers, will manifest clinically. Therefore, almost all molecular changes occurs probabilistic. The effect of molecular changes is defined by the risk that the changes to make different diseases. Often in order to manifest clinical, molecular changes require the presence of predisposing factors to act a very long time. Meanwhile asymptomatic chronic diseases can evolve and molecular changes may cause irreversible damage. The phenomena occur as though behind molecular changes would be some hidden parameters that control these changes. One of the hidden parameters has been found to be represented by epigenetic factors that can stimulate or block the activity of different genes depending on the environment conditions. But no epigenetic factors can explain the nonlinearity between the molecular changes and their clinical manifestations. So for example, no epigenetic factors can explain how come the selection of genes which are activated or inhibited, in various diseases, or why they need so long time to manifest clinically. Therefore, we have pointed out that in addition of the substantial aspect of molecular changes there is an informational aspect, represented by molecular information carried by different molecules. The information is actually that ensures control of many biological and pathological processes, and information may not be confused with the molecule that carries. Molecular information it can explain much better the nonlinearity relations between molecular changes and their clinical manifestations. Therefore we have shown that in addition of the molecular pathology there is an informational pathology too, that could open new ways for prevention and treatment in the very complicated diseases we

are faced.

Method: To discover the causes of nonlinearity between molecular changes and their clinical manifestations, we studied the etiology and pathogeny of some diseases, very common and very serious, as atherosclerosis, diabetes, Alzheimer's disease, and cancer. We studied these diseases because they have a molecular substrate very well known, both in terms of genetic and epigenetic view. But also because in these diseases there is a nonlinearity between molecular changes and their clinical manifestations. Therefore the clinical manifestations of various molecular changes can be expressed only by means of probabilities. In this reason, in these diseases is more talk about some genetic predisposition and other risk factors not known precisely how probability works.

Results: Studying these diseases, we found that the nonlinearity between molecular changes and their clinical manifestations is expressed by a great variability of clinical manifestations, which can go to the absence of any clinical manifestations. We also found that as the research evolves molecular substrate of these diseases is increasingly more and more complicated. So for example, after 20 years ago it was discovered four genes, in recent years have discovered more than 10 genes that are involved in the onset of Alzheimer's disease. This situation is true in the case of atherosclerosis, diabetes and cancer, where it is discovered more and more candidate genes. And candidate genes require the presence of risk factors that occur sometimes and sometimes not produce disease. On the other hand, risk factors need a very long time to be clinically evident, which brings into question the role of regulatory mechanisms in the disease. Also the treatment action we use in these diseases is probabilistic nature. And the result of treatments varies from one individual to another. Therefore they speak of necessity personalization of treatment depending on the particularities of each patient. The phenomena occur as if between molecular changes and their clinical manifestations would intervene and other factors that can sometimes help and sometimes can stop, the action of molecular modifications.

Conclusions: After analyzing nonlinear relations between molecular changes and their clinical manifestations, we concluded that in addition to substantial aspect of molecular changes, it is involved also an informational aspects. As is known that in addition of the substance and energy, all the molecules have a molecular information, represented by the spatial structure of the molecule. And this information plays an important role in the recognizing process of different molecules and in the control process of many biological and pathological reactions. Therefore the body has become a very complicated communication system, which transmits over a lot of chemical messengers. Molecular information they transmit these molecules can better explain the nonlinearity of relations between molecular changes and their clinical manifestations, because in information systems acts an informational causality. This means that information not determine some effects, but only triggers some effects, which depend on the operating program of the recipient, such is the cells on they act. Molecular information and informational causality can explain better the relations between molecular changes and their clinical manifestations. But this means that behind of molecular pathology there is a hidden informational pathology, which could open new ways for prevention and treatment in very complicated diseases we are faced.

DE LA PATOLOGIA MOLECULARĂ LA PATOLOGIA INFORMAȚIONALĂ

Introducere: Marile progrese ale geneticii, ale biochimiei, ale imunologiei şi ale altor ştiințe fundamentale, au transferat întreaga patologie umană de la nivelul organic la nivelul molecular. Astfel noi cunoaştem astăzi foarte bine substratul molecular al aproape tuturor bolilor cu care suntem confruntați. De aceea diagnosticul şi tratamentul acestor boli se face astăzi pe baza modificărilor moleculare pe care le prezintă. Cu toate acestea între modificările moleculare şi manifestările lor clinice nu există o relație liniară deoarece ele nu produc întotdeauna boala respectivă. Nu întotdeauna mutațiile unor gene, sau prezența unor markeri moleculari, se manifestă clinic. Aproape toate modificările moleculare se manifestă probabilistic. Efectul unei modificări moleculare se defineşte prin riscul acelei modificări de a face boala respectivă. De multe ori pentru a se putea manifesta clinic, modificările moleculare au nevoie de prezența unor factori favorizanți care să acționeze un timp foarte îndelungat. În tot acest timp bolile cronice pot să evolueze asimptomatic, iar modificările moleculare pot să producă tulburări ireversibile. Fenomenele se petrec ca şi când în spatele modificărilor moleculare s-ar mai afla nişte parametrii ascunşi, care controlează modificările respective. Unul dintre parametrii ascunşi s-a dovedit a fi reprezentat de factorii epigenetici care pot stimula sau bloca activitatea unor gene în funcție de condițiile de mediu. Dar nici factorii epigenetici nu pot explica neliniaritatea dintre modificările moleculare şi manifestările lor clinice. Aşa spre exemplu, nici factorii epigenetici nu pot explica cum se face selecționarea genelor care sunt

activate sau inhibate in diferite boli, sau de ce ei au nevoie de un timp atît de lung pentru a se manifesta clinic. Noi am arătat în repetate rânduri că pe lângă aspectul substanțial al modificărilor moleculare mai intervine şi aspectul informațional, reprezentat de informația moleculară pe care o transportă diferitele molecule. Informația şi nu substanța, este de fapt cea care asigură controlul numeroaselor procese biologice şi patologice. De aceea informația nu poate fi confundată cu molecula care o poartă. Iar informația moleculară ne poate explica mult mai bine neliniaritatea relațiilor dintre modificările moleculare şi manifestările lor clinice. De aceea noi am arătat că pe lângă patologia moleculară mai există şi o patologie informațională, care ar putea deschide noi căi de prevenire şi tratament în bolile extrem de complicate cu care suntem confruntați.

Metodă: Pentru a descoperii cauzele neliniarității dintre modificările moleculare şi manifestările lor clinice, noi am studiat etiopatogenia unor boli, aşa cum ar fi ateroscleroza, diabetul, boala Alzheimer şi cancerul. Am studiat aceste boli deoarece ele au un substrat molecular foarte bine cunoscut, atît din punct de vedere genetic cât şi epigenetic. Dar şi pentru faptul că în aceste boli există o neliniaritate între modificările moleculare şi manifestările lor clinice. Aceasta face ca manifestările clinice ale diferitelor modificări moleculare să nu poată fi exprimate decât prin intermediul unor probabilități. De aceea în aceste boli se vorbeşte mai mult despre nişte factori genetici predispozanți şi despre nişte factori de risc, care nu se ştie precis de ce acționează probabilistic.

Rezultate: Studiind aceste boli, noi am constatat că neliniaritatea dintre modificările moleculare şi manifestările lor clinice se exprimă printr-o mare variabilitate a manifestărilor clinice, care poate să meargă până la lipsa oricăror manifestări clinice. De asemenea am constatat că pe măsura ce cercetările evoluează, substratul molecular al acestor boli se complică tot mai mult. Aşa spre exemplu, după ce în urmă cu 20 de ani s-au descoperit 4 gene, în ultimii ani s-au mai descoperit peste 10 gene implicate în apariția bolii Alzheimer. Acelaşi lucru este valabil şi în cazul aterosclerozei, al diabetului şi al cancerului, în care se descoperă tot mai multe gene candidat, care uneori produc, iar alteori nu produc boala. Iar genele candidat au nevoie de prezența unor factori de risc, care uneori produc iar alteori nu produc boala. Pe de altă parte, factorii de risc au nevoie de un timp foarte lung pentru a se putea manifesta clinic, ceea ce aduce în discuție rolul mecanismelor de reglare în apariția bolii. De asemenea acțiunea tratametelor pe care le folosim în aceste boli este de natură probabilistă. Iar rezultatul tratamentelor variază de la un individ la altul. De aceea se vorbeşte de necesitatea personalizării tratementului în funcție de particularitățile fiecărui bolnav. Fenomenele se petrec ca şi când între modificările moleculare şi manifestările lor clinice ar mai interveni şi alți factori care uneori pot facilita, iar alteori pot bloca, acțiunea modificărilor respective.

Concluzii: În urma analizei relațiilor neliniare dintre modificările moleculare şi manifestările lor clinice, noi am ajuns la concluzia că pe lângă aspectele substanțiale şi energetice pe care le presupun aceste modificări, mai intervin şi nişte aspecte informaționale. După cum se ştie toate moleculele conțin pe lângă substanța şi energia din care sunt formate şi o anumită informație moleculară, reprezentată de structura spațială a moleculei respective. Iar informația moleculară joacă un rol deosebit în recunoaşterea moleculelor şi în reglarea numeroaselor procese biologice. De aceea organismul uman a devenit un foarte complicat sistem de comunicații, de-a lungul căruia se transmit o mulțime de mesageri chimici. Informația moleculară pe care o transmit aceste molecule poate explica mai bine relația dintre modificările moleculare şi manifestările lor clinice, deaorece în sistemele informaționale apare o cauzalitate informațională. Cauzalitatea informațională subliniază faptul că informația nu determină, ci declaşează anumite reacții care nu depind numai de natura semnalului, adică a moleculei respective, ci şi de programul de funcționare al destinatarului, adică al celulelor asupra cărora acționează. Astfel, substratul informațional şi cauzalitatea informațională pot explica mai bine neliniaritatea şi probabilitatea care intervin în realțiile dintre modificările moleculare şi manifestările lor clinice. De aceea noi am arătat că dincolo de patologia moleculară se află de fapt o patologie informațională, care ar putea deschide noi posibilități de prevenire şi tratament în bolile extrem de grave şi de complicate cu care suntem confruntați.

OP-34

MICRORNA PROFILE IN HIV-HBV COINFECTION

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Introduction: After the successful exploit of the interaction between hepatitis C virus and the liver enriched miR-122, which conducted to the development of the first anti-miR drug-Miravirsen, an antisense oligonucleotide that sequesters mature miR-122, there is a considerable interest in the role of miRNA in viral diseases. miR-34a was shown to be involved in HBV related liver fibrosis and development of hepatocellular carcinoma, a key regulator of tumor suppression genep53 and a promoter of HIV replication.The aim of this study was to investigate the possible relation between miR-34a expression and HBV progression in a HIV-HBV coinfected cohort.
Methods: Expression levels of miR-34a were measured by quantitative real-time PCR (Life Technologies - TaqMan® MicroRNA Assays) and levels found in HIV patients were normalized against those in age- matched healthy subjects. The correlation between miR-34a levels and the participants' HBV status, liver fibrosis and cytolysis were evaluated.
Results: 237 HIV positive participants (47.7% males, median age 24 years) were included. 64.6% (n=153) were anti-HBc positive, out of which 52.3% (n=80) were HBsAg positive with 16.3% (n=13) HBeAg positive patients. 31.4%(n=48) patients had HBV recovery markers (anti-HBc and anti-HBs positive) and 16.3% (n=25) maintained isolated anti-HBc antibodies. Only 12.5% (n=10) chronic HBsAg carriers had significant liver fibrosis (APRI>1). 21.3% (n=17) patients had high (>1000 copies/ml) HBV plasma viral load, out of which 82.4% (n=14) were infected with genotype A and 52.9% (n=9) patients had Lamivudine resistance mutations. No difference in miRNA expression was found between HIV monoinfected and HIV-HBV coinfected study participants. Patients with HBsAg had significant higher miR-34a expression than those with resolved HBV infection (median miR-34a expression 0.31 vs 0.08, p=0.05). Among chronic HBsAg carriers an upregulated expression of miR-34a was present in patients with liver fibrosis (0.87 vs 0.22, p=0.02) as well as in those with hepatic cytolysis (0.48 vs. 0.14, p=0.01). In HBsAg carriers, miR-34a was overexpressed in patients with detectable HIV viral load (0.40 vs. 0.22, p=0.05), immunosuppression (1.20 vs. 0.40, p=0.05) andhigh CD4 Nadir cell number (p=0.04).
Conclusion: miR-34a expression seems to be a correlated with parameters of active HBV infection and hepatocellular injury and can constitute a marker for the progression of liver disease, as well as a potential therapeutic target.

PROFILUL MIRNA IN COINFECTIA HIV-VHB

Introducere: Dupa utilizarea cu succes a interactiunii dintre virusul hepatitei C si miR-122, care a dus la dezvoltarea primului medicament anti-miR - Miravirsen, o oligonucleotida antisens care sechestreaza miR-122, a crescut considerabil interesul pentru clarificarea rolului miRNA in bolile virale. miR-34a s-a dovedit a fi implicatin progresia fibrozei hepatice si aparitia carcinomului hepatocelular in infectia cuVHB, un reglator cheie al genei supresoare tumorale p53 si un promotor al replicarii HIV. Scopul studiului de fata a fost acela de a investiga posibila relatie intre expresia miR-34a si progresia VHB intr-o cohorta de pacienti coinfectatiHIV-VHB.
Materiale si metode: Expresia miR-34a a fost masurata prin real-timePCR (Life Technologies - TaqMan® MicroRNA Assays)si normalizata fata de un grup de subiecti sanatosi. A fost evaluata relatia intreexpresia miR-34a si statusul VHB al participantilor, fibrozasi citoliza hepatica.
Rezultate: In studiu au fost inclusi 237 de participanti HIV pozitivi (47,7% barbati, varsta mediana 24 de ani). 64,6% (n = 153) au prezentat anticorpi anti-HBc, dintre care 52,3% (n = 80) au prezentat AgHBs pozitiv. Studiul a inclus 16,3% (n = 13) pacienti cu AgHBe prezent. 31,4% (n = 48) dintre pacienti au prezentat markeri de vindecare VHB (anticorpi anti-HBc si anti-HBs pozitivi), iar 16,3% (n = 25) au mentinut anticorpi anti-HBcizolati.

Doar 12,5% (n = 10) purtatori cronici de AgHBs au prezentat fibroza hepatica (APRI>1). 21,3% (n = 17) dintre pacienti au prezentat incarcare virala VHB semnificativa (> 1000 copii/ml plasma), dintre care 82,4% (n = 14) au fost infectati cu genotipul A si 52,9% (n = 9) au prezentat mutatii de rezistenta la Lamivudina. Nici o diferenta in ceea ce priveste expresia miRNA nu a fost gasita intre participantii HIV pozitivi si cei coinfectati HIV-VHB. Purtatorii cronici de AgHBs au avut expresia miR-34a semnificativ crescutacomparativ cu pacientii ce au prezentat markeri serici de vindecare (expresie mediana miR-34a 0,31 vs 0,08, p = 0,05). In grupul purtatorilor cronici de AgHBs expresia miR-34a a fost crescuta la pacientii cu fibroza(0,87 vs 0,22, p = 0,02) si citoliza (0,48 vs 0,14, p = 0,01) hepatica.De asemeni, miR-34a a fost supraexprimat la pacientii cu incarcatura virala HIV detectabila (0,40 vs 0,22, p = 0,05), imunosupresati (1,20 vs 0,40, p = 0,05) si cu CD4 nadir crescut (p = 0,04).
Concluzii: Expresia miR-34a pare sa fie corelata cu afectarea hepatocelulara siinfectia activa VHBsi poate constitui un marker pentru progresia bolii hepatice, precum si o potentialatinta terapeutica.

OP-35

EXTRACELLULAR VESICLES IN THE TREATMENT OF CANCERS

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Background and Aims: The cancer microenvironment plays a central role in cancer development, growth and homeostasis. This paradigm suggests that cancer fibroblasts support cancers, probably in response to stimuli received from the cancer cells. We aimed at investigating if extracellular vesicles (EVs) can shuttle microRNA (miR) species between cancer associated fibroblasts and cancer cells.
Methods: We extracted EVs according to published protocols. EVs were studied for their miR content by qRT-PCR. EVs were transfected with select miR species and utilized in vitro as well as in vivo in a rat model of cholangiocarcinoma.
Results: miR-195 is downregulated in cholangiocarcinoma cells, as well as in adjoining fibroblasts. Furthermore, we report that EVs shuttle miR-195 from fibroblasts to cancer cells. Lastly, we show that fibroblast-derived EVs, loaded with miR-195, can be administered in a rat model of cholangiocarcinoma, concentrate within the tumor, decrease the size of cancers, and improve survival of treated rats. Conclusions. EVs play a salient role in trafficking miR species between cancer cells and cancer associated fibroblasts in human CCA. Understanding of these mechanisms may allow devising of novel therapeutics.

OP-36

COMMON GENETIC VARIANTS: PREDICTION OF PHENOTYPE AND THERAPEUTIC RESPONSE IN MAJOR PSYCHOSES

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Background: The accumulation of large-scale genome-wide association studies (GWAS) intended to discover the genetic basis of various diseases stimulated the interest in clinical application of common genetic variantsfor 1) phenotype specification and prediction and 2) treatment response prediction and personalization. As a member of two international consortia [Psychiatric Genomics Consortium (PGC)and ConLiGen] we participate to the global effort of identifying the genetic basis of major psychoses and of their treatment.
Objectives: 1) Due to the already demonstrated genetic overlap between the two major psychoses - schizophrenia (SCZ) and bipolar disorder (BP) - in multinational samples (PGC, Lancet, 2013), we analyzed the usefulness of

the polygenic risk scores (PRS) derived from genome scans of BP and SCZ in predicting the risk for BP and BP-subtypes in the Romanian population. 2) We describe the identification of genetic markers underlying the response to lithium maintenance treatment in BP replicated for the first time in European and Asian patients and the prediction of treatment response based on polygenic risk scores (PRS).
Results: 1) In the Romanian sample PRS based on genome-wide significant SCZ-SNPs correctly differentiated the patients from controls (p=0.003) and marginally predicted two BP subphenotypes: the BP with incongruent psychosis (OR=1.39) (p=0.05) and the familial BP with loading of BP, SCZ, and unipolar major depression as opposed to the sporadic BP (OR=1.20), (p=0.05). This was in line with the clinical expectation. The prediction of a third subphenotype, the age-of-onset was ambiguous, with sub-threshold values. Three SNP-clusters emerged with different frequency in age-of-onset bands. 2) The ConLiGen discovered two genes located on chromosome 21 with influence on the positive response to lithium maintenance treatment in BP (Hou et al, Lancet, 2016). For the first time the two genes were replicated in 22 samples of European and Asian ancestry (2563 patients including 152 Romanian patients) and were confirmed in a prospectively followed up sample of patients on lithium monotherapy. The hypothesis that the influence of the two new genes on treatment response might have been conditioned by the general genetic risk for BP as measured by PRS based on genome scans of BP was not confirmed, since the prediction of treatment response based on this PRS-set was not significant.
Discussion: The PRS derived from GWAS significantly differentiated between cases and controls in our sample, but less accurate was the prediction of psychosis subphenotypes. Contrary to the expectation, but similar two other European samples (Bigdeli et al, 2015), the prediction of subphenotypes was better when the significance level of the SNPs included in PRS was lower. This is the consequence of the mix of SNPs with variable effect that buildPRS at the current discovery stage, which leads to limited predictive power.Maher (2015) concludes"while p-values and odds ratios are useful for discovery of novel SNPs influencing disease risk, they are inadequate for describing the potential clinical utility of PRS."
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VARIANTE GENETICE COMUNE: PREDICTIA FENOTIPULUI SI A RASPUNSULUI TERAPEUTIC IN PSIHOZELE MAJORE

Introducere: Acumularea de studii de asociere genome-wide pe esantioane mari destinate descoperirii bazei genetice a diferitelor boli a stimulat interesul pentru aplicarea clinica a variantelor genetice comune in scopul: 1) specificarii si predictiei fenotipului si 2) al predictiei si personalizarii raspunsului la tratament. In calitate de membru a doua consortii internationale [Psychiatric Genomics Consortium (PGC) si ConLiGen] noi participam la efortul global de identificare a bazei genetice a psihozelor majore si a tratamentului lor.
Obiective: 1) Pornind de la suprapunerea deja demonstrata a bazei genetice a celor doua psihoze majore - schizofrenia (SCZ) si boala bipolara (BP) – in esantioane multinationale (PGC, Lancet 2013), am analizat utilitatea scorului de risc poligenic derivat din scanari de genom ale pacientilor schizofreni si bipolari pentru predictia riscului de BP si a sub fenotipurilor BP in populatia romaneasca.2) Identificarea unor gene care conditioneaza raspunsul la tratamentul de intretinere cu saruri de litiu in BP si replicarea lor in esantioane de origine europeana si asiatica si predictia raspunsului la tratamentul cu litiu pe baza scorului de risc poligenic.
Rezultate: 1) In esantionul romanesc de pacienti BP scorul de risc poligenic bazat pe SNP-uri semnificativ asociate cu SCZ a diferentiat corect pacientii de martorii normali psihic (p=0.003) si a prezis, cu semnificatie marginala, doua subfenotipuri BP, si anume: BP cu trasaturi psihotice incongruente (OR=1.39) (p=0.05) si BP familial cu incarcatura de BP, SCZ si depresie majora unipolara in contrast cu BP sporadica (OR=1.20).Aceste rezultate au confirmat asteptarile.Predictia celui de-al treilea subfenotip, BP cu debut precoce, a fost ambigua, neatingand pragul de semnificatie. Insa au fost evidentiate trei clustere de SNP, care au avut frecvente diferite in diferite grupe de varsta de debut, ceea ce indica eterogenitate genetica. 2) ConLiGen a descoperit doua gene situate pe cromozomul 21 care influenteaza raspunsul la tratamentul de intretinere cu litiu in BP (Hou et al, Lancet, 2016). Pentru prima data cele doua gene au fost replicate in 22 esantioane de origine europeana si asiatica (2563 pacienti incluzand 152 pacienti romani) si au fost confirmate in studiul prospectiv de 2 ani a 73 pacienti tratati numai cu litiu. Ipoteza ca actiunea celor doua gene ar fi putut fi

conditionata de scorul global de risc poligenic pentru BP masurat printr-un set de SNP derivate din scanare de genom a pacientilor BP a fost infirmata, deoarece predictia raspunsului terapeutic bazata pe acest scor nu a fost semnificativa.

