

**COMENIUS UNIVERSITY IN BRATISLAVA
JESSENIUS FACULTY OF MEDICINE
MARTIN**



XXXVI. STUDENT SCIENTIFIC CONFERENCE

PROGRAM and ABSTRACTS

April 29, 2015

Martin, SLOVAK REPUBLIC

XXXVI. Student Scientific Conference
Jessenius Faculty of Medicine
Comenius University
Martin

The conference is supported by Dr. Jozef Lettrich Foundation

Dr. Jozef Lettrich Foundation

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PROGRAM AT GLANCE

Date: April 29, 2015

Place: Aula A Novomeského 7 (next to the student hostel)
Aula B Novomeského 7 (next to the student hostel)

Registration:

April 29, 2015 - 7.30
- before the beginning of your section

Opening ceremony (Aula A – Novomeského 7) **08.00 – 08.10**

Aula A

A1: Section of Theoretical Disciplines 08.15 – 09.45

- coffee break

A2: Section of Clinical Disciplines 10.00 – 12.25

Aula B

B1: Section of Non-Medical Study 08.15 – 09.30

- coffee break

B2: Section of Preclinical Disciplines 09.45 – 11.00

- coffee break

B3: Section of Molecular Oncology 11.15 – 12.45

Closing ceremony (Aula A – Novomeského 7) **13.00**

Duration of lectures (Slovak or English language):

8 minutes, discussion – 4 minutes

PROGRAM IN DETAILS

„AULA – A“

SECTION OF THEORETICAL DISCIPLINES

(8.15 – 9.45)

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Department of Neurology, UHM and JFM CU Martin

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Department of Histology and Embryology, JFM CU Martin

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APPLICATION OF "GO - NO - GO" TEST IN PSYCHOPHYSIOLOGY

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Department of Physiology, JFM CU Martin

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EFFECTS OF PRIMARY AND SECONDARY AFFERENT PATHWAYS MODULATION ON COUGH

Lucia Babálová and Ivan Poliaček
Institute of Medical Biophysics, JFM CU Martin

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Department of Neurology, UHM and JFM CU Martin

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Department of Internal Medicine I, UHM and JFM CU Martin
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Department of Pathophysiology, JFM CU Martin
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Kristián Varga, Marián Kollárik and Alžbeta Trančíková
Department of Pathophysiology, JFM CU Martin

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Department of Pathology, UHM and JFM CU Martin

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Department of Molecular biology, JFM CU Martin

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Martina Višňovská and Jozef Višňovský
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Amanda Jensen Einungbrekke and Lukáš Plank
Department of Pathological anatomy, UHM and JFM CU Martin

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ABSTRACTS

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ASSOCIATION OF RS703842 GENE POLYMORPHISM IN CYP27B1 GENE WITH SUSCEPTIBILITY AND PROGRESSION TO MULTIPLE SCLEROSIS

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BACKGROUND: Dihydroxycholecalciferol (calcitriol), an active form of vitamin D is a product of the long metabolic pathway. One of its physiological functions is regulation of the immune system response through vitamin D receptor. It is known that by these effects, vitamin D deficiency can be the risk factor of development of multiple sclerosis (MS) or deterioration of the clinical course of MS. The product of the CYP27B1 gene, 25-hydroxyvitamin D-1 α -hydroxylase, is an enzyme involved in the conversion of inactive form of vitamin D to its active form. Gene polymorphism rs703842 in CYP27B1 gene can potentially alter this activation of vitamin D and thus also its proposed immunomodulatory effects. The aim of our study was to uncover potential role of this gene polymorphism of CYP27B1 gene in MS susceptibility and disease progression. **MATERIALS AND METHODS:** In our study we genotyped 267 clinically diagnosed MS patients and 291 healthy controls. The relapsing – remitting form of MS was present in 85,02% cases and secondary progressive form in 14,98% cases. DNA was isolated from peripheral white blood cells. Genotype analysis was performed by PCR and restriction analysis. **RESULTS:** We found significantly decreased frequency of allele C in MS patients when compared to controls (28,46 % vs. 36,25 %, $p = 0,0055$). The incidence of homozygotes CC was also significantly decreased 8,24 % in MS patients in comparison to 16,15 % in healthy individuals ($p = 0,0045$). **CONCLUSION:** In our study, we observed significant differences in distribution of alleles and genotypes in MS patients when compared to healthy individuals. Significantly lower frequencies of allele C and genotype CC of rs703842 in CYP27B1 gene suggest for their potential protective role against MS development.