Discutia rezultatelor: Scorul de risc poligenic diferentiaza semnificativ pacientii BP de martorii normali, dar predictia subfenotipurilor BP nu este precisa. Contrar expectatiei, dar similar altor esantioane europene (Bigdeli et al., 2015), predictia subfenotipurilor a fost mai buna cand pragul de semnificatie al SNP-urilor incluse in scorul poligenic a fost mai redus. Aceasta este consecinta amestecului de SNP cu efect variabil, care sunt incluse in scor in stadiul actual de cunoastere a bazei genetice a psihozelor majore, ceea ce conduce la valoare predictiva limitata. Maher (2015) considera ca "valorile P si odds ratio sunt utile in descoperirea de noi SNP care influenteaza riscul de boala, dar sunt neadecvate pentru potentiala utilizare clinica a scorului poligenic". Aceasta observatie este valabila in prezent nu numai pentru bolile psihice, ci pentru toate categoriile de boli.

Referinte: Maher B.S. Current Epidemiology Reports, 2015, 2: 239–244. Hou L. et al.Lancet.2016, January 21, e-pub.

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OP-37

MOLECULAR DETERMINATIONS (ANTIGENIC EPITOPES) IN FOODCROSS ALLERGIES DIAGNOSIS WITH ORAL LOCALIZATION

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Cross allergies are reactions to a given substance in a patient with sensitization to another substance that has a similar molecular structure (common epitope or similar) and frequent oral clinical manifestations. Causal allergens in cross sensitization are: fruits, vegetables and pollens; frequently polisensitization. Cross sensitization to food-pollens with oral localization or/and general is due to the existence of common or similar epitopes: profilins, polcalcins, vicilins, beta expansins, lipid transfer proteins. The prevalence and incidence of cross allergy pollens, fruits, vegetables is estimated between 55% and 93%. Tests with food epitopes assure a more precocious and finesse diagnostic. It is recommended to determine the 40 allergen epitopes, obtained by genetic mechanisms. The benefit/cost ratio must be considered.

DETERMINARI MOLECULARE (EPITOPI ANTIGENICI) IN DIAGNOSTICULALERGIILOR ALIMENTARE INCRUCISATE CU LOCALIZARE ORALA

Alergiile incrucisate sunt reacții la o substantadata, la un pacient cu o sensibilizare la alta substanta care are o structura moleculara asemanatoare (epitop comun sau asemănător) si frecventa manifestare clinica la nivel oral. Alergenii cauzali in sensibilizarea incrucisată sunt: fructe, legume, polenuri; - frecvent polisensibilizare. Sensibilizarea incrucisata la alimente - polenuri cu localizare orala sau/si generala se datorează existentei unor epitopi comuni sau asemanatori: profiline, polcalcine, viciline, beta expansine, lipide de transfer ale proteinelor.Prevalenta si incidenta alergiilor incrucișate polenuri, fructe, legume se apreciaza intre 55% şi 93%. Testele cu epitopi alimentarine asigura un diagnostic precoce si mai de finete.Se recomanda determinarea celor 40 de epitopi alergenici, obtinuti prin mecanisme genetice. Este necesară aprecierea raportului beneficiu/cost.

OP-38

MOLECULAR MECHANISMS IN RHEUMATOID ARTHRITIS

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The bone is submitted all the life to a permanent renewal by the action of osteoclasts.and osteoblasts.In RA the distructive osteoclast activity is augmented which explains the cartilage distruction and bone erosions inside the involved joints. The synoviocytes-resident cells become FLS-fibroblast like synoviocytes-and there is a hyperplasia of these cells and pannus is formed.The pannus has pseudotumoral characteristics: the cells are resistant to apoptosis and are responsable for the production of metalloproteinases and cathepsins which destroy cartilage and bone. Macrophages and lymphocytes T CD4 + are infiltrating the pannus and activate synovial and FLS cells.The main producers of cytokines are the macrophages.These cytokines: TNF alpha, Il-6, Il-1 act on osteoblasts and RANKL (receptor activator NF-kB ligand) is released.The RANKL binds the osteoclast receptor RANK -stimulating the osteoclast to make bone erosions. The osteoclasts are also stimulated by a soluble RANKL(derived from FLS),and by Il-17 (sintetised by Th 17). As we can see that the inflammatory process is a complex one, involving many immune cells and cytokines.The permanent information delivered by genetics, immunology made possible the better understanding of the inflammatory process and the intrduction of a targeting treatment against these cytokines. Infliximab,a chimeric monoclonal antibody is the first medication used to block the action of TNF alpha. Etanercept, a soluble receptor for TNF alpha compets with TNF alpha receptor blocking the action of the cytokine. Adalimumab, a human monoclonal antibody has the same action as infliximab. Tollicizumab inhibits Il-6 receptor. All these monoclonal antibodies have not only antiinflammatory effect but also a direct antiresorbtive effect.

OSUL ESTE RECONSTRUIT PE TOT PARCURSUL VIETII PRIN ACTIUNEA OSTEOCLASTELOR SI OSTEOBLASTELOR

In artrita reumatoida activitaatea distructiva osteoclastica este exacerbata explicand distructia cattilajului si eroziunile osoas prezente in articulatiile afectate de procesul reumatoid. Sinoviocitele-celulele rezidente se hiperplaziaza transformandu-se in FLS fibroblast like synoviocyte-si constituie panusul sinovial;acesta are caracteristici pseudotumorale: FLS sunt rezistente la apoptoza si secreta metaloproeinaze si catepsine distugatoare ale cartilajului si osului. Macrofagele si limfocitele Th-CD4+ infiltreaza panusul activand sinoviocitele-FLS. Principalul producator dr citokine este macrofagul.Citokinele eliberate : TNF alfa, Il 6, Il 1 actioneaza pe osteoblast care sintetizeaza RANKL.-receptor activator nuclear factor kB ligand. RANKL osteoblastic se leaga de receptorul RANK al osteoclastelor pe care le actveaza si in final ele produc eroziunile osoase. Osteoclastele sunt de asemenea activate de RANKL solubil produs de FLS si de catre Il 17 produsa de Th 17. Dupa cum se poate observa procesul inflamator implicat in patogenia AR este complex implicand multe celule imune si citokinele produse de catre ele. Informatiile furnizate de biologia moleculara genetica si imunologie au permis introducerea de terapii tintite anticitokine. Infliximab, anticorp monoclonal este prima substanta utilizata pentru blocarea TNF alfa. Etanerceptul -receptor solubil al TNF alfa intra in competitie cu receptorul TNF alfa blocand actiunea citokinei. Adalimumabul este un anticorp monoclonal in totalitate uman avand acelesi mod de a actiona ca si infliximabul. Tocillizumabul are rol de a inhiba receptorul IL-6. Toti acesti anticorpi monoclonali au nu numai o puternica actiune antiinflamatorie ci si un putrnic efect anti resorbtiv direct.

OP-39

PRECISION MEDICINE - MULTIOMIC APPROACHES

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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, epigenomic, 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. The interaction between the genome-transcriptome-proteome profile of the patient and the environmental perturbations influences processes such as, inflammation, thrombosis, fibrosis, immune response, cell proliferation, apoptosis, necrosis, and often generates distinct pathophenotypes, clinical syndromes and diseases. Omics profiling of transcriptomes, proteomes, cytokinome, kinome, metabolomes, and autoantibodies has revealed a wide variation of the molecular components during the progression of the disease. In this regard, an integrated analysis of multi-omic data can lead to enhanced prediction of disease risk and evolution. The development of therapeutic agents that target molecular mechanisms is driving innovation in clinical-trial strategies. One of the main challenges is represented by heterogeneous information which must be integrated into personalized predictive models.
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MEDICINA DE PRECIZIE – O ABORDARE MULTIOMICA

Medicina de precizie se bazeaza pe tehnologii avansate OMICE cum ar fi secvențiere de ultimă generație, microarray de gene si proteine, microdisectie cu laser, ceea ce presupune integrarea datelor de genomica, epigenomica, proteomica, metabolomica și clinica ale fiecarui pacient. Dezvoltarea tehnologiilor de genotipare, de multiplex, si de hightroughput permite analiza genomului individual al pacientului din sangele periferic sau din materialul biopsic. Interactiunea din tregenomului-transcriptomul-proteomul pacientului si variatiile de mediu in fluenteaza procese cum ar fi, inflamatia, tromboza, fibroza, raspunsulimun, proliferarea celulara, apoptoza, necroza si adesea genereaza patofenotipuri, sindroame clinice si boli distincte. Analiza OMICAa transcriptomului, proteomului, citokinomului, kinomului, metabolomului si a autoanticorpilora scos la iveala o variatie mare a componentelor moleculare in timpul progresiei bolii. In acest sens, o analiza integrativa a datelor multi-Omicepoate duce la predictive sporita a riscului de imbolnavire si evolutie. Dezvoltarea unor agenti terapeutici care vizeaza mecanismele molecular reprezinta o directive inovatoare in strategiile studiilor clinice. Provocarea majora in domeniul abordarilor omice este reprezentata de eterogenitatea in formatiilor ce necesita a fi integrate in modele predictive personalizate.
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OP-40

OF MEN AND WORMS: TREATMENT WITH HELMINTHS IN MULTIPLE SCLEROSIS

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In the last 50 years, environmental factors such as helminth infections have been proposed to explain why

autoimmunity is less prevalent in the developing world (hygiene or 'old friends' hypothesis). The epidemiology of multiple sclerosis (MS) shows an inverse correlation with helminth infections. Positive effects of helminths in animal models of MS and observational studies in people with MS naturally infected with helminths suggest that those organisms can act as immune regulators and led to clinical trials of helminth therapy. The goal of helminth therapy is to introduce parasitic organisms into people with MS in a controlled and predictable fashion, and to prevent immune-mediated disease without increasing the risk of pathology with high parasite load. The mechanisms by which helminths induce immunomodulation are complex and not completely understood. Treatment of the animal model of MS with helminths generates an immunoregulatory response beyond the classical Th2 response. These multimodal effects, which involve tolerizing stimulation of B cells and dendritic cells by helminth-derived molecules, induction of Tregs, and production of TGF-β and IL-10 could explain why helminth treatment modulates both Th1- and Th2-driven conditions.I will review current data regarding the rationale and the current state of research in the field of helminth therapies in MS including the first phase II double blind controlled trial of hookworm in MS, which is ongoing.

DESPRE OAMENI SI VIERMI: TRATAMENTUL CU PARAZITI INTESTINALI (HELMINTI) IN SCLEROZA MULTIPLA

Date solide epidemiologi ce arata o raspandire inversa, o excludere mutuala intre bolile autoimune – inclusiv scleroza multipla (SM)- si infectiile cu paraziti intestinali. Date provenind din studii pe animale si studii pilot asupra unor pacienti cu SM sugereaza existenta unui mecanism imunomodulator eficace si secundar infectiei controlate cu viermi intestinali. SM este considerata ca fiind o afectiune disimuna in care celulele Th1 au un rol important, iar infectia helmintica modifica profilul Th1 spreunul de tip Th2. Imunomodularea realizata de parazitii intestinali este insa mult mai complexa de atat si implica celulele T reglatore, celulele prezentatoare de antigen si probabil si alte mecanisme. Intelegerea imonomodularii secundare prezentei helmintilor in intestin ofera date noi cu privire la mecanisme imunomodulatoare care pot fi tinte pentru noi terapii. Voi discuta perspectivele, limitele si implicatiile terapiei helmintice in SM, precum si integrarea cunoasterii oferite de aceasta in tabloul complex al fenomenelor imune din SM. Ma voi referi la primul studiu clinic de faza II dublu orb controlat cu Necatoramericanus in SM.

OP-41

THE SIGNIFICANCE OF MOLECULAR DIAGNOSTIC IN INFECTIONS WITH RESPIRATORY SYNCYTIAL VIRUS

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Introduction: Respiratory syncytial virus (RSV) is an important respiratory pathogen that often causes severe and even fatal infections, particularly in children aged under 6 months.
Several studies have shown that other population groups may be vulnerable to severe RSV infections; for example, pregnant women, immunocompromised individuals and patients with chronic medical conditions. The clinical presentation usually does not differ from that caused by other respiratory pathogens, and RSV often co-circulates with other respiratory viruses including influenza.Result of this research, the importance of diagnosis as accurate and precise due to RSV infections, the more there are ways through preventive vaccination (at this time there are many viral preparations that are verified by clinical studies).
Materials and methods: Nasal and throat swabs and aspirations are the samples used to detect RSV. The protocols used for this purpose are similar as in the case of detection of influenza viruses and they can be used in the collection, storage and transport of samples. Immunofluorescence techniques are still quite widespread

in detecting RSV especially in laboratories from low and medium developed countries. These techniques are especially low sensitivity in samples collected from children. To gain sensitivity actual we use polymerase chain reaction. Genotyping and sequencing RSV is very important for understanding the pathogenicity of this infection which currently is poorly understood. Laboratory techniques used in our study were (1) Real-time RT-PCR (Reverse Transcription-Polymerase Chain Reaction) for the detection of influenza viruses type A and type B; (2) Multiplex RT-PCR with Commercial Kit One-Step ACE RT-PCR Seeplex (Seegene) for the detection of other respiratory viruses (RSV type A and type B, Parainfluenza viruses types 1,2,3 and 4,Metapneumovirus, Adenovirus, Rhinovirus , Enterovirus, Bocavirus) and (3) sequencing of a fragment of 278 pairs of bases of the type RSV N gene and a fragment of 212 pairs of bases of the type B RSV SH gene to confirm and identify RSV.
Results: Detection of influenza viruses type A and B were negative. (2) detection of patient samples aged 2.9 years died with RSV infection caused by type B, of patient died 0.5 months with RSV infection type A with associated chronic diseases and patients aged 1 month 3 months and 2 months with congenital heart malformation and coming from the community were positive with Seegene kit. Because studies show that RSV type B infections give small or medium severity were (3) sequenced lung samples from patients died 2.9 months and who have not diseases associated to confirm the presumptive diagnosis of infection caused RSV type B. The sequencing showed 100% identity between the two pieces of lung and 97-98% identity with sequences from GenBank (eg human RSV B / Homo sapiens / USA / 90E-181-01 (gi/ 727 880 010); human RSV B / GZ / 13-730 (gi / 733 370 580); Kilifi_9697_16_RSV human RSV B (gi / 75.151450). Conclusions The rate of hospitalization is usually about 0,3% RSV disease and the fatality rate is very small (less than 1% in otherwise healthy children) and the Type B is generally milder. But in this particular case, thehRSV type B infection induced a severe illness with fatal result to a 2y9m old infant with no other associated disease or special conditions. In patients with associated chronic disease, RSV can cause either death or serious infections. Infections like influenza and infections due to RSV can spread easily in community. RT-PCR method proves to be a highly sensitive and specific for the diagnosis of infections due to RSV. For understanding pathogenicity mechanisms of this infection are neededmore studies in RSV genotyping and gene sequencing.