This work was supported by grants VEGA 1/0213/12, 2012/30-UKMA-7 Biological and molecular markers of MS.

EFFECT OF MOBILE PHONE CALLING IN DIFFERENT ENVIRONMENTS

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INTRODUCTION: The aim of this study was to reveal intensity of electric field changes near the cellular phone's (CP) antenna during calling in shielded places. We compared areas with low and high GSM signal strength. **METHODS:** We used hand held broadband field meter Narda 550 NBM to measure intensity of electric field (E) with 100 kHz – 3 GHz E probe attached. According to ICNIRP, the value of E reference limit is 58.34 V/m for general public exposure to high frequency EMF at 1800MHz and needs to be averaged over any 6-min period. We used a pocket CP Sony Ericson D750i with its SAR value 0.99W/kg given for head area. We attached CP directly to the E probe. Two different positions (front and back) were measured to determine differences between E on head side and E on hand side of CP. Background values were measured for comparison to the CP active stand-by mode. We chose ordinary office on the 2nd floor, biophysics practical room A on the 3rd floor, civil defence shelter located in the basement and area outside near the building of Jessenius Medical Faculty. **RESULTS:** We observed significant increase of E ($p > 0.01$) in shielded area with poor signal strength in the shelter ($61.51 \pm 0.25 \text{ V/m}$; mean \pm SD) and in the office ($35.02 \pm 0.11 \text{ V/m}$) comparing to outside area ($8.67 \pm 1.78 \text{ V/m}$) for hand side. Similarly, E ($30.24 \pm 0.3 \text{ V/m}$) was found in the shelter, ($14.47 \pm 1.07 \text{ V/m}$) at the office and also in the practical room ($10.04 \pm 0.53 \text{ V/m}$), comparing to outside area ($4.91 \pm 1.82 \text{ V/m}$) for head side of CP. **CONCLUSION:** Some of our results exceeded the given ICNIRP limit for E emitted by CP under poor signal conditions. Our recommendation is to avoid or decrease the call length and control CP signal intensity inside the buildings or other shielded places.

ACCUMULATION OF 4-HYDROXYNONENAL PROTEIN ADDUCTS IN RAT HEARTS DURING AGING

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Cumulative oxidative damage is proposed as the major cause of the aging process. Lipids are major targets of oxidative attack and this causes formation of lipid peroxidation products (LPO). 4-hydroxy-2-nonenal (HNE) is a major end-product of LPO of n-6 polyunsaturated fatty acids. This highly toxic aldehyde exhibits great reactivity towards proteins. Formation of HNE-protein adducts usually results in altered structure and deterioration of protein function. The present study was designed to identify HNE-modified proteins in heart mitochondria of adult (6 months), old (15 months) and senescent (26 months) male Wistar rats. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis revealed 13 bands with HNE-protein adducts. Using matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry we identified ADP/ATP translocase, creatine kinase and enzymes of electron transport chain (ETC), citric acid cycle and fatty acid oxidation. Of these only subunits of ETC complex III and V and ADP/ATP translocase exhibited altered HNE-modification with aging. Moreover, amounts of HNE-protein adducts did not increase progressively with age, but culminated in old rats and declined in senescent rats. These results suggest that modification of proteins by HNE is possibly not the major cause of mitochondrial dysfunction but may contribute to altered function of some enzymes and transporting proteins during aging.

EPIGENETIC MODIFICATIONS INDUCED BY HOMOCYSTEINE ALTER THE GENE EXPRESSION IN HUMAN GLIAL CELLS

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Homocysteine (Hcy) is an intermediate of the S-adenosylmethionine cycle, which sustains methylation reactions in cells. The methylation of proteins regulates their function and stability, while the methylation of DNA and histones is involved in the epigenetic regulation of gene expression. Increased levels of Hcy are linked with neurodegeneration. Hcy acts as a neurotoxic and gliotoxic agent, although the detailed pathomechanism is still unclear. On a molecular level, Hcy is feedback inhibitor of methylation reactions.

The aim of this study was to evaluate the hypothesis that Hcy may alter epigenetically the gene expression in human glial cells by affecting the methylation status of histone H3.

We used the T98G glioblastoma cell line as study model. The cells were incubated in medium supplemented with Hcy (100 μ M) for 48 or 72 hours. The methylation status of histone H3 at Lys 4 and the levels of p53, BAX and caspase-3 were estimated by immunoblotting methods. The specific enzymatic activity of lactate dehydrogenase (LDH) was determined by biochemical assays.

The incubation with Hcy for 48 hours suppressed the methylation of histone H3 and reduced the levels of p53 and BAX in the cells, but stimulated the expression of caspase-3. LDH activity was also decreased. After 72-hour, the level of histone H3 methylation, p53 and BAX returned to the control values, while the level of caspase-3 remained increased.

Our results confirm that Hcy inhibits the methylation of histone H3, which is a key epigenetic mark. This correlates with the altered expression of pro-apoptotic proteins and LDH. The modifications in gene expression induced by Hcy may play a role in the etiopathogenesis of neurodegeneration.

This work was supported by the projects VEGA 1/0242/13, and “Competence center for research and development in diagnosis and therapy“ code: 26220220153 co-financed from EU sources and European Regional Development Fund.

EFFECT OF INDUCED HYPERHOMOCYSTEINEMIA TO NEURODEGENERATIVE PATHOLOGICAL CHANGES UNFOLDING IN EXPERIMENTAL MODEL

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Diseases of the central nervous system are found in patients with severe hyperhomocysteinemia (hHcy). Epidemiological studies show a positive, dose-dependent relationship between mild-to-moderate increases in plasma total homocysteine concentrations (Hcy) and the risk of neurodegenerative diseases, such as Alzheimer's disease (AD). Alzheimer's disease (AD) commonly co-occurs with stroke. Therefore, we investigated the effect of ischemia-reperfusion injury (IRI) in combination with hHcy to neurodegeneration in rat brains. We have studied neurodegeneration as well as post-translation changes in MAPK (mitogen-activated protein kinase) pathways after global IRI in rat brain in association with hHcy and possible unfolding of AD. Global forebrain ischemia was induced by 4-vessels occlusion. In the concrete, 15 min of ischemia followed with reperfusion period of 72h and 7 days. hHcy was induced by methionin diet (0.2g/kg) in duration of 30 days. We demonstrated occurrence of degeneration of selectively vulnerable neurons after induced IRI as well as after hHcy. Western blot study and immunohistochemical analysis suggested that IRI and also hHcy down-regulates the p-ERK protein which is associated with survival of neural cells. On the other hand, both stressors up-regulates p-p38 protein, which is well known neurodisturbant. These findings suggest that IRI after induced hHcy could have a neurodegenerative role on global brain ischemia in rats. Our results also indicate that the model of combined insults could lead to progression of AD-like pathological features.

Supported by VEGA 1/0229/15 and 1/0213/12 and by project: " IDENTIFICATION OF NOVEL MARKER IN DIAGNOSTIC PANEL OF NEUROLOGICAL DISEASES" co-financed from EU sources and European Regional Development Fund.

APPLICATION OF "GO - NO - GO" TEST IN PSYCHOPHYSIOLOGY

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"Go-no-go" test is neuropsychological test designed for attention and impulsivity measurement, thus, it can be used in diagnosis of mental disorders. The complex analysis of autonomic nervous system (ANS) reactivity during the test are rare. AIM: To study complex ANS response to "go-no-go" test in healthy students. METHODS: Twenty students (9 women, age: 24.6 ± 0.8 yr.) were examined. The continuous ECG signal and electrodermal activity (EDA) were monitored during rest (T1), go-no-go test (T2) and rest (T3). Each interval lasted 6 minutes. The evaluated parameters of heart rate variability (HRV): RR interval, rMSSD, spectral power in high frequency band (HF) reflecting cardiac vagal control; symbolic dynamics - 0V% as a potential index of cardiac sympathetic regulation. The amplitude of EDA (μ S) was considered as an index of cholinergic sympathetic activity. The evaluated "go-no-go" test parameters: mean reaction time (RT-mean), commission (EC) and omission errors (EO). RESULTS: RR interval was significantly shortened during T2 compared to T1 ($p < 0.001$), and EDA was significantly higher during T2 and T3 compared to T1 ($p = 0.03$) No significant differences were found in HF-HRV, rMSSD and 0V%. Go-no-go test parameters: RT-mean=269.806 ms, EC=1.0 and EO=0.6. CONCLUSION: Our results revealed sympathetic arousal indexed by higher EDA, and tachycardic reaction in response to go-no-go test. Interestingly, HRV parameters were without significant changes. Quantitative characteristic of test performance reflected good attention and low impulsivity potentially related to activation of reticular formation, which may result in specific sympathetic response associated with subjective behavioral characteristics in response to specific stressor. Our results could contribute to understanding of autonomic reactivity in response to go-no-go test in psychophysiological research. This method may help to better differential diagnosis of mental disorders.