IMPORTANTA DIAGNOSTICULUI MOLECULAR IN INFECTIILE CU VIRUS RESPIRATOR SINCITIAL

Introducere: Virusul Respirator Sincitial (VRS)este un patogen respirator important care determina deseori infectii severe si chiar decese indeosebi la copiii mai mici de 6 luni. Exista studii care au demonstrat ca sunt anumite grupe populationale care pot fi vulnerabile la infectii grave cu VRS, ca de exemplu, gravidele, persoane imunocompromise si pacienti cu afectiuni cronice. De obicei tabloul clinic nu difera de cel determinat de alte virusuri respiratorii si de multeori VRS cauzeaza co-infectii cu alte virusuri respiratorii. Rezulta din aceste cercetari, importanta unui diagnostic cat mai precis si exact al infectiilor datorate de VRS, cu cat mai mult ca exista mijloace de preventive prin vaccinare (existand la aceasta ora mai multe preparate virale ce sunt verificate prin studii clinice).
Materiale si metode: Tampoanele nazale si faringiene precum si aspiratele sunt cele mai folosite probe pentru detectia VRS. Protocoalele utilizate in acest scop sunt similare ca in cazul detectiei virusurilor gripale si ele pot fi folosite in colectarea, stocarea si transportul probelor. Tehnicile de imunofluorescenta sunt inca destul de larg raspandite in detectia VRS, in special in laboratoarele din tarile slab si mediu dezvoltate. Aceste tehnici prezinta o sensibilitate scazuta mai ales in cazul probelor colectate de la copii. Pentru a castiga in sensibilitate se foloseste actual reactia de polimerizare in lant. Genotiparea si secventierea VRS este foarte importanta pentru intelegerea patogenitatii acestei infectii care la ora actuala este putin inteleasa. Tehnicile de laborator utilizate in studiul nostru au fost (1) Real-time RT-PCR (Reverse Transcription-Polymerase Chain Reaction) pentru detectarea virusurilor gripale tip A si tip B; (2) Multiplex RT-PCR cu Kit-ul commercial RT_PCR One-Step ACE Seeplex (Seegene) pentru detectia altor virusuri respiratorii (VRS tip A si tip B, Virusuri paragripale tipurile 1,2,3 si 4, Metapneumovirus, Adenovirus, Rhinovirus, Enterovirus, Bocavirus) si (3) secventierea unui fragment de 278 perechi de baze a genei N pentru VRS tip A si a unui fragment de 212 perechi de baze a genei SH pentru VRS tip Bpentruconfirmareasiidentificarea VRS.
Rezultate: (1) Detectia virusurilor gripale tip Asi B au fost negative. (2) Detectia probelor pacientului in varsta de 2,9 ani decedat cu infectie cauzata de VRS tip B, a pacientului de 0,5 luni decedat cu infectie cu VRS tip

Asi cu afectiuni cornice associate si a pacientilor cu varste de 1 luna, 3 luni si 2 luni cu malformatii congenitale de cord si proveniti din colectivitate, au fost positive cu Kit-ul Seegene. Deoarece studiile arata ca tipul B al VRS da infectii de severitate mica sau mijlocie s-au (3) secventiat probele de plaman provenite de la pacientul decedat de 2,9 luni si care nu avea boli associate pentru a se confirma diagnosticul prezumtiv de infectie cauzata de VRS tip B. Secventierea a aratat 100% identitate intre cele 2 fragmente de plaman si 97-98% identitate cu cateva secvente din GenBank (de exemplu VRS umanB/Homo sapiens/USA/90E-181-01 (gi/727880010); VRS uman B/GZ/13-730(gi/733370580);VRS uman Kilifi_9697_16_RSV B (gi/75151450).
Concluzii: Desi rata de spitalizareeste in general in infectiile cu VRS de 0,3% si rata de decese este foarte mica in tarile dezvoltate (mai mica de 1%) si tipul B de VRS da forme clinice usoare in cazul copilului de 2,9 ani, VRS de tip B a indus o forma clinica severa soldata cu deces. La pacientii cu boli cronice asociate, VRS poate provoca fie deces, fie infectii grave. Ca si infectiile gripale si infectiile datorate de VRS se pot transmite cu usurinta in colectivitate. RT-PCR se dovedeste a fi o metoda sensibila si cu specificitate inalta pentru diagnosticul infectiilor datorate de VRS. Pentru intelegerea mecanismelor patogenitatii acestei infectii sunt necesare mai multe studii de genotipare si secventiere a genelor VRS.

OP-42

ANTIRETROVIRAL RESISTANCE IN A COHORT OF EXTENSIVELY - TREATED HIV - INFECTED ROMANIAN PATIENTS

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Background: HIV drug resistance can lead to treatment failure and could be a risk factor for HIV-associated neurocognitive impairments. Our goal was to assess the prevalence of drug resistance and its impact on neurocognitive impairments in a cohort of extensively-treated HIV-infected Romanian patients, long term survivors, parenterally infected in early childhood.
Methods: HIV viral load was tested by quantitative RT-PCR (Cobas TaqMan HIV-1 Test, Roche Molecular Systems). Pol gene sequencing was performed using the ViroSeq HIV-1 Genotyping System (Abbott Laboratories, USA) for patients with more than 1000 copies HIV RNA/ml. HIV subtype was determined using the REGA database. The neurocognitive impairments were assessed using a comprehensive, seven-domain neuropsychological battery and a global deficit score (GDS) was calculated for each participant (cut- off 0.5). Results. 219 HIV-1 infected patients (median age: 23 years, males: 47%) were analyzed. The median duration on cART was 13 years and 50.45% had an AIDS-defining event (stage C). Current median CD4 cell count was 463 cells/mm3 (range: 4- 1956 cells/mm3), 16.2% presenting severe immunosuppression (CD4 count< 200 cells/ml). Median plasma viral load was 2.11 log10 HIV-1 RNA copies/ml (0- 5.65 log10); more than half of the patients (59.9%) had undetectable viral load, and only 24.3% had active viral replication (HIV viral load > 1000 copies/ml). HIV-1 subtype analysis indicated that all strains belonged to the F subtype. The rate of antiretroviral resistance was 15%, with 6% of the subjects presenting resistance to two drug classes, 4% having triple class resistance and 1% having multiple resistance mutations to all currently available drugs. Patients infected with HIV-resistant variants had lower CD4 T-cell counts and nadir CD4 counts, longer exposure to cART and monotherapy regimens comparing with those infected with wild type HIV. No correlation was found between the global deficit score (GDS) and the presence of HIV drug resistance. There were no significant differences between subjects carrying resistant viruses and those infected with wild type in term of neurocognitive and functional deficits for the seven evaluated domains (verbal fluency, speed of information processing, executive functioning, learning, memory, attention/working, motor skills).
Conclusions: We report a low rate of ARV resistance in a cohort of extensively-treated HIV-infected Romanian patients, long term survivors. Our data suggest that HIV drug resistance do not influence

neurocognitive impairments (NCI); a longitudinal follow-up of these patients is needed in order to assess its impact on the progression of NCI.

REZISTENTA LA ANTIRETROVIRALE INTR-O COHORTA DE PACIENTI INFECTATI HIV, POLIEXPERIMENTATI TERAPEUTIC

Background: Rezistenta HIV la antiretrovirale poate determina esec terapeutic si poate reprezenta un factor de risc pentru afectarea neurocognitiva asociata infectiei HIV. Studiul nostru evalueaza prevalenta rezistentei la antiretrovirale si impactul acesteia asupra deficitului neurocognitiv intr-un grup de pacienti infectati HIV politratati, supravietuitori de lunga durata, infectati parenteral in copilarie.
Metode: Incarcarea virala HIV s-a realizat prin tehnica RT-PCR cantitativ (Cobas TaqMan HIV-1, Roche Molecular Systems). Pentru pacientii cu ARN HIV >1000 copii/ml s-a realizat secventierea genei pol utilizand ViroSeq HIV-1 Genotyping System (Abbott Laboratories). Subtipul HIV a fost definit conform algoritmului bazei de date REGA. Afectarea neurocognitiva a fost evaluata cu ajutorul unei baterii neuropsihologice ce vizeaza 7 domenii cognitive si un scor de deficit global (GDS) a fost calculat pentru fiecare participant (cut off- 0,5).
Rezultate: 219 subiecti HIV-1 pozitivi (varsta mediana: 23 ani, barbati: 47%) au fost inclusi in studiu. Durata mediana a terapiei cART a fost de 13 ani, iar 50,45% au prezentat un eveniment definitoriu SIDA (stadiul C). Valoarea mediana curenta a numarului de celule CD4 a fost 463 celule/mm^3, 16,2% prezentand imunosupresie severa (CD4 <200 celule/mm^3). Valoarea mediana a incarcaturii virale a fost 2.11 log10 copii ARN HIV-1/ml; majoritatea pacientilor (59,9%) au avut incarcare virala nedetectabila, si numai 24,3% au prezentat replicare virala activa (ARN HIV-1> 1000 copii/ml). Analiza subtipului HIV-1 a indicat ca toate tulpinile studiate apartin subtipul F. Rata rezistentei HIV la antiretrovirale a fost de 15%, cu 6% dintre subiecti prezentand rezistenta la doua clase de antiretrovirale, 4% prezentand rezistenta tripla si 1% avand mutatii multiple de rezistenta la toate ARV disponibile in prezent. Pacientii infectati cu variante HIV rezistente au prezentat o valoare mai mica a numarului de limfocite CD4 si CD4 nadir si o expunere mai indelungata la cART si regimuri monoterapeutice comparativ cu cei infectati cu tipul salbatic. Nu a existat nicio corelatie intre scorul global de deficit neurocognitiv (GDS) si prezenta rezistentei la antiretrovirale. Nu au fost identificate diferente semnificative intre subiectii purtatori de tulpini HIV rezistente si cei infectati cu tulpini tip salbatic in ceea ce priveste deficitele neurocognitive si functionale pentru cele 7 domenii evaluate (fluenta verbala, viteza de procesare a informatiei, functia executiva, capacitatea de invatare, memoria, atentia, abilitatile motorii).
Concluzii: Raportam o rata redusa a rezistentei la ARV intr-un grup de pacienti romani infectati HIV poliexperimentati terapeutic, supravietuitori de lunga durata. Datele noastre sugereaza ca rezistenta la antiretrovirale nu influenteaza deficitul neurocognitiv (NCI) asociat HIV; este necesara o urmarire longitudinala a acestor pacienti pentru a evalua impactul rezistentei HIV asupra progresiei NCI.

OP-43

PATHOPHYSIOLOGICAL IMPLICATION OF TRP ION CHANNELS IN PANCREATIC CANCER

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Transient receptor potential melastatin is a membrane ion receptor participating to cold sensation in peripheral neurons. TRPM8 is over-expressed in several solid tumours such as breast, kidney and lung. In pancreatic adenocarcinoma (PDA) this membrane protein was related to tumour size and stage, migration and proliferation of PDA cells. With this study we bring new insights in the structure and function of TRPM8 which shows that the form expressed in the pancreatic tumoural cell line Panc-1 is un-glycosylated as compared to the glycosylated protein

over-expressed in Human Embryonic Kidney 293(HEK) cells. We studied the roles of TRPM8 in cell migration, Ca2+ uptake and cell proliferation. We found that the un-glycosated form determine a slight but significant decrease in pancreatic cell migration. Moreover, the uptake of calcium depends of the presence of tunicamicyn a specific inhibitor of N-linked glycoslylation. Thus, our observations suggest that un-glycosylation of TRPM8 may be protective against cell migration and proliferation in PDA cells.

OP-44

ENDOTHELIAL MICROPARTICLES AND INFLAMMATORY BIOMARKERS IN HEART FAILURE

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Rational: Endothelial-derivedmicroparticles (EMP) are circulating submicron-sized membranous vesicles released from activated or apoptotic endothelial cells. They are active messenger in pathophysiological responses and play a key role in coagulation, inflammation, endothelial function, and angiogenesis and they serve as surrogate marker of endothelial function. It is known that heart failure (HF) is associated with endothelial dysfunction.
Objective: The purpose of this study was to determine the profile distribution of EMPsin heart failure (HF) patients compared to healthy subjects and to identify possible correlations between diversity of the circulating MPs concentration and inflammatory biomarkers.
Methods: We enrolled 40 HF patients with New York Heart Association (NYHA) class II or more, with stable disease within 6 months (age 65.9±10.5 years). The control group consisted of 20 healthy individuals (age-gender matched).EMPs were investigated by flow-cytometry for the expression ofdifferent antigens ($CD31^+$, $CD42b^-$, $CD62E^+$). The following biomarkers were evaluated: paraoxonase (PON),myeloperoxidase (MPO) and plasma lipoprotein-associated phospholipase A2 (Lp-PLA2) activity.
Results: Total EMP levels were significantly higher in patients with HF compared with healthy subjects ($p< 0.001$). In HF patients the EMP levels of subpopulation expressing the following antigens $CD31^+/CD42b^-$, $CD31^+/CD62E^+$ and $CD31^+/CD42b^-/CD62E^+$ were 2.5, 5.8 and respectively 6.2-fold higher than the one detected in the normal population. Between HF and control group the most marked increase was noticed for $CD62E^+$ EMP levels ($p<0.001$). Lp-PLA2 activity was higher in HF patients group (413±67 U/L), compared to healthy subjects (225.65±20.8 U/L) ($p< 0.001$). EMPs ($CD31^+/CD42b^-$ and $CD31^+/CD62E^+$) levels were positively correlated with MPO and negatively correlated with PON. None correlation had statistical significance.
Conclusions: When incorporated in a multi-marker strategy, the detection and quantification of EMPs phenotypemay be a valuable biomarker of endothelial dysfunction which could be used in HF risk stratification. EMPs may not only reflect the presence of HF but also play a causative role in its development. Additional larger scale studies will be needed in order to identify the additive power of EMP levels to the actual biomarkers used in HF risk stratification and evolution.

MICROPARTICULELE ENDOTELIALE SI BIOMARKERI INFLAMATORI IN INSUFICIENTA CARDIACA

Introducere: Microparticulele endoteliale sunt vezicule membranoase circulante, de dimensiuni sub-micronice, eliberate de catre celule endoteliale activate sau aflate in apoptoza. Aceste microparticule sunt mesageri activi ai raspunsurilor fiziopatologice si joaca un rol important in coagulare, inflamatie, functie endoteliala, angiogeneza si totodata reprezinta un marker-surogat al functiei endoteliale. Pe de alta parte asocierea dintre insuficienta cardiaca si disfunctia endoteliala este cunoscuta.
Obiectiv: Scopul acestuistudiu este determinarea profilului distributiei microparticulelor endoteliale la pacientii cu insuficienta cardiaca comparativ cu subiectii sanatosi si identicarea unor posibile corelatii intre

concentratiile diferitelor microparticule circulante si altibiomarkeri ai inflamatiei.
Material si Metoda: Au fost inrolati 40 de pacienti cu insuficienta cardica cronica (clasaNYHA II-IV), cu o evolutie stabila a bolii in ultimile 6 luni (varsta medie : 65.9±10.5 ani). Grupul control a fost alcatuit din 20 de subiecti sanatosi. Microparticulele endoteliale au fost masurate utilizand citometria de flux pentru detectia diferitelor antigene ($CD31^+$, $CD42b^-$, $CD62E^+$). Urmatorii biomarkeri au fost evaluati : paraoxonaza (PON), mieloperoxidaza (MPO) si activitatea fosfolipazei A2 asociata LDL (Lp-PLA2)
Rezultate: Nivelele totale de microparticule endoteliale au fost crescute in mod semnificativ in cazul pacientilor diagnosticati cu insuficienta cardiaca, fata de subiectii sanatosi($p>0,001$). Comparativ cu subiectii sanatosi, pacientii cu insuficienta cardiaca au prezentat nivele crescute ale microparticulelor endoteliale din subpopulatiile ce au exprimate urmatoarele antigene $CD31^+/CD42b^-$, $CD31^+/ CD62E^+$si$CD31^+/ CD42b^-/ CD62E^+$ de 2.5, 5.8 respectiv 6.2 ori. Intre grupul diagnosticat cu insuficienta cardiaca si grupul control, cea mai marcanta crestere a fost observata in cazul microparticulelor endoteliale avand antigenul $CD62E^+$($p>0.001$). Activitatea Lp-PLA2 a fost mai mare in cazul grupului de pacienti cu insuficienta cardiaca (413±67 U/L) decat in cazul subiectilor sanatosi (225.65±20.8 U/L) ($p<0.001$).S-a identificat o corelatie pozitiva intre nivelul microparticulelor endoteliale ($CD31^+/CD42b^-$ and $CD31^+/CD62E^+$) si MPO, respective o corelatie negative cu PON. Niciuna dintre aceste corelatii nu a prezentat semnificatie statistica.
Conclusions: Prin incorporarea intr-o strategie multimarker, datele referitoare la detectarea si cuantificarea fenotipului microparticulelor endoteliale pot fi utile in stratificarea riscului de aparitie a insuficientei cardiace. Microparticulele endoteliale nu numai ca pot indica prezenta insuficientei cardiace, dar au si un rol cauzativ in dezvoltarea acestei patologii. Sunt necesare studii ulterioare implicand o populatie mai mare pentru a identifica puterea aditionala pe care nivelul de microparticule endoteliale o adauga la cea a biomarkerilor utilizati actual la stratificarea si evolutia riscului de insuficienta cardiaca.

OP-45

DYNAMICS OF BIOMARKERS AFTER CORONARY STENT PROCEDURE

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Rational: Although drug-eluting stents (DES) have drastically reduced the incidence of in-stent restenosis (ISR), this remains an important clinical problem. The key pathophysiological players in appearance of ISR are the aggressive neointimal proliferation and neoatherosclerosis.
Objective: The aim of this study was to identify pre-interventional markers with the capacity to predict in-stent restenosis (ISR).
Methods: 80 patients with significant stenosis proven through angiography underwent percutaneous coronary intervention (PCI). For all patients the following biomarkers were evaluated: creatin kinase isoenzyme MB (CK-MB), C reactive protein (CRP), Ischemia-modified albumin (IMA), CD40 ligand, plasma adiponectin (APO), paraoxonase (PON),myeloperoxidase (MPO) and plasma lipoprotein-associated phospholipase A2 (Lp-PLA2)activity. All biomarkers were evaluated before and after PCI, at established time points. Post procedure, biomarkers were measured at 24, 48, 72 hours, and at 1, 3, respectively at 6 months. ISR was evaluated at 6 months after stenting procedure by coronary angiography, and it was defined as > 50% stenosis of the target lesion. Based on the initial CRP value, measured before PCI a cut off value of 3 mg/L was established, and the subjects were divided in 2 groups (Group 1 PCR≤3mg/L, Group 2 PCR>3mg/L). Each group was subdivided, based on type of stent used, bare metal stent (BMS), respectively drug eluting stent (DES).
Results: 6 month after PCI, the ISR was present in 33.75% cases. As expected, the ISR rate was lower in DES compared to BMS cases, the difference being significant just for Group 1 ($p<0.001$), but not for Group 2, were the initial inflammatory status had higher intensity. For both groups, regardless of type of stent used, baseline APO plasma concentration, measured before PCI, was lower in ISR patients than those without ISR [3.97

(±1.05) vs 6.65 (±2.95) μg/ml respectively, p<0.001]. SimilarAPO plasma concentration pattern was mentioned at 6 month [5.05 (±1.75) vs 7.52 (±3.02) μg/ml respectively, p<0.005].ROC curve showed an APO cut-off valueof 4.9 μg/ml, at discharge, being significant in detection of patients susceptible to develop ISR post PCI(odd ratio, 4.27; 95% CI, 1.56-11.72,P< 0.001). APO and PON values varied similar during the study. ISR rate was independent from any otherinvestigated inflammation markers baseline values (CK-MB, CRP, IMA, CD40 ligand, MP, Lp-PLA2).

Conclusions: The persistence of a low APO plasma level post PCI, at discharge, may be used as a clinically useful marker for ISR prediction in patients undergoing PCI. Further studies, involving higher number of participants are needed for confirming our results.

DINAMICA BIOMARKERILOR POST IMPLANTARE STENT

Introducere: Chiar daca aparitiastenturilor active farmacologic a redus dramatic incidenta restenozei in stent, aceasta ramane o problema clinica importanta. Principalele cauze fiziopatologice in aparitia fenomenului de restenoza in stent sunt reprezentate de proliferarea neointimala agresiva si de neoateroscleroza.

Obiectiv: Scopul acestui studiu este de a identifica preinterventional markerii capabili sa prezica fenomenul de restenoza in stent.

Material şi metoda: 80 de pacienti cu stenoza coronariana semnificativa, dovedita prin angiografie au fost supusi angioplastiei coronariene percutane(PCI). Pentru toti acesti pacienti, urmatorii biomarkeri au fost evaluati: creatin-kinaza izoenzima MB (CK-MB), proteina C-reactiva (CRP), albumina modificata de ischemie (IMA), ligandul CD40, adiponectina plasmatica (APO), paraoxonaza (PON), mieloperoxidaza (MPO) siactivitatea fosfolipazei A2 asociata LDL (Lp-PLA2). Toti acesti biomarkeri au fost evaluati atat inainte cat si dupa PCI, la intervale de timp prestabilite. Postprocedural, biomarkerii au fost evaluati la 24, 48 si 72 de ore, iar apoi dupa 1, 3 si respectiv 6 luni de la efectuarea procedurii. ISR a fost evaluata angiografic la 6 luni de la procedura de implantare a stentului si a fost definita ca stenoza de >50% a leziunii tinta. Luand in considerare valoarea initiala a proteinei C-reactive, masurata inainte de PCI, s-a stabilit o valoare limita a nivelului de proteina C-reactiva de 3mg/L, iar subiectii studiului au fost impartiti in doua grupuri (Grupul 1 CRP≤3mg /L, Grupul 2CRP>3mg /L). Fiecare grup a fost mai apoi subdivizat in functie de tipul de stent folosit, stent metalic simplu (BMS), respectiv stent activ farmacologic (DES).

Rezultate: La 6 luni dupa postinterventional, restenoza in stent a aparut la 33.75% dintre cazuri. Dupa cum era de asteptat, rata incidentei aparitiei restenozei a fost mai mica in cazul utilizarii DES decat in cazul BMS, difenta fiind semnificativa din punct de vedere statistic numai pentru Grupul 1 (p<0,001), nu si pentru Grupul 2, unde nivelul inflamator initial era mult mai mare.Pentru ambele grupuri, indiferent de tipul de stent folosit, valorile plasmatice initiale ale APO, masurate preinterventional, erau mai mici in cazul pacientilor ce au experimentat restenoza in stent decat in cazul celor care nu au prezentat acest fenomen [3.97 (±1.05) vs 6.65 (±2.95) μg/ml, p<0,001]. Un pattern similar a fost observant pentru valorile plasmatice ale APO la 6 luni post interventional [5.05 (±1.75) vs 7.52 (±3.02) μg/ml, p<0.005]. Curba ROC a evidentiat o valoare cut-off pentru APO de 4,9 μg/ml, la externare, cu semnificatie in detectia pacientilor susceptibili la aparitia restenozei in stent postinterventional (OR 4,27; 95% CI, 1,56-11,72, p<0,001). Valorile APO si PON au variat similar pe durata studiului. Incidenta aparitiei restenozei in stentnu a fost corelata cu valorile initiale ale altui biomarker investigat (CK-MB, CRP, IMA, CD40 ligand, MP, Lp-PLA2).

Concluzii: Prezenta unuinivel plasmatic scazut ale APO postinterventional, prezent la externare, poate fi utilizat ca un marker importat din punct de vedere clinic in predictia fenomenului de restenoza in stent la pacientii ce au fost supusi unei angioplastii coronarienepercutane. Pentru a putea confirma rezultatele noastre, sunt necesare studii ce vor include un numar mai mare de pacienti.

OP-46

THE NEUROSCIENCES OF STRESS

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This communication presents an integrative "top-down" approachon the mechanisms of reception and processing ofthe stress factors, as well as the mechanisms for the modulation of perception and immediate and long-term response from neurobiological and epigenetic perspectives.

STRESUL DIN PERSPECTIVA NEUROSTIINTELOR

Comunicarea prezintă o abordare integrativă "top-down" privind mecanismele receptării și procesării factorilor stresanți, precum și ale percepției și modulării răspunsului imediat și de lungă durată din perspectiva neuro-biologică și epigenetică.

P-01

STRUCTURAL ANOMALIES OF CHROMOSOME 11 IN MYELOID AND LYMPHOID MALIGNANCIES

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Reciprocal translocations, chromosomal deletions and gene amplifications are recurrent anomalies in both myeloid and lymphoid acute leukemias (ALs). Chromosome 11 represent a frequent target for genetic lesions in ALs. Although 11q23 translocations, involving KMT2A gene, are the most frequently reported anomalies of chromosome 11, many other lesions are described across all ALs subtypes. We report on 14 adult patients with ALs associated with chromosome 11 aberrations at first presentation, selected out of 101 ALs patients. Bone marrow aspirate and/or peripheral blood was used for morphological, cytochemical, flow cytometry and chromosomal studies. Cytogenetic investigations were performed on GTG-banded slides. FISH with locus specific probes (commercial and „home-made") was applied for molecular characterization. Our patient group included 9 acute myeloid leukemias, 4 acute lymphoblastic leukemias and one biphenotypic acute leukemia. Reciprocal translocations involving the KMT2A gene were the most common anomalies (7 out of 14). Two patients had interstitial deletions of 11q. KMT2A amplification, with a variable number of copies per cell (3-12), was detected in 4 patients. The amplification occured in different cytogenetic forms (ring chromosome 11, unbalanced translocations). Chromosome 11 anomalies were detected as part of complex karyotypes in 6 patients. In our ALs patient group the chromosome centered evaluation detected a significant complexity of genetic aberrations of chromosome 11. Molecular cytogenetic investigations played an important role in refining the abnormalities and, ultimately, in patient clinical management. The cytogenetic complexity and heterogeneity points towards the need of an individualised approach for patients with ALs.

ANOMALII STRUCTURALE ALE CROMOZOMULUI 11 IN NEOPLASMELE MIELOIDE SI LIMFOIDE

Translocatiile reciproce, deletiile cromozomiale si amplificarile genice sunt anomalii recurente in leucemiile acute mieloide si limfoide (LA). Cromozomul 11 reprezinta o tinta frecventa pentru leziuni genetice in LA, fiind descrise numeroase defecte genetice asociate. Dintre acestea, translocatiile 11q23 implicand gena KMT2A sunt cele mai frecvent raportate. Prezentam 14 pacienti selectati dintr-un grup de 101 pacienti adulti cu LA, la care au fost detectate anomalii ale cromozomului 11 la diagnostic. Evaluarea morfologica, citochimica, citometria in flux si analiza cromozomiala au fost efectuate pe probe de maduva osoasa si / sau sange periferic. Pentru investigatiile citogenetice au fost utilizate preparate bandate GTG. Tehnica FISH cu sonde locus specifice (comerciale sau preparate in laborator) a fost utilizata pentru caracterizare moleculara. Noua pacienti au prezentat leucemie acuta mieloblastica, 4 pacienti leucemie acuta limfoblastica si 1 pacient leucemie acuta bifenotipica. Cele mai frecvente defecte au fost translocatiile reciproce implicand gena KMT2A (7 din 14 pacienti). Doi pacienti au prezentat deletii interstitiale 11q. La 4 pacienti au fost detectate amplificari ale genei KMT2A cu numar variabil de copii/celula (3-12). Aceste amplificari s-au prezentat in forme citogenetice variate (cromozom inelar 11, translocatii neechilibrate). La 6 pacienti anomaliile cromozomului 11 au fost detectate ca parte a unor cariotipuri cu modificari complexe. In grupul nostru de studiu a fost observata o heterogenitate semnificativa a aberatiilor genetice ce implica cromozomul 11, frecvent detectate in cariotipuri complexe. Investigatiile citogenetice moleculare au avut un rol important in caracterizarea anomaliilor detectate si managementul clinic al pacientilor. Datele prezentate subliniaza, o data in plus, necesitatea abordarilor individualizate la pacientii cu LA.

P-02

ELTD1, A CANDIDATE BIOMARKER AND THERAPEUTIC TARGET IN GLIOBLASTOMA

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Epidermal growth factor, latrophilin and 7 transmembrane domain-containing protein 1 (ELTD1), an orphan adhesion GPCR (G-protein coupled receptor) in considered to be a regulator of normal angiogenesis, also potentially involved in cancer progression and development. Discovered approximately 14 years ago by Nechiporuk et al, ELTD1 was shown to be highly expressed in cardiomyocytes and smooth muscle cells of both blood vessels and bronchi, in murine models. In a recent study by Wallgard et al., the ELTD1 gene levels were substantially more expressed in the endothelial cells of the small blood vessels of the brain in humans (levels were 254 higher in comparison to other areas of the brain). Higher ELTD1 gene levels were also reported in rodent high grade glioma samples in comparison to low grade glioma samples, by Towner et al. Another study of other types of cancer (renal, ovarian and head and neck cancers) has also shown that tumors samples presented higher ELTD1 protein levels in comparison to normal tissue of the same origin. We also found that ELTD1 was expressed in tumour tissues of patients with glioblastoma. In addition, our study shows that ELTD1 silencing induced cell death in glioblastoma cells, suggesting that this receptor may be important in glioblastoma treatment. In our study, we demonstrated the expression of ELTD1 in tissue belonging to glioblastoma patients, using immunohistochemical techniques. To assess importance of ELTD1in tumoral cell development, the receptor expression was blocked using siRNA in a glioblastoma cell culture and the viability of the cells was analysed by MTT.

ELTD1, UN POTENTIAL BIOMARKER SI TINTA TERAPEUTICA IN TRATAMENTUL GLIOBLASTOMULUI

Proteina 1 continand factorul de crestere epidermal, latrofilina si un domeniu 7 transmembranar (ELTD1), un membru orfan al familiei de receptori care leaga protein G(GPCR) este considerat ca fiind un reglator al angiogenezei normale, el fiind de asemenea implicat in aparitia si dezvoltarea unor forme de cancer. Descoperit acum aproximativ 14 ani de Nechiporuk si colaboratorii, s-a demonstrat ca receptorul ELTD1 este puternic exprimat la nivelul cardiomiocitelor si a celulelor musculare netede de la nivelul vaselor mici de sange si bronhiilor, in modele murine. Intr-un studiu mai recent elaborate de Wallgard si colaboratorii, niveluri substantial mai ridicate ale genei ELTD1 au fost descoperite la nivelul vaselor mici de sange ale creierului uman (de 254 de ori mai puternic exprimate in comparatie cu alte arii ale creierului). Towner si colaboratorii au aratat c gena ELTD1 este supraexprimata in gliomele de inalta malignitate in comparative cu gliome de malignitate scazuta, provenite de la rozatoare. Un alt studiu asupra altor forme de cancer (renal, ovarian si al capului si gatului) a aratat niveluri mai ridicate alte proteinei ELTD1 in comparatii cu mostre prevelate din tesuturi sanatoase. Studiul nostru a demonstrat ca ELTD1 este exprimat la nivelul tesuturilor provenind de la pacienti cu glioblastom. De asemenea, rezultatelenostru aratata ca inhibarea receptorului ELTD1 produce moartea celulelor de glioblastom, sugerand ca acest receptor ar putea fi important in tratamentul glioblastomului. In studiul nostru am demonstrat prin tehnici de imunohistochimie prezenta receptorului ELTD1 la nivelul tesuturilor prelevate de la pacienti cu glioblastom. Pentru a analiza importanta receptorului ELTD1 in dezvoltarea celulii tumorale, am blocat receptorul folosing siRNA intr-o cultura celulara de glioblastom iar apoi am analizat supravietuirea celulara folosind metoda MTT.

P-03

CROHN'S DISEASE – MORPHOLOGICAL PARTICULARITIES

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Crohn's disease is an idiopathic inflammatory disorder which can affect any segment of the digestive tract. Generally considered uncommon and often underestimated, it can endanger the patient's life due to its local and systemic complications. In this article we present the case of a 67-year-old male patient who was admitted for cramping abdominal pain, nonbloody diarrhea, fever and anorexia. He described a 5-year history of similar episodes composed of the same symptoms for which he was admitted. In the past no diagnosis was confirmed and he received no treatment, due to the fact that the episodes were autolimited and the patient didn't ask for medical attention. In this case surgery was required and the diagnosis of Crohn's disease was histopathologically confirmed, thus leading to a proper choice of treatment to avoid possible complications.

BOLA CROHN – PARTICULARITATI MORFOLOGICE

Boala Crohn este o afecțiune inflamatorie idiopatică, care poate afecta orice segment al tubului digestiv. În general, considerata mai puțin frecventa și adesea subestimata, poate pune în pericol viața pacientului datorită complicațiilor sale locale și sistemice. În acest articol vom prezenta cazul unui pacient de sex masculin în vârstă de 67 de ani , care a fost internat pentru crampe abdominal , diaree, febra si anorexie. El a descris un istoric de 5 ani de episoade similare reprezentate de aceleasi simptome. In trecut nici un diagnostic nu a fost confirmat și nu a primit nici un tratament, iar datorită faptului că episoadele au fost autolimitate, pacientul nu a solicitat îngrijiri medicale. În acest caz, a fost necesară o intervenție chirurgicală și diagnosticul bolii Crohn a fost confirmat histopatologic , conducând astfel la o alegere corectă a tratamentului pentru a evita posibilele complicații.

P-04

EPIGENETIC MODIFICATIONS IN DIFFERENTIATED THYROID CANCER

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Thyroid carcinoma is the most common endocrine malignancy worldwide with the most common histological type - papillary thyroid cancer (PTC). Aberrant methylation of tumor suppressor genes (TSG) is a hallmark for many types of cancers. We aim to profile tumor samples and evaluate the methylation status for 22 TSG promoters (APC, BRCA1, CDH1, CDH13, CDKN2A, DAPK, ESR1, FHIT, GSTP1, MGMT, MLH1, NEUROG1, PDLIM4, PTEN, RARB, RASSF1, RUNX3, SOCS1, TIMP3, TP73, VHL,WIF1), to correlate methylation level with biological phenotypes. We performed a methylation analysis (Human TSG EpiTect Methyl II Signature PCR Array-Qiagen) in PTC samples compared with normal thyroid tissue. Preliminary, we evaluated the promoter methylation for TP73, PDLIM4, WIF1 genes in 120 patient's samples, consisting of 60 PTC and follicular adenoma specimens and their adjacent normal tissue, with qMS-PCR using bisulphite treated DNA samples. The methylation percentage was found to be increased in PTC samples than control ($p<0.001$). Higher methylation percentage (MP) values were found for TP73 (85.16%) BRCA1 (76.94%), WIF1 (75.8%), PDLIM4 (67.74%). The methylation level was correlated with tumor grade. TP73 gene promoter methylation seem to be a characteristic for follicular

adenoma samples and PTC follicular variant. Methylation of PDLIM4 gene promoter was found from the incipient state of neoplasia, also found in a higher percentage in patients with PTC and PTC follicular variant. These results illustrate the involvement of epigenetic alterations in thyroid oncogenesis. We consider that the TSG promoter's methylation profile may be a starting point for diagnosis, prognosis of PTC.
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MODIFICARI EPIGENETICE IN CARCINOMUL TIROIDIAN DIFERENTIAT

La nivel mondial carcinomultiroidian este cel mai frecvent cancer endocrine. Tipul histologic cel mai frecvent este cancerul tiroidian papilar (PTC). Metilareaaberantă a genelorsupresoare-tumorale (TSG) este un semn distinctiv pentru multe tipuri de cancer. Scopul nostru este de astabili profilul de metilarepentru 22 de promotoriin probele tumorale (STG APC, BRCA1, CDH1, CDH13, CDKN2A, DAPK, ESR1, FHIT, GSTP1, MGMT, MLH1, NEUROG1, PDLIM4, PTEN, RaRb, RASSF1, RUNX3, SOCS1, Timp3, TP73, VHL, WIF1), si de a corelanivelul de metilare cu fenotipuribiologice. Am efectuat o analiză de metilare (STG Human EpiTect de metil II Semnătură PCR Array-Quiagen) înprobele PTC, comparativ cu țesutul normal tiroidian. Preliminar, am evaluat metilarea promotorului pentru trei gene TP73, PDLIM4, WIF1 în 120 de probe de pacienti, constând din 60 PTC și specimene de adenoma follicular și țesutullor normal adiacent, cu ajutorul tehnicii QMS-PCR folosind tratate-mentul cubisulfit de Naal probelor ADN. Procentul de metilare sa dovedit a fi crescut în probele PTC de control (p <0,001). Un procent demetilare(MP) mai mare a fost gasi tpentru TP73 (85.16%) BRCA1 (76,94%), WIF1 (75,8%), PDLIM4 (67.74%). Nivelul de metilare a fost corelat cu gradul tumorii. Metilarea promotorului genei TP73 pare a fi o caracteristică pentru probele de adenomfolicular si papilar variant foliculară PTC. Metilarea promotorului genei PDLIM4 s-a constatatin stadiul incipient al neoplaziei. De asemenea, a fost găsit într-un procent mai mare la pacienții cu PTC și PTC varianta foliculară. Aceste rezultate ilustrează implicarea modificărilor epigenetic în oncogeneză tiroidiana. Noi considerăm că profilul de metil are promotorului STG poate fi un punct de plecare pentru diagnosticul, prognosticul PTC.
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P-05

CORRELATIONS BETWEEN IMMUNOPHENOTYPE AND GENETIC ABNORMALITIES IN ACUTE LEUKEMIAS

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Background: Acute leukemia is a significant challenge for diagnosis and treatment, regarding quick and accurate diagnosis and adapted treatment to prognosis, because of high risk of refractory response to treatment in relapse.
Aim: Our study proposes to identify specific features in acute leukemia by immunophenotyping, in correlations to genetic abnormalities in a group of patients analyzed over a period of two years.
Methods: There were analyzed 160 newly diagnosed patients with acute leukemia. Other features were analyzed: sex, hematological parameters, morphology, immunophenotype, genetic abnormalities by cytogenetic and molecular biology methods. Immunophenotyping and cytogenetic analysis was performed from fresh bone marrow aspirate, and molecular biology from peripheral blood.
Results: In the cases of acute lymphoblastic leukemia (ALL), 11 of 28 were found with coexpression of KOR-SA and the correlation with presence of Philadelphia chromosome (Ph cr) was 100%. 1 was pre-B ALL and 1 were mature B cell ALL, and the others 9 cases were pro-B ALL subtype. Two cases had coexpression of myeloid markers, strong suggested for 11q23 abnormality, and MLL gene. 14 of cases were suggestive for FLT3/ITD

mutation because of aberrant CD7 expression, and 5 cases of AML1/ETO expression because of association of CD34+ CD56+ CD19+ expression on blasts. 9 from AML cases were suggestive for MLL gene related with CD56 aberrant expression. Ambiguous acute leukemia cases were not expressed aberrant markers.
Conclusion: Multiparameter analysis by immunophenotyping by flowcytometry of acute leukemia cases was high informative for further genetic abnormalities and is an important tool to discriminate different prognosis subtypes. Quick results by FCM is an important tool to evaluate at diagnosis the prognosis and for therapeutic decision making in acute leukemias.

CORELATII IMUNOFENOTIP - ABERATII GENETICE IN LEUCEMIILE ACUTE

Introducere: Leucemia acută este o provocare pentru diagnostic și tratament, în ceea ce privește diagnosticul rapid și precis și tratamentul adaptat in functie de prognostic, din cauza riscului ridicat de raspuns refractar la tratament în recidiva.
Scop. Studiul nostru își propune să se identifice trăsături specifice în leucemia acută analizate prin imunofenotipare, în corelație cu anomaliile genetice într-un grup de pacienți diagnosticati cu leucemie acuta pe o perioadă de doi ani.
Material si metoda: Au fost analizati 160 de pacienți nou diagnosticați cu leucemie acută. Au fost analizate urmatoarele caracteristici: sex, parametrii hematologici, morfologie, imunofenotip, anomalii genetice prin metode de citogenetice si de biologie moleculara. Imunofenotiparea și analiza citogenetică a fost realizată din aspiratul de măduvă osoasă proaspăt, și biologia moleculară din sângele periferic.
Rezultate: În cazurile de leucemie limfoblastică acută (LAL), 11 din 28 au fost găsite cu coexpresie de KOR-SA și corelatia cu prezența cromozomului Philadelphia (Ph cr) a fost de 100%. Un caz a fost LAL pre-B și unul a fost LAL cu celule B mature, iar celelalte 9 cazuri au fost LAL pro-B subtip. Două cazuri au avut coexpresie de markeri mieloizi, sugestiv pentru aberatia 11q23, și gena MLL. 14 de cazuri au fost sugestive pentru mutatia FLT3 / ITD datorită expresiei aberante a CD7, iar 5 cazuri pentru prezenta genei de fuziune AML1 / ETO datorită asocierii CD34 + CD56 + CD19 +. 9 cazuri de LAM au fost sugestive pentru gena MLL cu coexpresie aberantă CD56. Cazurile leucemie acută ambigua nu au exprimat markeri aberanti.
Concluzie: Analiza multiparametrica prin imunofenotipare prin citometrie in flux a cazurilor de leucemie acută adus o informatii importante pentru estimarea unor anomalii genetice si este un instrument important pentru a evidentia subtipuri diferite de prognostic genetic. Rezultatele rapide obtinute prin imunofenotipare constituie un instrument important pentru a evalua la diagnostic prognosticul și pentru stabilirea deciziei terapeutice în leucemiile acute.