SUPPORT: VEGA 1/0087/14.

EFFECTS OF PRIMARY AND SECONDARY AFFERENT PATHWAYS MODULATION ON COUGH

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The role of myelinated nerve fibers in the vagus and superior laryngeal nerves (SLN) in the execution of cough, sneeze and swallow was studied in pentobarbitone-anesthetized, spontaneously breathing cats. The nerve conduction was reduced by cooling of the nerves ($<6^{\circ}\text{C}$). Cough reflex was induced by mechanical stimulation in the intrathoracic trachea, sneeze in the nose, swallow by injection of water into the oropharynx (3ml). Electromyograms of the diaphragm, abdominal, cricopharyngeus, thyroarytenoid, and styloglossus muscles together with blood and esophageal pressures were recorded.

Unilateral vagal cooling reduced the number of coughs (related to 10s stimulus) as well as the inspiratory and expiratory cough efforts ($p<0.01$). Vagus nerve on one side usually dominated the initiation of cough. Vagal cooling also resulted in prolonged inspiratory and expiratory phase, the total and active portion of the cycle, and the distance between diaphragm and abdominal maxima during cough as well as in reduced respiratory rate. Unilateral cooling of SLN had no effect on cough, however, bilateral block of SLN reduced cough expiratory efforts ($p=0.05$). Cooling of the SLN also reduced the swallowing response. No appreciable differences in heart rates and mean arterial blood pressures as well as in sneeze reflex were seen. Computational modeling confirmed significant contribution of reduced afferent drive into the cough central pattern generator to the cough pattern changes observed after vagal cold block. Experimental data are consistent with selective, but complex processing of primary and secondary afferent signaling in the execution of various behaviors involved in airways defence.

This work was supported by the VEGA 1/0126/12 (30%) and and Development Agency under the contract No. APVV-0189-11 (40%). The project is co-financed from EU source - Martin biomedical center (BioMed Martin), ITMS code: 26220220187 (30%).

THE DIFFERENCE IN CLINICAL PICTURE AND A PROFILE OF OLIGOCLONAL BANDS IN PATIENTS WITH MULTIPLE SCLEROSIS IN BULGARIA AND SLOVAKIA

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INTRODUCTION: Multiple sclerosis is a chronic inflammatory autoimmune disease of the central nervous system, directed against the myelin sheath, which leads to demyelination and axonal loss. This study have analyzed the clinical difference of the patients in two groups, Bulgarian and Slovak. **METHODS:** Data were collected retrospectively in a period of 1 year between 2011-2014. We have been focused on the cerebrospinal fluid oligoclonal bands(OCB) status in the two groups, clinical picture measured by the Expanded Disability Status Scale (EDSS). In this study we have statistically evaluated a group of Slovak patients with Multiple Sclerosis(MS), describe the relations in the group, and correlate them with the Bulgarian MS results. We have used PAST- statistical tests. **RESULTS:** This study was based on 22 patients from Slovakia and 20 patients from Bulgaria. The patient' s negativity of OCB in the Slovak study group has been found in 45.5% (n=10), and in 15% (n=3) in Bulgarian group. In the Slovak group we have noticed that OCB positivity correlate with IgG index ($r = 0.72$, $p=0.0000$ Kendall's Tau), but OCB did not match with EDSS or relapses. In Bulgarian group we have proved association between EDSS and IgG index ($r=0.39$, $p=0.014$ Kendall's Tau). When looking at both populations (Slovak and Bulgarian) we could determine that they didn't differ in many factors such as age, age of onset, and duration of disease. We have detected a difference in the OCB status ($p=0.036$) and the EDSS. Bulgarian patients had lower EDSS ($p = 0.015$ Mann-Whitney) than Slovak MS patients despite relatively more frequent relapses ($p=0.07$ Mann-Whitney*), and positivity of OCB. * There was a trend towards higher frequency of relapses in Slovak group. **CONCLUSION:** In Slovakia the high frequency of patients have shown negative OCB in comparison with the Bulgarian group and the rest of the world. Our results support an assumption that Bulgarian patients, no matter the OCB status, show milder disease course. In conclusion, our study demonstrates the difference between the two patient groups, and the knowledge that the OCB status cannot be used as prognostic factor between countries.