P-06

THE STUDY OF COLORECTAL CANCER STEM CELLS USING THE CONFOCAL LASER ENDOMICROSCOPY

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Background: Colorectal cancer is the third most common diagnosed malignancy in the world. Recent studies propose colorectal cancer stem cells (CSC) as responsible for the treatment failure and relapse. Although the research regarding CSC has been amplified during the last years, their complex biology limited the possibility of describing clear identification and isolation strategies. Hence, we conducted a prospective study with the aim of evaluating the expression of CD 44, a marker of CSC, using a state of the art endoscopy method, confocal laser endomicroscopy (CLE). It allows real time assessment of the colorectal mucosa at cellular level, through the use of topic or systemic contrast agents.
Methods: Our study involved 13patients previously diagnosed with primary colorectal adenocarcinoma during routine colonoscopy procedures, with pathology confirmation. Minimum two paired fresh biopsies, with a diameter of approximately 3 mm, were collected from tumor and normal tissue, incubated, stained

with fluorescent anti-CD44 antibodies and scanned using a dedicated CLE system. The CLE images were analyzed offline using the image processing software Image J. Minimum 5 images for each sample were selected considering the most pronounced fluorescent signal and a clear display of the tissue and CD44 positive cells. The CD44 imunomarking protocol was confirmed through imunohistochemistry techniques.
Results: A number of 537 slides presented a distinct fluorescent signal, after imunomarking the fresh biopsies with CD44 antibody. The most 65 relevant images were selected, stacked and analyzed for cell counting. Standard biopsy sections from normal mucosa showed a well defined layout of the mucosal structures, with normal size and symmetric distribution of the colonic crypts, associated with a diminished number of CD44 positive cells. These characteristic were lost among the tumor samples, the crypts presenting an altered architecture of the structures, distortion of the crypts and a higher representation of C44 positive cells. CLE evaluation of the selected images revealed 34,92±16 cells/slide for the tumor tissue and 22,77±11,65 cells/slide for the normal tissue (p=0,037). Regarding the correlation between the CD44 positive cells quantified through CLE and colorectal cancer T, N staging, the majority of the patients were staged as T3/4, N0 or N1. The CD44 positive cells was higher in the tumor tissue when compared to normal mucosa, the difference between the two types being statistical relevant for T3/4: p= 0,028, r= 0,622, while for N0: p=0, 1, r= 0, 92 and for N1: p= 0, 1, r= 0, 8.
Conclusion(s): This is the first study utilizing CLE as an imagistic image for assessing colorectal CSC markers. Targeted CLE based on fluorescent anti-CD44 antibodies was found to be feasible for real-time imaging diagnosis and evaluation of putative cancer stem cells in CRC patients, although further extended studies are required, involving a larger number of patients and a large spectrum of CSC biomarkers. Furthermore, the translation of imunoendomicroscopy in vivo is promising real time results which might facilitate the process of patient selection for personalized therapies, contributing also to the assessment of treatment efficiency and patient evolution.

STUDIUL CELULELOR STEM DIN CANCERUL COLORECTAL UTILIZAND ENDOMICROSCOPIA CONFOCALA LASER

Introducere: Cancerul colorectal (CRC) reprezinta al treilea cel mai frecvent diagnosticat cancer din lume. Studii recente propun celulele stem canceroase (CSC) ca fiind responsabile de esecul terapeutic sau de recidiva tumorala din CRC. Cu toate ca cercetarile privind CSC s-au amplificat pe parcursul ultimilor ani, complexitatea biologiei acestora nu a permis pana in prezent descrierea unei strategii incontestabile de identificare si izolare. Prin urmare, scopul studiului nostru a fost reprezentat de evaluarea expresiei biomarker-ului CD 44 al CSC, utilizand o tehnica endoscopica de ultima generatie, endomicroscopia confocala laser (CLE). Aceasta permite evaluarea histologica in timp real a mucoasei colorectale, prin utilizarea unor agenti de contrast cu administrare topica sau sistemica.
Material si metoda: Studiul nostru a inclus 13 pacienti diagnosticati cu adenocarcinom colorectal. Minimum doua biopsii pereche din țesut normal si tumoral, cu un diametru de aproximativ 3 mm, au fost prelevate in timpul colonoscopiei, incubate la intuneric cu anticorpi marcati fluorescent anti-CD44 si scanate utilizand un sistem dedicat de endomicroscopie Pentax. Secventele de endomicroscopie achizitionate au fost ulterior analizate utilizand software-ul de procesare a imaginii ImageJ. Pentru numararea CSC au fost selectate cel putin cinci imagini pentru fiecare fragment de tesut examinat, prezentand cea mai intensa fluorescenta. Protocolul de utilizare a CD44 a fost confirmat prin tehnici imunohistochimice.
Rezultate: Dupa imunomarcarea cu anticorpul anti-CD44 au fost salvate in total 537 de imagini ce au prezentat un semnal fluorescent distinct. Cele mai clare 65 de imagini au fost stocate, prelucrate si evaluate prin numararea manuala a celulelor stem. Biopsiile standard din țesutul normal au relevat un aspect bine definit al structurilor mucoasei, cu cripte de dimensiuni normale si distributie simetrica asociat unui numar redus de CSC. In tesutul tumoral caracteristicele descrise mai sus s-au pierdut, arhitectura prezentand un aranjament neregulat, cu cripte distorsionate si structuri greu reconoscibile, prezenta celulelor CD44 pozitive fiind semnificativ crescuta. Evaluarea CLE a imaginilor selectate a relevat 34,92 ± 16 celule/sectiune în țesutul tumoral şi 22,77 ± 11,65 celule/secțiune in cazul tesutului normal (p=0,037). In ceea ce priveste corelatia dintre numărul de celule stem cuantificate prin CLE si stadializarea T si N a cancerului colorectal, majoritatea pacientilor au fost incadrati in stadiul T3, N0 sau N1. Numărul de celule marcate CD44 a fost mai ridicat in tesutul tumoral comparativ cu

tesutul normal, diferenta dintre cele doua tipuri de tesuturi fiind relavanta din punct de vedere statistic in stadiul T3/4: p= 0,028, r= 0,622, pe cand pentru N0: p=0, 1, r= 0, 92 si pentru N1: p= 0, 1, r= 0, 8.
Concluzii: Acesta este primul studiu ce utilizeaza CLE ca metoda imagistica de evaluare a unor markeri ai CSC din CCR. CLE este o metoda fezabila de examinare a formatiunilor tumorale, insa pentru optimizarea tehnicii sunt necesare studii suplimentare implicand un spectru mai larg de biomarkeri ai CSC si un numar mai mare de pacienti. Mai mult, translatarea imunoendomicroscopiei in vivo promite rezultate in timp real care ar putea facilita procesul de selectare a pacientilor in vederea aplicarii terapiilor personalizate, contribuind deasemenea la evaluarea raspunsului la tratament si a evolutiei pacientului.

P-07

CORRELATIONS BETWEEN MOLECULAR AND CYTOLOGICAL ASPECTS IN CHRONIC GENITAL INFECTION WITH CHLAMYDIA TRACHOMATIS

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Introduction: Genital infection with Chlamydia trachomatis is a scourge of the modern world with major material and psychological influences on families who want children. Detection and its treatment is necessary.
Methods: The study was conducted during 12 months in SC ProDiagnostic SRL and SCJU "Sf Andrei" Constanta, by correlating the results obtained from patients who have performed Babes Papanicolaou cytology and PCR detection of DNA Chlamydia trachomatis.
Results: After analyzing the results of investigations it was identified a positive statistical correlation between changes suggestive of infection with Chlamydia trachomatis described on cytology and positive results obtained in PCR testing for DNA Chlamydia trachomatis. A small percentage of patients didn't present any cytology abnormality but with positive DNA Chlamydia trachomatis,or cytologic changes in the absence of Chlamydia trachomatis DNA detection.
Conclusions: Infection with Chlamydia trachomatis is suggested by cytologic changes, but due to the influence of factors related to experience and method of harvesting can occur false negative results that require confirmation by DNA PCR detection of Chlamydia trachomatis. In cases of couples who want children and can not conceive them naturally, PCR testing to identify Chlamydia trachomatis DNA becomes mandatory.

CORELATII INTRE ASPECTELE MOLECULARE SI CITOLOGICE IN INFECTIA GENITALA CRONICA DATA DE CHLAMYDIA TRACHOMATIS

Introducere: Infectia genitala cu Chlamydia trachomatis reprezinta un flagel al lumii moderne cu influente majore materiale si psihologice asupra familiilor timere ce isi doresc copii. Detectia si tratarea ei fiind necesare.
Material si metoda: Studiul s-a elfectuat pe parcursul a 12 luni, in cadrul SC ProDiagnostic SRL si SCJU "Sf Apostol Andrei" Constanta, prin corelarea rezultatelor obtinute de la paciente ce au efectuat examen citologic Babes-Papanicolaou si detectie PCR a ADN Chlamydia trachomatis.
Rezultate: In urma analizei rezultatelor investigatiilor s-a identificat corelarea statistica pozitiva intre modificarile sugestive pentru infectia cu Chlamydia trachomatis descrise in urma examenului citologic si rezultatele pozitive obtinute la testarea PCR pentru ADN Chlamydia trachomatis. Un procent mic de paciente a prezentat fie absenta modificarilor citologice dar cu prezenta ADN Chlamydia trachomatis, sau prezenta modificarilor citologice in absenta detectiei ADN Chlamydia trachomatis.
Concluzii: Infectia cu Chlamydia trachomatis este sugerata de modificari citologice, dar datorita influentei factorilor ce tin de experienta si mod de recoltare, pot apare rezultate fals negative ce necesita confirmare

prin detectarea PCR a ADN Chlamydia trachomatis. In cazurile cuplurilor care doresc copii si nu ii pot concepe pe cale naturala, testarea Chlamydia trachomatis prin identificare PCR a ADN devine obligatorie.

P-08

CYTOTOXIC STUDIES ON SPECIFIC ACTIVITIES OF SAPROPELIC MUD FOR THERAPEUTICAL POTENTIAL ASSESSMENT

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Introduction: Extracts of mud sludge is a valuable therapeutic aid and therapeutic alternatives to synthetic drugs, particularly in chronic diseases such as arthritis, osteoarthritis, etc. Use of mud extracts contribute to the long-term stability of the therapeutic effects, thus avoiding the drawbacks common to the conventional medicines, such as resistance, therapeutic and adverse effects. The purpose of this study is to investigate the cytotoxicity and therapeutic efficacy of the active fractions obtained from the therapeutic mud, using in vitro methods.
Material and methods: Cytotoxicity-testing was performed in vitro using ATCC-CRL-9855 cell cultures, in standard conditions and at different times of exposure at concentrations of 75 mM, 15 mM, 6 mM and 3 mM using the MTS and LDH assays.
Results: In the first part of our study, we focused to establish if these 13 mud extracts have cytotoxic effects (MTS assay) and to what extent. The extracts were provided by Pell-Amar Cosmetics as spray-dried powders. For this purpose we used different concentrations - ranging 3 to 75 mM (considering an "average" MW of 90 for extracts), at different cell densities (5000/10000 cells) and incubation times (48/72h). Preliminary results showed - for 10000 cells incubated for 72 hours – IC50 were 247 mM for sample 1, 386 mM for sample 3, 410 mM for sample 5 and 373 mM for sample 7. For 5000 cells at 72 hours – IC50 were 440 mM for samples 3 and 5. IC50 could not be calculated for 48 hrs exposure, although a dose-effect relation could be observed. Our results indicated the relatively low-cytotoxic effects of mud extract analyzed. Preliminary results of anti-inflammatory effects of these extracts demonstrated a decrease of pro-inflammatory cytokines release.
Conclusions: Using a combination of in vitro assays, mud extracts could be classified and ranked for their cytotoxiciy and specific activity, providing an effective screening system for the discovery of potential therapeutic compounds.
Acknowledgement: This work is partially supported by the PNII 265/2014 and PN 16.22.04.01.

STUDII DE CITOTOXICITATE PRIVIND ACTIVITATILE SPECIFICE ALE NAMOLULUI SAPROPELIC IN VEDEREA EVALUARII POTENTIALULUI TERAPEUTIC

Introducere: Extractele de namol sapropelic reprezinta adjuvanti terapeutici valorosi, cat si alternative terapeutice la medicamentele sintetice, in special in boli cronice precum artrita, osteoartrita etc. Utilizarea extractelor de namol contribuie la o stabilitate pe termen lung a efectelor terapeutice, evitand astfel inconvenientele comune ale medicamentelor conventionale, cum ar fi instalarea rezistentei terapeutice si a efectelor adverse. Scopul acestui studiu este de a investiga citotoxicitatea si eficacitatea terapeutica a fractiunilor active obtinute din namol sapropelic folosind metode in vitro.
Material si metode: Testarea in vitro a citotoxicitatii a fost realizata pe culturi celulare ATCC-CRL-9855, in conditii standard si la diferite momente de expunerii, la concentratii de 75 mM, 15 mM, 6 mM and 3 mM, utilizand testele MTS și LDH.

Rezultate: Teste de citotoxicitate: Prima parte a studiului nostru a fost axata pe determinarea efectelor citotoxice (test MTS si LDH) pentru 13 extracte de namol. Extractele au fost furnizate de Pell-Amar Cosmetics sub forma de pulbere uscata prin pulverizare. In acest scop, am folosit diferite concentratii, variind de la 3 pana la 75 mM (considerând o "medie" de greutate moleculara de 90 pentru toate extracte), la diferite densitati celulare (5000/10000 celule) si diferiti timpi de incubare (48/72 ore). Rezultatele preliminare au aratat urmatoarele: pentru 10000 celule incubate timp de 72 de ore - IC50 au fost 247 mM pentru proba 1, 386 mM pentru proba 3, 410 mM pentru proba de 5 si 373 mM pentru proba 7. Pentru 5000 celule la 72 de ore - IC50 au fost 440 mM pentru probele 3 si 5. Desi a fost observata o relatie doza-efect la expunerea de 48 de ore, IC50 nu a putut fi calculata. Rezultatele noastre indica existenta unor efecte citotoxice relativ scazute pentru extractele de namol analizate. Rezultatele preliminare ale efectelor anti-inflamatorii ale acestor extracte au demonstrat o scadere a eliberarii citokinelor pro-inflamatorii.
Concluzii: Folosind o combinatie de analize in vitro, extractele de namol ar putea fi clasificate si ierarhizate pentru citotoxicitatea si activitatea specifica, furnizand un sistem eficient de screening pentru descoperirea de potentiali compusi terapeutici.
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P-09

STEPS TOWARDS A PERSONALIZED PREVENTIVE MEDICINE: POPULATION ALLELIC FREQUENCIES OF CANDIDATE GENES ASSOCIATED WITH GLUCO-LIPIDIC METABOLISM

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Introduction: Knowledge about the personal genetic profile has the potential to influence medical treatment, nutritional and life-style choices. However, most hereditary pathology has polygenic as well as non-genetic causes (e.g. type 2 diabetes, obesity). While it may be difficult to pinpoint individual contributing genes for complex diseases, combinations of particular sets of alleles (haplotypes or genetic profiles) may help identify elevated risk cases. Here we report SNP allele frequencies of four candidate genes often tested in associations with gluco-lipidic metabolism and obesity – pPAR-gamma, ADRB2, APOA2 and FABP2, in a group of normal population. We aim to estimate SNP allelic frequencies and haplotype combinations for Romanian population, and evaluate their usefulness as metabolic syndrome risk-markers in an extensive pannel of personal genetic tests.
Material and method: Over 100 consent-informed volunteers (1:1 sex ratio, age 31 12.48, range 19 to 75, most under 35) were genotyped on five SNP-s (rs1801282, rs1042713, rs1042714, rs5082, rs1799883), using DNA extracted from saliva and PCR-RFLP, ARMS PCR and tetra ARMS PCR methods. PCR products were migrated on agarose gel, post-stained with ethidium bromide and photographed in UV. Genotypes and allele frequencies were compared to those reported on other European populations reported on NCBI. Regression and odds-ratio analyses were applied to determine possible BMI (body mass index) by genotype differences, controlling for influences of age and sex.
Results: There were no significant differences of allele frequencies reported for European populations in our sample. While sex and age were powerful predictors of BMI – (males and older were heavier; R2adj. = 34.6, p<10-3), none of the tested SNPs were associated with weight gain, either when tested separate, together, or controlling for age and sex.
Conclusions: Our preliminary study indicates that SNP allele frequencies in the Romanian population are mirroring those of European origin. A larger sample is necessary to distinguish genetic from age/sex effects, especially for rare alleles (pPAR-gamma, ADRB2), and to obtain haplotypes of risk.

PASI CATRE O MEDICINA PREVENTIVA PERSONALIZATA: FRECVENTE ALELICE POPULATIONALE ALE UNOR GENE CANDIDAT ASOCIATE METABOLISMULUI GLUCO-LIPIDIC

Introducere: Datele profilului genetic personal au potentialul de a influenta tratamentul medical, nutritia si stilul de viata. Totusi, cele mai multe boli ereditare au cauze poligenice si non-genetice (de ex. diabetul de tip 2, obezitatea). In timp ce identificarea unor gene singulare cu influenta asupra bolilor complexe este dificila, combinatii ale unui set particular de alele (haplotipuri sau profile genetice) ar putea ajuta la identificarea cazurilor cu risc ridicat. In studiul de fata raportam frecventele alelice SNP a patru gene candidat deseori testate in asocieri cu metabolismul gluco-lipidic si obezitatea – pPAR-gamma, ADRB2, APOA2 si FABP2, pe un esantion normal de populatie. Urmarim sa estimam frecventele alelice SNP si combinatii ale acestora (haplotipuri) in populatia din Romania si sa evaluam utilitatea acestora ca markeri de risc ai sindromului metabolic, parte a unui panel extins de teste genetice personalizate.
Material si metoda: Peste 100 de voluntari consimtamant-informati (1:1 sex ratio, varsta 31 12.48, intre 19 si 75 ani, cei mai multi sub 35) au fost genotipati pe cinci SNP (rs1801282, rs1042713, rs1042714, rs5082, rs1799883), utilizand ADN extras din saliva, cu metode PCR-RFLP, ARMS PCR si tetra ARMS PCR. Produsii PCR au fost migrati pe gel de agaroza, marcati cu bromura de etidiu si fotografiati in UV. Genotipurile si frecventele alelelor au fost comparate cu cele raportate din alte populatii europene in NCBI. Analize de regresie si "odds-ratio" au fost utilizate pentru a evalua diferente ale IMC (indicele de masa corporala) in functie de genotip, controlind factori precum varsta si sexul.
Rezultate: In esantionul curent nu au existat diferente semnificative ale frecventelor alelice fata de cele raportate in alte populatii europene. In timp ce varsta si sexul constituie predictori puternici ai IMC – (barbatii si varstinicii au scoruri mai mari; R2adj. = 34.6, p<10-3), nici unul din SNP genotipate nu au fost asociate cu cresterea in greutate, fie testate separat, impreuna, sau controland statistic pentru sex si varsta.
Concluzii: Acest studiu preliminar sugereaza o identitate intre frecventele alelelor SNP testate in populatia din Romania si in altele de origine europeana. Un esantion extins este necesar pentru a distinge efectele genetice de cele induse de sex/varsta, in special in cazul alelelor rare (pPAR-gamma, ADRB2), si pentru a putea evidentia haplotipurile cu risc ridicat.

P-10

SYNDROME 46,X,I(X)(Q10) – CASE REPORT

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We present here a 28-year-old patient with a rare variant of Turner syndrome (TS), with some atypical clinical features that should be thoroughly investigated because of potential complications. Short stature and primary amenorrhea were the chief complaints at the time of referral. Chromosome analyses were performed using GTG banding and her karyotype was 46,X,i(X)(q10). The FISH studies, using probes specific to chromosome X (XIST gene, Xq13.2 and SHOX gene, Xp22.33), confirmed the presence of isochromosome Xq10. Comparing our patient phenotype with isochromosome Xq with individuals who have the 45,X type of TS, we noted presence of moderate mental retardation (it's not characteristic), short stature and skeletal manifestations (attributed to haploinsufficiency of the SHOX), widespread nipples, obesity and hirsutism. We consider that chromosomal analysis for all suspected cases of TS should be promptly done at childhood in order to design an appropriate management plan early in life.

SINDROM 46,X,I(X)(Q10) – STUDIU DE CAZ

Prezentăm cazul unei paciente în vârstă de 28 de ani diagnosticată cu o variantă rară de sindrom Turner (ST)

și manifestări clinice atipice, care a necesitat investigații pentru punerea diagnosticului și datorită riscului de complicații. Statura mică și amenoreea primară au fost principalele acuze. Initial am realizat analiza cromosomilor folosind bandare GTG, iar cariotipul a fost 46,X,i(X)(q10). Ulterior, prin tehnica FISH, folosind probe specifice pentru cromosomul X (gene XIST, genele Xq13.2 și SHOX, Xp22.33), am confirmat prezența isocromosomului Xq10. Comparând fenotipul pacientei noastre cu cel al pacientelor cu sindrom Turner și cariotip 45,X, am notat prezența retardului mintal moderat (o manifestare care nu este caracteristică), statura mica și manifestări scheletice (atribuite haploinsuficienței genei SHOX), torace plat și mameloane îndepartate, obezitate și hirsutism. Apreciem că trebuie făcută analiza cromosomială în toate cazurile suspecte de sindrom Turner, încă din copilărie, pentru un management de caz adecvat.

P-11

PROGNOSTIC VALUE OF GENE PROFILE IN NEUROENDOCRINE TUMORS

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Introduction: Neuroendocrine tumors (NETs) are neoplasms arising mainly from the gastrointestinal tract, pancreas and the bronchial tree. Histopathological and immunohistochemical diagnosis and classification of NETs play a role in establishing the therapeutic management of these tumors. Therapeutic means limited to surgery in the past, have been improved through the use of new therapies such as rapamycin and somatostatin analogs. Effective methods of diagnosis are needed for the rigorous selection of cases with greater likelihood of therapeutical response. RT-PCR is a more accurate quantitative method which can replace the routine immunohistochemical testing.
Materials and methods: We studied tumoral and peritumoral fragments fixed in formalin and embedded in paraffin from 26 patients diagnosed with NET (pulmonary, gastrointestinal and pancreatic) by histopathological and immunohistochemical techniques. In order to assess gene expression a PCR-array method was used (Qiagen) which identified relative expression of 18 genes of interest by comparing the tumor with the peritumoral tissue, the normalization being carried out by means of two reference genes. The quality control test was performed using primers for the control of genomic DNA contamination, reverse transcription control, and control of the efficiency of amplification.
Results: In cases of pulmonary NETs, VGF, MGMT and SSTR1 gene were strongly over-expressed, GAST, SYP, and CCND1 genes were moderately overexpressed while MTOR, INS, CHGA, MKI67, SSTR5 and SLIT2 genes had a low overexpression. In gastrointestinal NETs, CHGA and SSTR1 genes were moderately overexpressed, while GRPR, mTOR, SSTR3 and SSTR5 genes had a low overexpression. SSTR2 and MKI67 genes were downregulated. In pancreatic tumors, GAST, VGF and TYMS genes were moderately overexpressed, SSTR3 and SSTR1 genes had a low overexpression and MGMT and SLIT2 genes were downregulated. We identified different gene profiles for the three anatomical locations of NET studied. Somatostatin receptor (SSTR) overexpression represents a predictive marker for the therapy with somatostatin analogues. According to the literature, the expression of somatostatin receptors, especially SSTR1, is positively correlated with the patient survival rate.
Conclusions: Identification of gene profile may represent a sensitive tool for diagnosis and therapy in NETs. A better survival rate of patients with SSTRs overexpression justifies the use of these receptor genes as prognostic markers in NETs.
This work was supported by project PN-II-PT-PCCA-2011-3.2.0623 – „qPCR evaluation of differentiation, drug sensitivity and prognosis of neuroendocrine tumors"

VALOAREA PROGNOSTICA A PROFILULUI GENIC IN TUMORILE NEUROENDOCRINE

Introducere: Tumorile neuroendocrine (NET) sunt neoplasme care provin în principal din tractul gastro-

intestinal, pancreas și arborele bronșic. Diagnosticul histopatologic, imunohistochimic si clasificarea NET au rol in stabilirea conduitei terapeutice a acestor tumori. Abordarile terapeutice, limitate în trecut la intervenția chirurgicală, au fost îmbunătățite prin utilizarea unor agenti terapeutici noi cum ar fi rapamicina și analogii de somatostatină. Pentru selectarea riguroasa a cazurilor cu probabilitate mai mare de raspuns terapeutic este necesara identificarea unor metode performante de diagnostic. RT-PCR reprezinta o metoda cantitativa de mai mare precizie care poate inlocui testarea imunohistochimica uzuala.

Materiale si metode: Au fost luate in studiu fragmente tumorale si peritumorale fixate in formol si incluse in parafina de la 26 pacienti diagnosticati histopatologic si imunohistochimic cu NET având localizare pulmonara, gastro-intestinala sau pancreatica. Pentru evaluarea expresiei genice s-a utilizat metoda PCR array (Qiagen) si s-a identificat expresia relativă a 18 gene de interes, comparand tesutul tumoral cu cel peritumoral, normalizarea realizandu-se prin intermediul a 2 gene de referinta. Controlul de calitate al testului s-a efectuat utilizand primeri pentru controlul contaminarii cu ADN genomic, controlul revers-transcrierii si controlul eficientei amplificarii.

Rezultate: In cazul NET cu localizare pulmonara s-a constatat că genele VGF, SSTR1 si MGMT sunt puternic supraexprimate, genele GAST, SYP, MTOR si CCND1 sunt moderat supraexprimate, iar INS, CHGA, MKI67, SSTR5 și SLIT2 sunt slab supraexprimate. Genele CHGA și SSTR1 sunt moderat supraexprimate, genele GRPR, MTOR, SSTR5 și SSTR3 sunt usor supraexprimate, SSTR2 si MKI67 sunt subexprimate in cazul tumorilor cu localizare gastro-intestinala. Tumorile de pancreas au prezentat genele GAST, VGF si TYMS moderat supra-exprimate, genele SSTR3 si SSTR1 usor supraexprimate, iar genele MGMT si SLIT2 subexprimate. Analizand rezultatele obtinute pentru cele trei tipuri de localizari se constata existența profilurilor genice diferite. Existenta supraexpresiei receptorilor pentru somatostatina (SSTR) reprezinta un marker predictiv pentru terapia cu analogi de somatostatina. Conform datelor din literatura, expresia receptorilor pentru somato-statina, in special SSTR1, se coreleaza pozitiv cu rata de supraviețuire a pacientilor.

Concluzii: Identificarea profilului genic poate reprezinta un instrument sensibil pentru diagnostic si terapie în NET. Asocierea supraexpresiei SSTR cu existenta unei rate de supravietuire mai bună a pacientilor ofera posibilitatea utilizarii SSTR ca markeri de prognostic în NET.

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P-12

PLANT PRODUCTION OF HBV-DERIVED ANTIGENS FOR VACCINE DEVELOPMENT

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HBV (Hepatitis B Virus) infection remains a major health problem especially in developing countries, affecting more than 300 million people. An efficient recombinant vaccine is available on the market, but its use in mass immunization programs is limited because of the high costs. Furthermore, 10% of the vaccinated population has no response or low response to the current HBV vaccine. In this context, our study has focused on developing new viral antigens in order to produce a more immunogenic vaccine in plants, as a low-cost production system. HBV surface antigens have been produced previously in different plants, but the properties of the newly obtained antibodies were poorly characterized, especially in neutralization studies. The current vaccine is based on the small surface antigen, HBsAg, which has the ability to self-assemble into highly immunogenic subviral particles (SVPs). Our strategy takes advantage of this property and proposes the investigation of new antigens by fusing the small envelope protein (S) with an immunogenic fragment from the large protein (L). We have generated two chimeric proteins by insertion of the 21-47 L-derived peptide at different positions of S protein. Both chimeras and the wild-type protein were produced in green plants (Nicotiana benthamiana) and in mammalian cells, as reference. Expression of the antigens was investigated in both production systems and properties like protein folding, dimerization, N-glycosylation were analysed. The ability of the antigens to self-

assemble into SVPs allowed their purification by ultracentrifugation on sucrose gradient from both plant and mammalian cells. The purified antigens were used for immunization studies in BALB mice and the sera obtained had neutralization capacity against HBV infection in vitro. To conclude, the novel plant-derived antigens represent promising candidates as low-cost alternative for vaccine development against HBV.
The research leading to these results has received funding from EEA Financial Mechanism 2009-2014 under the project contract no 5SEE/2014.

PRODUCEREA DE ANTIGENE VHB ÎN PLANTE PENTRU DEZVOLTAREA DE VACCINURI

Infecțiile cu Virusul Hepatitei B (VHB) constituie în continuare o problemă majoră de sănătate, mai ales în țările aflate în curs de dezvoltare, fiind afectate peste 300 de milioane de persoane. Există un vaccin comercial eficient, însă utilizarea sa în programele de imunizare este limitată de costurile ridicate. În plus, 10% din populația vaccinată dezvoltă un răspuns imun foarte scăzut la acest vaccin. În acest context, studiul de față are în vedere dezvoltarea de noi antigene virale pentru producerea unui vaccin mai imunogen în plante – ca sistem de producție cu costuri mai mici. Antigenele de suprafață ale VHB au fost produse în diferite plante, dar proprietățile anticorpilor obținuți au fost slab caracterizate, mai ales în studii de neutralizare. Vaccinul curent are la bază proteina de suprafață S, care prezintă abilitatea de a se autoasambla în particule subvirale puternic imunogene. Ținând cont de această proprietate, studiul propune investigarea unor noi antigene obținute prin fuziunea proteinei S cu un fragment imunogen din proteina de suprafață L. Am generat două proteine himere prin inserția peptidei 21-47 din proteina L în diferite poziții ale proteinei S. Atât proteinele himere, cât și proteina S au fost produse în plante (Nicotiana benthamiana) și în celule mamaliene, drept control. Expresia antigenelor a fost investigată în ambele sisteme de producție și au fost analizate plierea, dimerizarea, N-glicozilarea proteinelor. Abilitatea antigenelor de a se autoasambla în particule subvirale a permis purificarea lor prin ultracentrifugare pe gradient de sucroză, atât din plante, cât și din celule mamaliene. Antigenele purificate au fost folosite pentru a imuniza șoareci BALB, iar serul obținut a avut capacitate neutralizantă față de infecția cu VHB in vitro. În concluzie, noile antigene produse în plante constituie candidați ca alternativă economică pentru dezvoltarea de vaccinuri împotriva infecției cu VHB.
Cercetările care au condus la aceste rezultate au primit finanțare din partea Mecanismului Financiar al Spațiului Economic European 2009-2014 prin contractul nr. 5SEE/2014.

P-13

AMYLOID PRECURSOR PROTEIN DETECTION IN MEMBRANE MACROMOLECULAR COMPLEXES, ISOLATED FROM MOUSE BRAIN CORTEX

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Introduction: Amyloid precursor protein is a type I transmembrane protein, involved in Alzheimer's disease pathology. Although the pathologic cleavage towards formation of detrimental amyloid plaques is extensively studied, data regarding physiological role of the protein is still lacking.
Aim: To investigate the presence of macromolecular complexes involving amyloid precursor protein in mouse normal brain
Material and method: Biologic material: C57 Bl6 mice brain cortex; electrophoretic methods: 2D electrophoresis (isoelectric electroforesis, followed by PAGE electrophoresis) and native, non-denaturating electrophoresis.
Results and discussion: distribution of proteins in the mouse brain cortex was analysed by 2D electrophoresis. Thus, we were able to observe that most high molecular weight protein spots were located in the low pH region.

Next, we tested several non-denaturating electrophoresis conditions, in both low PAGE concentration gradients (4% , 7,5%) and agarose gels (2.5% and 5%). We were able to determined that best non-denaturating conditions involve 2.5% agarose gels, in imidazole-based running buffer, at low voltage.
Conclusions: We were able to determine the correct electrophoresis conditions to visualize high molecular weight molecules. Next step is to determine the correct non-denaturating protein extraction conditions.
Acknowledgement: This work was partially supported by PN-II-RU-TE-2014-4- 1534, PN 16.22.04.01.

INVESTIGAREA EXISTENTEI PROTEINEI PRECURSOARE A AMILOIDULUI IN COMPLEXE PROTEICE MACROMOLECULARE MEMBRANARE, IZOLATE DIN CORTEXUL CEREBRAL DE SOARECE

Introducere: Proteina precursoare a amiloidului este o proteina transmembranara tip I, implicatain patologia bolii Alzheimer. Cu toate caclivajul patologic, care favorizeaza formarea placilor de amiloid, este studiat extensiv, datele referitoare la rolul fiziologic al proteinei sunt inca incomplete.
Scop: Investigarea prezenței complexelor macromoleculare care implica proteina precursoare de amiloid in creierul normal de soarece.
Material si metoda: Material biologic: cortex cerebral desoareci C57 BL6; Metode electroforetice: electroforeza 2D (izoelectrofocusarea urmată de electroforeză PAGE) si electroforezanativa, non-denaturanta.
Rezultate si discutii: distributia proteinelor in cortexul cerebral de soarece a fost analizata prin electroforeză 2D. Astfel, am putut observa că cele mai multe spoturi cu greutate moleculară mare au fost localizate in regiunea cu pH scazut. In continuare, am testat mai multe conditii electroforeza non-denaturanta, in gradienti de concentratie PAGE(4%, 7,5%) cat si in geluri de agaroză (2,5% și 5%). Am constatat ca cele mai bune conditii de electroforeza non-denaturanta implica 2,5% geluri de agaroza, in tampon de migrare pe baza de imidazol, la joasa tensiune.
Concluzii: Am fost in masura sa determinam conditiile corecte de electroforeza pentru a vizualiza molecule cu greutate moleculara ridicata. Urmatorul pas este de a determina conditiile optime de extractie non-denaturantaa proteinelor din cortexul cerebral.
Acknowledgement: Aceasta lucrare a fost sustinuta partial de PN-II-RU-TE-2014-4-1534, PN 16.22.04.01.

P-14

GENE POLYMORPHISMS INVOLVED IN GASTRIC CANCER

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Background: Gastric cancer remains a major health problem worldwide and despite the advances in surgery and chemotherapy it is still the second leading cause of cancer death across the world. Currently it is accepted a multifactorial model in gastric carcinogenesis where environmental factors, diet and genetic susceptibility interact in producing the host disease. NOD2 is known as nucleotide-binding oligomerization domain containing protein 2 and is a gene located on long arm of chromosome 16 at position 21 (16q21). It offers instructions for the synthesis of proteins implicated in immune system function such as macrophages or monocytes. NOD2 protein is involved in several processes that defend the body by recognizing foreign invaders and by stimulating the immune system. Our study aimed to assess the frequency of NOD2 rs2066844C/T Arg702Trp polymorphism in a group of 72 gastric cancer patients and in a group of 250 healthy persons forming the control group. In this matter we tried to verify the risk of cancer gastric associated with NOD2 gene variation.
Material/Methods: Genomic DNA (Deoxyribonucleic acid) was extracted from blood leukocytes using Wizard Genomic DNA Purification Kit (Promega, Madison, WI), following the manufacturer protocol. NOD2 Arg702Trp polymorphism was genotyped by Real-Time PCR (polymerase chain reaction) using specific TaqMan probes for each allele (rs2066844, assay C__11717468_20, Applied Biosystems Foster City, CA). RealTime PCR was performed on a ViiA™ 7 Real Time PCR System (Life Technologies, Carlsbad, USA) and

components of reaction were: DNAsample, Universal Master Mix (Applied Biosystems, Foster City, CA), TaqMan SNP Genotyping Assay 40x (Applied Biosystems, Foster City, CA) and DNase-free, sterile-filtered water.
Results: No statistically significant difference was observed between gastric cancer patients and controls when we compared one genotype with other genotype (the CC genotype serves as reference) (OR 0.45, 95% CI: 0.10 - 2.05) or when we compared allele frequencies (the C allele serves as reference) (OR 0.46, 95% CI: 0.11 - 2.04).
Conclusion: NOD2 Arg702Trp polymorphism is not correlated with gastric cancer risk in Romanian population and further investigations are needed to elucidate the contribution of NOD2 gene in gastric carcinogenesis.
Acknowledgement: Financed by the project IMANG, UEFISCDI, No. PN-II-RU-TE-2014-4-2289

POLIMORFISME GENICE IMPLICATE ÎN CANCERUL GASTRIC

Introducere: Cancerul gastric rămâne o problemă majoră de sănătate la nivel mondial, în pofida progreselor în tratamentul chirurgical şi chimioterapie, fiind în continuare a doua cauză de mortalitate prin cancer la nivel global. Actualmente este acceptat un model multifactorial în carcinogeneza gastrică, unde factorii de mediu, dieta şi susceptibilitatea genetică a gazdei interacționează în producerea bolii. NOD2 este o genă localizată pe brațul lung al cromozomului 16, la poziția 21 (16q21). Aceasta oferă instrucțiuni pentru sinteza proteinelor implicate în funcționarea sistemului imun, Proteina NOD2 este implicată în mai multe procese care apară organismul prin recunoașterea moleculelor bacteriene și prin stimularea sistemului imunitar. Studiul nostru a vizat evaluarea frecvenței polimorfismului NOD2 rs2066844C/T Arg702Trp într-un grup de 72 de pacienți cu cancer gastric si într-un grup de 250 de persoane sănătoase care formează grupul de control. În acest sens, am încercat să verificăm riscul de cancer gastric asociat cu variația genei NOD2.
Material și metodă: ADN-ul genomic (acid dezoxiribonucleic) a fost extras din leucocite din sângele pacienților utilizând kit-ul de purificare Wizard Genomic DNA (Promega, Madison, WI). Polimorfismul NOD2 Arg702Trp a fost genotipat prin PCR (reacție de polimerizare în lanț) în timp real, folosind sonde specific TaqMan pentru fiecare alelă (rs2066844, C__11717468_20 test, Applied Biosystems Foster City, CA). PCR în timp real a fost realizată pe un system ViiA™ 7 Real Time PCR System (Life Technologies, Carlsbad, USA) și componentele de reacție au fost: probe ADN, Universal Mașter Mix (Applied Biosystems, Foster City, CA), test de genotipareTaqMan SNP 40x (Applied Biosystems, Foster City, CA) și apăsterile fără ADNază.
Rezultate: Nu am observant diferențe semnificative între cele două grupuri, atunci când am comparat un genotip cu alt genotip (genotipul CC drept referință) (OR 0,45, 95% CI: 0.10-2.05) sau când am comparat frecvențele alelelor (alela C drept referință) (OR 0,46, 95% CI: 0.11-2.04).
Concluzii: Polimorfismul genic NOD2 Arg702Trp nu este corelat cu riscul de cancer gastric în grupul studiat și sunt necesare investigații suplimentare pentru a elucida contribuția genei NOD2 în carcinogeneza gastrice.
Acknowledgement: Rezultatele cercetării sunt obținute în cadrul proiectului IMANG, UEFISCDI, Nr contract PN-II-RU-TE-2014-4-2289

P-15

MUTATIONAL SPECTRUM OF DMD GENE AND NNOS EXPERSSION

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Introduction: Duchenne and Becker muscular dystrophy (DMD) are the most common muscular dystrophy characterized by progressive muscle wasting caused by the absence of dystrophin protein. An important function of dystrophin is to recruit nNOS to the sarcolemma. Mutations in DMD gene are associated with the absence/reduction of nNOS from sarcolemal level. Selective loss of NOS in dystrophinopathies seems to be directly correlated with mutational spectrum of DMD gene.