SUMMARY OF DIETARY HABITS AND THEIR SIGNIFICANCE IN RELATION TO SELECTED DISEASE PARAMETERS IN PATIENTS WITH MULTIPLE SCLEROSIS

Jana Copková and Ema Kantorová*

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Introduction: Multiple sclerosis (MS) is a chronic neurological autoimmune disease. Factors maintaining the autoimmune inflammation are not fully known. In our work we have focused on mapping of the dietary habits, examination of lipid metabolism, and body weight, suggested to be potential co-factors of the disease.

Methods: We examined 66 patients with MS (11 men and 55 women) treated in the MS Center. Control group consisted of 69 healthy subjects of the same age (14 men and 55 women). Information were attained via questionnaires focusing on the dietary habits (amounts of food with high saturated fat content (ST), vegetables (V), fruit (F) in diet, and fatigue [0 to 10]). Other factors studied were: body mass index (BMI), cholesterol (CH), triglycerides (TAG), and „Expanded disability status scale“ (EDSS).

Results: In the group of men, we have observed that the amount of SF, V and O does not vary between groups. Despite it, the MS patients have had higher BMI (27,4 vs 23,1. $p = 0,00015$), higher levels of TAG (2,36 vs 0,95. $p = 0,004$), and marginally higher CH levels. Fatigue was more intensive in MS patients than control subjects. EDSS did not correlate with any of the other parameters observed. Similarly, statistically we have not found differences in dietary habits in the group of women. We have not found any differences in the CH, and TAG levels. Fatigue was more intensive in MS patients in comparison with healthy women (5,1 vs 4,2. $p = 0,004$), while fatigue correlated with EDSS and age. We have also found correlation between fatigue and SF ($r = - 0,23$, $p = 0,012$).

Conclusion: Our patients with MS have shown lipid metabolism disturbances, higher body weight, as well as significantly higher fatigue in comparison with healthy individuals. Differences were gender-specific.

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FOCAL INFECTION IN ENT REGION IN CHILDREN

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The upper aerodigestive tract is the most common point of entry for pathogens and serves as the first step in interaction between microorganisms and immune system. Many disorders of immune system both inherited and acquired are manifested as infection of upper aerodigestive tract. On the other hand, those infections are also common in patients with an intact immunity, especially among children. AIM: The aim of this study was to evaluate microbial colonization of upper airway in children with focal infection in ENT region. METHODS: Fifty-eight children were enrolled in the prospective study. Differences in bacterial colonization of middle nasal meatus, nasopharynx and tonsils in children with adenoid vegetation, recurrent tonsillitis and control group were analysed. The influence of atopy and exposure of passive smoking on bacterial colonization of upper airway in children were investigated. The effect of surgery (adenotomy, tonsillectomy) on bacterial colonization was investigated. RESULTS: The most common isolated pathogens in upper airway were Haemophilus influenzae and Staphylococcus aureus in children with adenoid vegetation and Streptococcus pyogenes in children with recurrent tonsillitis. Identification of pathogenic bacteria in upper airway was significantly higher in children with adenoid vegetation and recurrent tonsillitis compared to control group ($P = 0.03$, $P = 0.02$, respectively). Presence of atopy and exposure to tobacco smoke were associated with significantly more often colonization of pathogenic bacteria in upper airways ($P = 0.04$, $P = 0.02$, respectively).

CONCLUSION: Adenoid vegetation and recurrent tonsillitis in children are associated with increased colonization by pathogenic bacteria in upper airways. Atopy and tobacco smoke exposure are related to increased colonization by pathogenic bacteria in upper airways.