Aims: The aim of the present study was find a relationship between the type of mutation in DMD gene and nNOS expression in order to find the importance of binding domain of nNOS to dystrophin.
Materials and Methods: We report a study of 40 patients with dystrophinopathies genetics characterized by MLPA for which we evaluate the expression of nNOS by fluorescence immunostaining and western blot.
Results: Our results confirm that a total absence of nNOS at the sarcolemmal level in dystrophic muscle, are associated with mutation in hot-spot region of DMD gene that disrupting the reading frame while mutation non-disrupting the reading frame are associated with a reduction of expression of nNOS. Also it was observed that the presence of nNOS in the cytosol is associated with a severe phenotype of dystrophinopathy.
Conclusions: DMD gene suffers mutations that removed a crucial region for binding nNOS to the dystrophin. Mutations occurring in DMD gene determine a variable expression of nNOS depending on the location of mutation in DMD gene. Also, nNOS expression analysis could also be useful in the differential diagnosis of dystrophinopathies.

SPECTRUL MUTATIONAL AL GENEI DMD SI EXPRESIA NNOS

Introducere: Distrofiile musculare Duchenne (DMD) și Becker (DMB) sunt cele mai frecvente boli musculare caracterizate prin pierdere musculare progresiva cauzate de lipsa proteinei numita distrofina. O functie importanta a distrofinei este de a atasa nNOS (nitric oxid sintaza de tip neuronal) la sarcolema. Mutatiile in gena DMD sunt asociate cu absenata sau reducerea nNOS la nivel sarcolemal. Pierderea selective a nNOS in distrofinoapatii se pare ca este in corelatie directa cu spectru mutational al genei DMD.
Obiective: Scopul studiului de fata a fost de a gasi o relatie intre tipul mutatiilor in gena DMD si expresia nNOS, pentru a determina importanta domeniilor de legare nNOS la distrofina.
Materiale si metode: in acest studiu raportam 40 de pacienti cu distrofinopatii caracterizati genetic prin MLPA (Multiplexl ligation-dependent probe amplification), pentru care am evaluat expresia nNOS prin imunofluorscenta si Western blot.
Rezultate: Rezultatele obtinute arata ca absenta totala a nNOS la nivelul sarcolemei in muschiul distrofic, este asociata cu mutatii de tipul deletiilor in regiunile hot-spot ale genei DMD si care perturba cadrul de citire, in timp ce mutatiile care nu schimba cadrul de citire sunt asociate cu o reducere a expresiei nNOS. Totodata s-a observat ca prezenta nNOS in citosol este asociata unui fenotip sever de distrofinopatie.
Concluzii: Mutatiile aparute in gena DMD determina o expresie variabila a nNOS in functie de localizarea mutatiei in gena. Totodata analiza expresiei nNOS ar putea fi utila si în diagnosticul diferential al distrofinopatiilor.

P-16

BIOCOMPATIBILITY ASSESSEMENT OF SOME NEW DENTAL ALLOYS DESIGNED TO IMPROVE THE ACUTE AND CHRONIC BIOLOGICAL POST IMPLANT RESPONSE

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Introduction: Many different types of alloys have been widely used for restorative prosthodontics and the common criteria for all these is the permanent existence in the oral cavity for prolonged time, leading to allergies or more acute complications after implant. In order to reduce the post-implant complications, traditional titanium alloys were replaced with CoCrMo alloys, but recent studies show that these alloys influence cell viability and produce allergies. Adding Nb and Mo ions in the composition of the commercial existing alloy can increase its biocompatibility and decrease the post-implant complications. In this view, the aim of our study was to evaluatein vitrothe biological performance of the novelCoCrNbMoZralloy in comparison with commercial HeraeniumCE alloy.

Materials and methods: The biocompatibility of theCoCrNbMoZr alloy was assessed in vitro in terms of human adipose stem cells (hASCs) morphology, viability and proliferation status.
Results: CoCrNbMoZrallowshASCs to quickly adhere and to acquire their characteristic spindle-like shape and better sustain cellular proliferation in comparison with the commercial alloy. The hASCs viability on CoCrNbMoZr was significant higher and the alloys cytotoxicity was very low compared with HeraeniumCE alloy.
Conclusions: CoCrNbMoZr alloy could be further employed for in vitro cell differentiation studies.

EVALUAREA BIOCOMPATIBILITATII UNOR NOI ALIAJE DENTARE CONCEPUTE PENTRU IMBUNATATIREA RASPUNSULUI BIOLOGIC ACUT SI CRONIC POST – IMPLANT

Introducere: Foarte multe tipuri de aliaje au fost utilizate pana in prezent in protetica dentara reparatorie, characteristic lor comuna fiind existent permanenta in cavitatea bucala pentru o perioada indelungata de timp, lucru ce poate conduce la alergii sau complicatii acute post-implant. Pentru a reduce complicatiile post-implant, aliajele traditionale de titan au fost inlocuite cu aliajele CoCrMo, dar studii recente au demostrat ca acestea influenteaza viabilitatea celulara si produc alergii. Adaugarea ionilor de Nbsi Mo in compozitia aliajului comercial existent poate mari biocompatibilitatea acestuia si poate descreste riscul aparitiei complicatiilor post-implant. Scopul acestui studiu a fost evaluarea in vitro a performantei biologice a noului aliaj CoCrNbMoZr in comparatie cu aliajul commercial HeraeniumCE.
Materiale si metode: Biocompatibilitatea aliajului CoCrNbMoZr a fost evaluate in vitro din punct de vedere al morfologiei, viabilitatii si statutului proliferarii celulelor stem adulte derivate din tesutul adipos (hASCs).
Rezultate: Aliajul CoCrNbMoZe permite hASCs sa adere rapid si sa adopte morfologia lor caracteristica si sustine mult mai bine proliferarea celulara comparativ cu aliajul comerical. Viabilitatea hASC pe CoCrNbMoZe a fost semnificativ mai mare si citotoxicitatea lui mult mai scazuta comparativ cu aliajul HeraeniumCE.
Concluzii: Aliajul CoCrNbMoZr poate fi implicat in viitor in studii de diferentiere celulara in vitro.

P-17

MARKERS OF HEPATITIS B VIRUS INFECTION IN PEOPLE WHO INJECT DRUGS

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Background: Before the introduction of routine HBV immunization of all newborns in 1995, Romania was an endemic country for HBV infection, mostly acquired perinatally. During the last 5 years an alarming increase in the number of injecting drug users (IDUs) co-infected with HIV and viral hepatitis was reported, fact that may impact the local HBV prevalence.
Objective: The aim of our study was to determine the prevalence of HBV infection among IDUs admitted in a single tertiary facility and to evaluate their epidemiological and virological characteristics.
Methods: A cross sectional study on 123 IDUs tested between 2011 -2015 for HIV infection and viral hepatitis serological markers and active viral replication.
Results: Out of 123 evaluated IDUs, 93 (75.6%) tested positives for anti-HBc IgG antibodies, among them 39.7% (37/93) were HBsAg carriers, 34.4% (32/93) were immune after natural infection and 25.8% (24/93) presented isolated HBcIgG antibodies. The majority of HBsAg carriers were young males (94.5%), with a median age at drug use initiation of 17 years; mostly using both heroin and ethnobotanical drugs (67.5%) for a median time of 8.5 years. The majority of HBsAg carriers, 91.8% (34/37), were also HCV co-infected and 62.1% (23/37) HIV co-infected. Overall, 64.8% (24/37) of the HBV chronic carriers presented detectable HBV-DNA, but active viral replication (ADN-VHB>1000 UI/mL) was found in only 35.1% (13/37), associated with

significantly higher hepatic cytolysis (ALT-1396 vs. 265 UI/mL). Among HIV-infected IDUs, HBsAg portage was more frequently associated with higher HIV viral loads (5.00 vs. 4.77 log10 copies/mL, p=0.18), but good immunologic status (median CD4 cell count >500/mm3 in 34.2% vs. 28.5%, p=0.34), and accompanied by active HBV viral replication (38.4% vs. 10%, p<0.001). In patients with active viral replication, the most commonly detected HBV genotype was D (8/13, 61.5 %) followed by genotype A (4/13, 30.7%). Patients with genotype D had lower HBV viral load (154000 vs. 8212500 UI/mL) and were more often HIV co-infected (72.2% vs. 33.3% p<0.001).

Conclusions: A high percentage of IDUs have markers of past or present HBV infection, frequently associated with both HCV and HIV co-infections. Nevertheless, active viral replication is present only in a third of the HBs chronic carriers, more frequently among those co-infected with HIV. HBV screening and vaccination among individuals in vulnerable groups are mandatory in order to reduce the transmission risk and liver disease progression.

P-18

PROTEOMIC STRESS INDUCED BY TNF ON HUMAN PANCREATIC ADENOCARCINOMA CELL LINE (BXPC-3)

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Introduction: Tumor necrosis factor (TNF) is a double-crosser in cancer. TNF can act as an endogenous tumor promoter, stimulating growth, proliferation, invasion, and metastasis of cancer cells, whereas in other instances TNF operates as a cytokine that induces tumor necrosis. The mechanisms involved in the dual action of TNF are worth studying due to their potential for clinical exploitation. The aim of the present study was to identify the proteins that are modulated by TNF in a human primary pancreatic adenocarcinoma cell line (BxPC-3) using a proteomic approach.

Materials and Methods: The BxPC-3 cell line was grown in the presence of 10ng/ml TNF (T) or in its absence (N – control) for 48 hours. The proteomic analysis was performed on protein lysates from the two cell groups in three technological replicates. Nano-liquid chromatography-electrospray ionization hybrid mass spectrometry (Nano-LC-ESI-MS/MS) and bioinformatics tools were used to identify the proteins. For the characterization of various properties of the identified proteins, the annotation based on gene ontology (cellular component, molecular function and biological process) group data set comparison was performed with Protein Center3.14 software. The label free relative quantification on the precursor level was done with SIEVE 2.1 software (Thermo Scientific).

Results: The mass spectrometric global analysis of the BxPC-3 cell homogenates led to the identification of 2288 proteins from the T group and 2192 proteins from the N groups. From the total identified proteins,18 molecules were demonstrated to be involved in the TNF signaling pathway (KEGG map 04010). In the BxPC-3 tumor cell line, TNF induced statistically significant changes in the expression of S100 family (A11, A16, A2, A9, P) and Hsp family (Hsp70, Hsp27) of proteins, results validated by Western blot methodology. In addition, the mass spectrometric evaluation showed that the enzyme cyclooxygenase 2 (COX-2) was up-regulated (T / N = 2.6802 ± 0.42 **) in the BxPC-3 activated cell line.

Conclusions: In human primary pancreatic adenocarcinoma cell line TNF induces significant changes in the expression of S100 and Hsp protein families. The expression pattern of these molecules could be correlated with the stage or type of cancer development with valuable information for precise diagnosis. In addition TNF induced the expression of COX-2, a pro-angiogenic factor; this is particularly interesting since its inhibition could be beneficial in the adequate designed treatment of pancreatic cancer. These preliminary results reveal that the proposed proteomic analysis is a promising strategy for the evaluation of pathological proteomic differences and heterogeneity in the pancreatic cancer cells.

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P-19

PROTEOMIC STUDY - A USEFUL TOOL IN CHRONIC KIDNEY DISEASE TREATMENT

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Introduction: Cardiovascular complications, through vascular calcifications (VCs), are the leading cause of mortality in patients with chronic kidney disease (CKD). VC isa common complication in CKD patients and is considered an active process with multiple factors and mechanisms involved in kidney-bone-vascular axis.
The aim of this proteomic study is to assess a novel biomarker panel, particularly useful for identification of VCs early phases in CKD patients, having important consequences on the therapeutic interventional strategies, prognosis, and life-expectancy of patients with CKD.
Material and Method: Serum samples from 88 CKD patients (stages II-IV, not undergoing dialysis) and 21healthy controls were analysed to simultaneously measure the level of 8 molecules (osteoprotegerin, osteocalcin, osteopontin, FGF-23, PTH, IL-6, IL-1β, TNFα - Milliplex MAP Human Bone Magnetic Bead Panel) using xMAP technology. Multiplexed data acquisition was performed on Luminex 200 platform using xPONENT 3.1 software. Fetuin A was assessed using Quantikine ELISA Human Fetuin A.
Results: Mineral metabolism candidate biomarkers and molecules that actively regulate VC process showed an increased circulating level compared with control. The pro-inflammatory cytokines level (TNFα, IL-6) was also increased in CKD patients compared with control, while for IL-1β no trend was visible so far. Reduced serum level of fetuin-A, inhibitor of pathologic calcification, was associated with CKD. A positive correlation between biomarkers level and the stages inside the CKD group has also been observed.
Conclusion: Although research efforts in the past decade have greatly improved the knowledge of the multiple factors and mechanisms involved in vascular calcification in patients with CKD, many questions remain unanswered. Detecting VC through its severe clinical manifestations may turnto be too late for any therapy to halt its progression or even to reverse it. The configuration of a specific biomarker panel for identification of early phases of VCs and for scoring its severity in CKD patients will allow rising further strategies to improve CKD patient management.
Acknowledgment: This work was partially supported byPNII 93/2012 and PN 16.22.04.01.

STUDIU PROTEOMIC IN CONDUITA TERAPEUTICA A BOLII RENALE CRONICE

Introducere: Complicatiile cardiovasculare reprezintaprincipala cauza de mortalitatein boala renala cronica (BRC). Calcificarea vasculara este o complicatie frecventa la pacientii cu BRCsi este considerata un proces activ cuimplicatii in axa os - rinichi - sistem cardiovascular.
Scopul acestui studiu proteomiceste de a descoperi un nou panelde biomarkeri, cu utilitatein identificarea precoce a calcificarilor vasculare la pacientii cu BRC, avand consecinte importante inabordarea terapeutica, prognosticul si speranta de viata a pacientilor cu BRC.
Material si Metoda: Probele de ser provenind de la 88 de pacienti cu boala renala cronica (stadiile II-IV, nesupusi dializei) si 21 de martori sanatosi au fost analizate pentru a masura simultan nivelul seric a 8 molecule (osteoprotegerin, osteocalcin, osteopontin, FGF-23, PTH, IL-6, IL-1β, TNFα - Milliplex MAP Human Bone Magnetic Bead Panel) folosind tehnologia de multiplexare xMAP array. Achizitiasi prelucrarea datelor a fost realizata pe platforma Luminex 200,utilizand software-ul xPONENT 3.1. Molecula Fetuin-A a fost evaluata utilizand kitul

Quantikine ELISA Human Fetuin A.
Rezultate: Biomarkerii candidati pentru metabolismul mineral si moleculele care reglează în mod activ procesul de calcificare vasculara au prezentat un nivel circulant crescut la pacientii cu BRC in comparatie cu martorii sanatosi. Nivelul circulant al citokinelor proinflamatorii (TNF, IL-6) a fost crescut la pacientii cu CKD, in timp ce pentru IL-1βnu s-a observat o tendinta evolutiva specifica. Nivelul seric redus al Fetuin-A, inhibitor al procesului de calcificare patologica, a fost asociat cu BRC. De asemenea s-a observat o corelatie pozitiva intre nivelul circulant al biomarkeriloranalizatisi stadiul BRC.
Concluzii: Desi nivelul de cunoastere a mecanismelor implicate in aparitia calcificarilor vasculare la pacientul cu BRC s-a imbunatatit considerabil in ultimele decenii, multe întrebari au ramas fara raspuns. Descoperirea calcificarilor vasculare prin prisma manifestarilor clinicesevere este considerata tardiva pentru succesul terapeutic. Configurarea unui panel specific de biomarkeri cu utilitate in identificarea precocea CV in BRC va permite dezvoltarea de strategii terapeutice noi in vederea imbunatatirii managementului pacientului cu BRC.
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P-20

INSULIN RESISTANCE AND HYPOVITAMINOSIS D IN NON-DIABETIC YOUNG HIV-SEROPOSITIVES UNDERGOING ANTIRETROVIRAL THERAPY

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Background: Vitamin D may impact insulin production and sensitivity and further glucose metabolism as both pancreatic and adipose cells express vitamin D receptors.The study objectives were to:1) evaluate the prevalence of insulin-resistance;2) evaluate the prevalence of hypovitaminosis D; 3) assess the correlation between vitamin D serum levels and insulin resistance in young HIV-infected adults, having undergone multiple combined-antiretroviral therapies (cART).
Methods: A cross-sectional study over non-diabetic cART-experienced HIV-seropositives in a tertiary care center was conducted over 6 cold-season monthsin 2014-2015. Insulin-resistance was defined as HOMA>2.6, where HOMA is homeostasis model assessment and is calculated as [insulinemia (µUI/mL) x glycemia (mg/dL)] / 405. Plasma vitamin D levels below 30 ng/mlwere considered suboptimal.
Results: Thirty-five patients were enrolled, with F:M ratio=1.7, median age of 25 years [IQR 25;26], having undergone 5 cART regimens [IQR 4;8] in 18 years since diagnosis [IQR 15.5;22.5]. Median CD4 was 518 c/mmc [302;719], while undetectable HIV viremia was found in 59.3%. Prevalence of insulin resistance was 29.6% and of vitamin D deficiency- 69%. In women, strong Spearman correlations were found between HOMA score and HIV viremia (p=0.02, rho=611); HOMA score and number of cART regimens (p=0.004, rho=815); and serum vitamin D and HIV load (p=0.02, rho=602). In men, vitamin D seemed inversely associated with age (p=0.02, rho=-657).Years from diagnosis were marginally inversely correlated with vitamin D levels in both sexes. Overall, insulin resistance seemed to be correlated with hypovitaminosis D (p=0.02), but with equivocal clinical interpretation in this particular small population.
Conclusions: The young Romanian HIV-seropositives are candidates for early polipathology related to HIV infection itself or the 2-decade of antiretroviral therapy so far.A third of them have insulin resistance, while two thirds have hypovitaminosis D. These results anticipatebone, metabolic and cardiovascular diseases. Supressing HIV viremia even in patients experiencing both resistance mutationsandfatigue as a result of life-longcART,and also managing the above-mentionedsubclinical changesmay bring complex benefits on long-term in young HIV-seropositives.

INSULINOREZISTENȚA ȘI HIPOVITAMINOZA D LA PACIENȚII TINERI NON-DIABETICI HIV POZITIVI AFLAȚI IN TRATAMET ANTI RETROVIRAL

Introducere: Vitamina D poate influența producția de insulină, sensibilitatea țesuturilor la acțiunea insulinei și metabolismul glucidic întrucât atât suprafața celulelor pancreatice cât și suprafața celulelor adipoase prezintă receptori pentru vitamina D. Obiectivele studiului au fost: 1) evaluarea prevalenței insulino-rezistenței; 2) evaluarea prevalenței hipovitaminozei D; 3) descoperirea unor corelații între nivelele serice ale vitaminei D și insulinorezistența la adulții tineriinfectați HIV multiexperimentați terapeutic.
Metode: Studiu transversal ce s-a desfășurat într-un centru terțiar de îngrijire, între 2014-2015 pe o perioadă de 6 luni, în timpul sezonului rece,asupra pacienților HIV pozitivi non-diabetici multi experimentați terapeutic. Insulino-rezistența a fost definite printr-un scor HOMA (homeostasis model assessment) >2.6 calculat cu formula: insulinemia (µUI/mL) x glicemia (mg/dL) / 450. Valorileplasmatice ale vitaminei D maimici de 30 ng/ml au fost considerate suboptimale.
Rezultate: Au fost înrolați 35 de pacienți cu raportul F:M=1.7, cu mediana vârstei de 25 de ani [IQR 25;26], care au urmat 5 scheme de tratament antiretroviral [IQR 4;8] în 18 ani de când au fost diagnosticați [IQR 15.5;22.5]. Mediana celulelor CD4 a fost de 518c/mmc [302;719], iar 59,3% dintre pacienți au avut viremie nedetectabilă. Prevalența insulino-restenței a fost de 29.6% iar a deficitului de vitamină D de 69%.În cazul femeilor s-au observat corelații tip Spearman semnificative între scorul HOMA și viremia HIV (p=0.02, rho=611); scorul HOMA și numărul de scheme ARV (p=0.004, rho=815); și între valoarea serică a vitaminei D și încătura virală HIV (p=0.02, rho=602). În cazul bărbaților vitamina D a fost corelată negativ cu vârsta (p=0.02, rho=-657). Numărul de ani de la diagnostic s-a corelat negativ cu vitamina D în cazul ambelor sexe. Per total insulino-rezitența pare să se coreleze cu hipovitaminoza D (p=0.02) în cazul acestei mici subpopulații.
Concluzii: Pacienții tineri HIV pozitivi din România sunt predispuși la a dezvolta precoce polipatologia asociată infecției HIV în sine sau tratamentului ARV. O treime dintre pacienți au insulino-rezitență iar două treimi au hipovitaminoza D. Aceste modificări preced afecțiunile osoase, metabolice și cardiovasculare. Suprimarea viremiei HIV chiar și la pacienții cu mutații de rezistență datorită ARV îndelungat și care suferă de complicațiile subclinice mai sus menționate aduce beneficii pe termen lung la pacienții tineri HIV-pozitivi.

P-21

MAMARY „IN SITU" AND INVASIVE DUCTAL CARCINOMA – HER2 STATUS

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Introduction: The role of HER2 amplification/overexpression in progression of in situ from invasive ductal carcinoma has not been yet clarified. Our study evaluated HER2 status in ductal carcinoma in situ associated to invasive breast ductal carcinoma.
Materials and methods: 80 cases diagnosed with ductal in situ breast carcinoma associated to invasive carcinoma has been studied. HER2 status was determined by immunohistochemistry, followed by chromogenic or fluorescent in situ hybridization for moderate positive immunohistochemical expression.
Results: Immunohistochemically 39% of cases were HER2 intense positive and 60% of cases were moderate positive. The cases with HER2 moderate expression presented in 20% high level gene amplification, 40% low level gene amplification and absence of amplification in 40% of cases. The concordance between in situ and invasive component of individual tumors was 90%. Two cases showed HER2 gene amplification in the associated ductal in situ carcinoma with no evidence of gene amplification in the invasive tumor.
Conclusions: Our study emphasize that not always HER2 gene amplification/ overexpression play a key role in the progression of ductal carcinoma in situ to invasive carcinoma and other molecular alterations may by more important in tumor progression.

CARCINOMUL MAMAR "IN SITU" ȘI DUCTAL INVAZIV – STATUS HER2

Introducere: Rolul amplificării sau supraexpreiei HER2 în progresia de la carcinoma ductal in situ la carcinoma ductal invaziv încă nu este pe deplin cunoscută. Prezentul studiu determină statusul genei HER2 din carcinoamele mamare ductale in situ asociate carcinoamelor ductale invasive.
Materiale și metode: 80 cazuri de carcinoame mamare ductale in situ asociate carcinoamelor ductale invazive au fost studiate. Statusul HER2 a fost deteminat imunoshistochimic, urmat de hibridizare cromogenica sau flurescenta in situ, pentru cazurile moderat pozitive conform protocoalelor furnizate de producator.
Rezultate: Imunohistocimic 39% din cazurile studiate au fost HER2 intens pozitive, iar 60% din cazuri au fost moderat pozitive. Cazurile moderat pozitive au prezentat în proporție de 20% amplificare genică cu grad crescut, 40% amplificare genică cu grad scăzut și absența amplificării pentru 40% din cazuri. Concordanța între statusul genei HER2 între componenta in situ și invazivă a carcinoamelor ductale a fost de 90%. Două cazuri au prezentat amplificarea cu grad crescut a genei HER2 pentru componenta intraductală și absența amplificării genei HER2 pentru componenta invazivă.
Concluzii: Studiul evidențiază faptul că nu întotdeauna supuraexpresia/amplificarea genei HER2 joacă un rol important in progresia de la carcinomul ductal in situ la carcinomul invaziv mamar, fiind implicate alte alterări moleculare în progresia tumorală.

P-22

EVALUATION OF TISSULAR TUMOR ANGIOGENESIS IN HUMAN HEPATOCELLULAR CARCINOMA

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Introduction: Hepatocellular carcinoma represents the most common primary malignancy of the liver, being one of the most prevalent and lethal cancer worldwide with more than 500,000 people newly-diagnosed every year. One important feature of hepatocellular carcinoma is its hypervascularity. Neoangiogenesis contributes to metastasis and poor prognosis. Objective: Our study aims to analyze the expression profile of the main proteins involved in angiogenesis processes in hepatocellular carcinoma. Materials and methods: Tumoral and adjacent non-malignant tissue fragments were obtained from 10 patients with hepatocellular carcinoma; each tumor was assigned a histological type and a depth grading of infiltration. All patients had recurrence at the time of the survey. Immunohistochemistry and dot blot techniques were performed to investigate the expression of several proteins involved in angiogenesis processes. Results: Immunohistochemistry results showed that VEGF-positive expression was found in 7 of 10 hepatocellular carcinoma patients. HCC tissues also showed positive staining for CD34. The results obtained from dot-blotting showed significant expression changes in tumor tissue compared with peritumoral tissue, in the case of several proteins involved in angiogenesis processes:basic FGF, IL-8 and VEGFwere amongst proteins with increased expression in tumor tissue compared to peritumoral tissue.Conclusion:The results obtained by immunohistochemistry and dot blot techniques highlighted the activation of several angiogenic factors, including VEGF, bFGF and IL-8 in hepatocellular carcinoma tumor tissues and these molecules may be potential targets for developing new therapeutical strategies in hepatocellular carcinoma through their effects on angiogenesis.

Acknowledgement: This study was financially supported by EEA-JRP-Romania-Norvegia nr.4SEE/30.06.2014.

EVALUAREA ANGIOGENEZEI ÎN CARCINOMUL HEPATOCELULAR

Introducere: Carcinomul hepatocelular reprezintă cea mai comună patologie malignă primară a ficatului, fiind una din cele mai prevalente și letale forme de cancer la nivel mondial, cu peste 500 000 persoane nou-diagnosticate anual. O caracteristică importantă a carcinomului hepatocelular este hipervascularizarea. Neoangiogeneza contribuie la metastazare și influențează nefavorabil prognosticul.Obiective: Studiul își propune analiza profilului de expresie al principalelor proteine implicate înprocesele de angiogeneză în carcinomul hepatocelular.
Materiale și metode: Au fost obținute fragmente de țesut tumoral și țesut non-malign adiacent provenind de la 10 pacienți diagnosticați cu carcinom hepatocelular. Fiecare tumoră a fost caracterizată în ceea ce privește tipul histologic și gradulde infiltrare. Toți pacienții prezenta urecurență la momentul urmăririi.Pentru a investiga profilul de expresie al proteinelor implicate înprocesul de angiogeneză s-au utilizat tehnici de imunohistochimie și dot-blot.
Rezultate: Prin tehnici de imunohistochimie (IHC),la7 din 10 pacienți s-a evidențiat prezența VEGF întesut tumoral. CD34, marker util de capilarizare malignă, a fost de asemenea prezentîn țesutul tumoral. Rezultatele obținute prin tehnici dot-blot arată modificări semnificativeîn expresia unor proteine implicate în angiogeneză în țesut tumoral comparativ cu țesutul peritumoral. Printre proteinele cu expresie crescută în țesutul tumoral se disting b FGF (basic fibroblast growth factor), IL-8 și VEGF (vascular endothelial growth factor A).
Concluzii: Rezultatele obținute printehnici de IHC și dot-blot evidențiază activarea unor factori angiogenici, printre care VEGF, bFGFși IL-8 întesutul tumoral; aceste molecule pot fi ținte potențiale în dezvoltarea de strategii terapeutice noi în carcinomul hepatocelular datorită efectelor acestora asupra angiogenezei.
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P-23

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL ASPECTS IN "MILK-LINE" NEVI

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Several international publications talk about some specific locations for melanocitic nevi that can present changes similar to melanoma or dysplasic nevi. Those sites include also the milk lines (axillary, breast, periombilical, inguinal and perinal).nevi from these sites can have common hormonal and embriological features, because of their origin from the milk lines. The study was performed in a period of 4 years, in the Pathology Department of Clinical County Emergency Hospital Constanta. It included a number of 198 nevi, selected from 924 cases. 10 morphologic parameters were performed, along with immuohistochemical examinations, for each lesion. The milk line nevi exhibit atypical morphologic features with a higher rate than other sites. Until present daysit is not known if these morphological changes are homon-related and this study, with ER negative expresion, does not bring any additional data. The diagnosis of nevi with site related atypias as well as dysplasic nevi remains a subjective one, and there is still no consens concerning the amount of morphological changes for considering a nevus from the above mentioned categories as being dysplasic.

ASPECTE MORFOLOGICE SI IMUNOHISTOCHIMICE ALE NEVILOR "LINIEI MAMARE"

În numeroase publicații internaționale s-a vorbit despre unele localizări ale nevilor melanocitari în care aceștia pot prezenta modificări similare celor întâlnite în melanom sau nevii displazici. Aceste localizări includ si liniile mamare (axila, sânul, regiunea periombilicală, inghinală și perineală). În aceasta categorie nevii pot avea trăsături hormonale și embriologice comune, facând parte din așa numitele linii mamare. Studiul a fost efectuat pe o perioadă de 4 ani, în cadrul Serviciului Clinic de Anatomie Patologică al Spitalului Clinic Județean de Urgență Constanța. Au fost incluși în studiu un număr de 198 nevi, selectati din 924 cazuri. S-au analizat 10 parametrii morfologici, impreuna cu examene imunohistochimice, pentru fiecare leziune. Nevii liniilor mamare prezintă modificări morfologice atipice cu o frecvență mai ridicată față de alte localizări. Până în prezent nu se știe dacă aceste modificări morfologice sunt hormono-dependente iar studiul de față, cu expresie negativa a ER, nu aduce informații suplimentare în acestă privință. Diagnosticul de nev cu atipii legate de localizare cât și al nevilor displazici rămâne unul subiectiv fara sa existe un consens cu privire la cantitatea de modificări necesară pentru a considera un nev dintr-una din categoriile de mai sus ca fiind cu caracter cert displazic.

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LICHENUS PLANUS – MORPHOLOGICAL AND MOLECULAR ASPECTS – CASE REPORT

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Lichen planus is a cellular immune response with unknown etiology and with cutaneous and mucosal involvement, rarely leads to nail dystrophy or cicatriceal alopecia. The typical cutaneous lesions are flat topped violaceous papules, and erythematous, pruritic macules, most frequent on the extremities. Currently, there are no prognostic markers to identify the lesions at increased risk for malignant transformation. Selected markers for cell proliferation, adhesion, apoptosis, and lymphocytic infiltration were assessed by immunohistochemistry in addition to static cytometry analyses for DNA content. DNA quantification of epithelial cells from atrophic lichen planus showed altered DNA content. DNA content was associated with proliferation activity, topoisomerase IIα, desmocollin-1 and infection with human papillomavirus. CD27+ and CD38+ lymphocytes were detected in inflammatory cell infiltrate, indicating an abnormal homing of B cells from blood circulation to tissue.

LICHENUL PLAN – ASPECTE MORFOLOGICE SI MOLECULARE – PREZENTARE DE CAZ

Lichenul plan reprezintã un rãspuns imun mediat celular cu origine necunoscutã care afecteazã tegumentul si mucoasele, si mai putin frecvent determinã distrofie unghialã sau alopecie cicatricealã. Leziunile cutanate tipice sunt reprezentate de papule turtite, violacee si macule eritematoase, pruriginoase, care apar mai frecvent la nivelul extremitãtilor. In prezent, nu exista markeri de prognostic pentru a identifica leziunile cu risc crescut de transformare malignă la cazurile cu lichen plan. Markerii selectati pentru proliferarea celulară, adeziunea, apoptoza, și infiltrarea limfocitară au fost evaluati prin imunohistochimie pentru determinarea conținutului de ADN. Cuantificarea ADN-ul celulelor epiteliale a prezentat un conținut de ADN alterat. Conținutul de ADN a fost asociat cu activitatea de proliferare și infecția cu papilomavirus uman. Limfocite CD27 + și CD38 + au fost detectate in infiltratul inflamator, ceea ce indică o localizare anormală a celulelor B din circulația sanguină la nivelul țesutului.

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SERUM PROTEOMIC PROFILE IN PROSTATE CANCER BY SELDI-TOF MASS SPECTROMETRY

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Background: Prostate cancer is the most commonly diagnosed cancer and a leading cause of cancer death in European men. Development of reliable biomarkers and a personalized clinical approach is critical in the management of clinically relevant prostate cancer.
Material and methods: Surface Enhanced Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (SELDI-ToF MS) was used to perform the proteomic biomarkers profile from serum samples (15 prostate cancer and 10 controls were applied to 2 types of protein chips: CM10 - weak cation exchanger and IMAC30-immobilized metal affinity.
Results: Spectra were selected on the basis of the largest number of peaks present at each pH (4, 9) for all chips and in terms of each peak's relative intensity. IMAC 30 at pH 7 was selected for serum sample analysis. The proteomic spectra obtained were compiled, normalized, and mass peaks with mass-to-charge ratios between 2 and 50 kDa identified. Peak information was analyzed using univariate statistics, 20 significantly different protein peaks were detected (selection of relevant clusters according to AUC, p-values. Biomarker Patterns Software (BPS) was applied to generate multiple biomarker correlation with clinical phenotype and accurate and reliable predictive models.
Conclusion: It was designed and experimented a methodology for identifying potential serum biomarkers with predictive value for the diagnosis of prostate cancer by proteomics technology SELDI – TOF MS followed by CART (classification tree pattern analysis).
Acknowledgment: PN II 192/2014 and PN 16.22.04.01.

PROFILUL PROTEOMIC SERIC IN CANCERUL DE PROSTATA PRIN SPECTROMETRIE DE MASA DE TIP SELDI-TOF

Introducere: Cancerul de prostata este cel mai frecvent tip de cancer diagnosticat si totodata cauza principala de deces prin cancer la barbati. Identificarea de noi biomarkeri si o abordare clinica personalizata sunt esentiale in managementul cancerului de prostata.
Material si metode: Platforma SELDI-TOF MS a fost utilizata pentru a efectua profilul proteic in 25 de probe de ser: 15 cazuri cu cancer de prostata si 10 martori. Probele au fost aplicate pe 2 tipuri de chip-uri de proteine: CM10 (Weak Cation Exchange ProteinChip CM10) si IMAC 30 (ProteinChip IMAC-30) realizandu-se mai multe variante experimentale.
Rezultate: Spectrele au fost selectate in functie de prezenta celui mai mare numar de picuri la fiecare pH (4, 9) pentru toate chip-urile si in functie de intensitatea fiecărui pic. A fost selectat pentru analiza probelor de ser chip-ul IMAC 30 (pH 7). Analiza spectrelor a presupus identificarea picurilor, normalizarea si alinierea spectrelor in intervalul de greutati moleculare 2-50 kDa. In urma analizei datelor a fost identificat un grup de 20 clustere serice in probele de ser (conditia de selectie a clusterelor relevante: $p<0,05$ si prezenta picului in cat mai multe probe). Biomarker Patterns Software (BPS) a fost aplicat pentru a corela biomarkeri multipli cu anumite fenotipuri, imbunatatind astfel sensibilitatea si specificitatea analizei fata de analiza pe biomarkeri individuali.
Concluzii: A fost proiectata si experimentata o metodologie de identificare a unor potentiali biomarkeri serici cu valoare predictiva in diagnosticul cancerului de prostata prin tehnologia proteomica SELDI-ToF MS urmata de CART (classification and regression trees).
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NON OCCUPATIONAL ETIOLOGICAL FACTORS IN SKIN DISCOLORATION. CASE PRESENTATION

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Introduction: Cutaneous pigmentary disorders found in workers exposed to occupational metal dusts can represent a challenge in diagnostic and therapeutic approach for both dermatology and occupational physicians.
Material and methods: We presentthe case of a 59 year old patient who has been working for 25 years in a gunpowder factory and has been exposed to metal dusts (silver, lead, iron). The patient presented with pigmentary lesions affecting the face, raising the presumptive diagnosis of occupational hyperpigmentation (occupational tattoo).
Results: The histopathological result excluded the occupational etiology (there weren't identified silver and lead particles) and it was suggestive for exogenous ochronosis. Exogenous ochronosis is a dyscromia generated by chronic use of topical products containing hidroquinone, a substance that affects melanogenesis metabolism.
Conclusion: The physician should rapidly recognize this pathology and discontinue the patient's treatment with hidroquinone.

FACTORI NON PROFESIONALI IN ETIOLOGIA DISCROMIEI. PREZENTARE DE CAZ

Introducere: Tulburarile de pigmentare tegumentara la lucratorii cu expunere profesionala la pulberi metalice pot reprezenta o provocare pentru medicul dermatolog si pentru medicul de medicina muncii in egala masura, atat in abordarea diagnostica, cat si terapeutica.
Material si metoda: Descriem cazul unei paciente de 59 ani care a lucrat la o fabrica de armament timp de 25 ani cu expunere la pulberi de metale (Argint, Plumb, Fier), directionata catre clinica de dermatologie pentru prezenta de leziuni hiperpigmentare la nivelul pliurilor fetei cu diagnostic prezumptiv de hiperpigmentare profesionala (tatuaj profesional).
Rezultate: In urma examenului histopatologic, am exclus etiologia profesionala (nu s-au identificat particule de argint, plumb etc.), diagnosticul fiind sugestiv pentru ocronoza exogena. Ocronoza exogena reprezinta o discromie generata de aplicarea cronica de produse topice cu continut crescut de hidroquinona, substanta care determina perturbarea metabolismului melanocitelor.
Concluzie: In concluzie, este necesara recunoasterea acestei patologii si stoparea aplicarii produselor care contin hidroquinona.

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GLYCOSYLATION MODULATES TRANSIENT RECEPTOR POTENTIAL CHANNELS IN PANCREATIC CANCER

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Transient receptor potential melastatin is a membrane ion receptor participating to cold sensation in peripheral neurons. TRPM8 is over-expressed in several solid tumours such as breast, kidney and lung. In pancreatic adenocarcinoma (PDA) this membrane protein was related to tumour size and stage, migration and proliferation of PDA

cells. With this study we bring new insights in the structure and function of TRPM8 which shows that the form expressed in the pancreatic tumoural cell line Panc-1 is un-glycosylated as compared to the glycosylated protein over-expressed in Human Embryonic Kidney 293(HEK) cells. We studied the roles of TRPM8 in cell migration, Ca2+ uptake and cell proliferation. We found that the un-glycosated form determine a slight but significant decrease in pancreatic cell migration. Moreover, the uptake of calcium depends of the presence of tunicamicyn a specific inhibitor of N-linked glycoslylation. Thus, our observations suggest that un-glycosylation of TRPM8 may be protective against cell migration and proliferation in PDA cells.