STICKY PLATELET SYNDROME IN PATIENTS WITH FETAL LOSS

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Sticky Platelet Syndrome (SPS) is defined as platelet hyperaggregability after low concentration of adenosine diphosphate (ADP) and/or epinephrine (EPI). It is associated with an increase incidence of arterial thrombosis, pregnancy complications and less often venous thromboembolism. After antiphospholipid syndrome, it is the second most frequent thrombophilia that causes fetal loss syndrome. SPS is a thrombophilic thrombocytopathy with familiar occurrence and probably autosomal dominant trait, although the exact genetic cause has yet to be identified. It has been suggested that the defects of the platelet membrane glycoproteins (e.g. GP6) or intracellular signal pathways involved in platelet activation and aggregation are responsible for the disorder. Several studies suggest a possible polygenic type of SPS heredity. METHODS: We examined 23 female patients with SPS and history of spontaneous abortion, and 42 healthy women. SPS is diagnosed by aggregometry. SPS is classified as type I (hyperaggregation after both ADP and EPI), type II (hyperaggregation after EPI alone – it is the most common), and type III (hyperaggregation after ADP alone). Then we were interested in 15 single nucleotide polymorphisms (SNPs) of GP6 gene (rs1654410, rs1671153, rs1654419, rs11669150, rs1613662, rs12610286, rs1654431, rs4281840, rs12981732, rs10417943, rs1671152, rs1654433, rs1671215, rs10418743, rs8113032) . RESULTS: We identified six SNPs of GP6 gene with higher occurrence in patients with SPS and abortion (rs1671153, rs1654419, rs1613662, rs1671152, rs1654433, rs1671215). We also identified four high-risk haplotypes of GP6 gene in our haplotype analysis that are connected to SPS, a possible cause of spontaneous abortions (CGATAG, CTGAG, CCGT, ACGG). CONCLUSION: Our results suggest that variability of the GP6 gene may be associated with platelet hyperaggregability in patients with SPS and fetal loss. These results have to be confirmed by further research.

THE EFFECT OF TYPE 2 DIABETES ON THE EFFICACY OF ADP RECEPTOR BLOCKER THERAPY IN PATIENTS WITH ACUTE ST ELEVATION MYOCARDIAL INFARCTION

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BACKGROUND: Several papers reported about a failure in antiplatelet response to ADP receptor blocker therapy which is connected with insulin resistance and type 2 diabetes (T2D). The aim of this preliminary study was to validate the impact of T2D on the efficacy of ADP receptor blocker in patients with acute ST elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (pPCI). **PATIENTS AND METHODS:** A single centre preliminary, prospective observational study in patients with acute STEMI and pPCI was performed. Totally 67 patients (37 men and 30 women) were enrolled. Among all study population, 33 patients received clopidogrel and 34 patients received prasugrel. The efficacy of ADP receptor blocker therapy had been tested in two time intervals using light transmission aggregometry (LTA) with specific inducer and vasodilator - stimulated phosphoprotein phosphorylation (VASP-P) flow cytometry assay. **RESULTS:** There were no significant differences in platelet aggregability after adenosine diphosphate (ADP) among T2D and nondiabetic (ND) group. Similarly, the platelet reactivity index of VASP-P did not differ significantly between T2D and ND group ($59.4 \pm 30.9\%$ versus $60.0 \pm 25.2\%$, NS and $33.9 \pm 25.3\%$ versus $38.6 \pm 29.3\%$, NS in second testing, respectively). The number of ADP receptor therapy non – responders tended to be higher in T2D patients; however the differences did not reach statistical significance. The time interval from ADP receptor blocker loading dosing to the blood sampling was similar in T2D and ND patients in both examinations (sample 1: 1.8 ± 0.9 hours versus 1.7 ± 0.9 hours; sample 2: 21.6 ± 2.2 hours versus 20.0 ± 1.9 hours). **CONCLUSION:** This preliminary study did not confirm the higher residual platelet reactivity on ADP receptor blocker therapy and higher prevalence of ADP receptor blocker non - responders in T2D acute STEMI patients.

NON-MOTOR SYMPTOMS OF PARKINSON'S DISEASE AND THEIR RELATION TO THE DEEP BRAIN STIMULATION

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Parkinson's disease (PD) is a progressive neurodegenerative disease with typical motor symptoms. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is effective treatment for motor symptoms. Nonmotor symptoms (NMSs) may also profoundly affect the quality of life in PD patients. AIM: To identify changes in NMSs of PD as a consequence of DBS in the patient's therapy half a year after surgery. METHODS: Specifically selected complex of questionnaires was administered to PD patients before and after bilateral DBS of STN. We analysed and compared results to find out significant differences in PD patients symptoms. RESULTS: Five PD male and female patients have been included in our study so far. In pre-surgical questionnaires we revealed wide spectrum of NMSs with various intensity in all cases. Six months after the surgery there was a significant reduction in urgent urination, excessive sweating, hot flushes, paraesthesia, palpitations and in some of the depressive symptoms. In two patients with breathing difficulties these completely disappeared. On the other hand aggravation of concentration with mild confusion was observed in two cases. We also observed flattened mood without fluctuations in three patients which appeared a year after the surgery, in one case together with paranoid thoughts. CONCLUSION: DBS is an important and successful method of PD treatment having significant effect not only on the motor symptoms of the disease. Changes of the NMSs have strong influence on the patient's life. With knowledge about these symptoms we can better prepare PD patients for the post-surgical adaptation. The major limitation of this study was the sample size. Further study on bigger cohort is needed.

COINCIDENCE OF HYPOTHERMIC DEATH AND ALCOHOL INTOXICATION

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In normal conditions, human being is able to maintain the body temperature at lower ambient temperatures. The heat loss is automatically reducible by peripheral vasoconstriction while the heat production is increased by shudder and thermogenesis in liver. In a case of impaired or unefficient ability to control body temperature in cold environment the body temperature decrease results in hypothermia. The cases of fatal hypothermia are primarily seen during the cold months but, overall, it is possible in room temperature, for example in unheated house at temperatures from 15 to 20°C or if the body surface is wet (due to increased conduction). Alcohol consumption, various diseases or exhaustion can intensify predisposition to hypothermia. Hypothermia combined with excessive alcohol consumption is often the cause of death of socially disadvantaged individuals. Characteristic morphological findings generally seen at external and internal autopsy are bright „cherrish“ red post-mortem spots, hemorrhagic erosions of the gastric mucosa (Wischnewsky spots), rarely pancreatic hemorrhage is found.

The purpose of our work was to evaluate the connection between hypothermic death and alcohol consumption based on the findings of Institute of Forensic Medicine and Medicolegal Expertises JLF CU in Martin in last five years, as well as to determine possible ethiology of hypothermic death and predisposing factors. Based on the results of statistics we can conclude that hypothermic death is the most often accidental with increased incidence in cold months. More than 50% of victims are intoxicated and the majority of the group are men (70%). Dying of hypothermia in warm seasons is often connected to higher levels of alcohol in blood sample. Wischnewsky spots, stated as specific sign at internal autopsy, were confirmed in 76% of cases, so they are not so specific sign for dying of hypothermia as was thought before.

ASSESSMENT OF INTRACRANIAL TRAUMATIC CHANGES IN PATIENTS WITH CRANIOCEREBRAL INJURY

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INTRODUCTION: Craniocerebral injuries are major cause of death or disability. Evaluation of intracranial traumatic changes is important for determination of patients' prognosis. **AIM:** Linking specific types of intracranial traumatic changes with poor outcome with consideration of selected characteristics of patients' condition in time of their admission.

METHODS: Prospective study of patients hospitalized at Clinic of Neurosurgery since November 2014. In order to obtain a wider survey, we also evaluated documentations of patients hospitalized in 2010. The final sample of 25 patients with traumatic intracranial changes was divided into five groups according to type of their injury. Further assessment of their CT scans was made with measurement of linear parameters and volume of the lesions and evaluation of perimesencephalic cisterns patency and midline shift. Age, sex, possible consumption of alcohol, photoreaction and GCS (Glasgow Coma Scale) score of the patients and their final non-/surgical treatment were also considered. These data were compared with GCS and GOS (Glasgow Outcome Scale) score in time of patients' discharge.

RESULTS: From 25 observed patients 2 had epidural haematoma (EDH), 8 had subdural haematoma (SDH), 2 had intracranial haemorrhage (ICH), 6 overcame cerebral contusion and 7 had subdural haematoma combined with intracerebral bleeding or/and cerebral contusion. Patients with ICH, SDH, EDH and brain contusion had mostly good outcome. On the other hand, from the group of 7 patients with SDH combined with ICH and/or brain contusion 2 died (GOS 1), 3 had GOS 3 and 2 had GOS 5. This was linked with alcohol consumption, obliterated perimesencephalic cisterns and midline shift above 16 mm.

CONCLUSION: Our current findings indicate that poor outcome is associated with SDH combined with brain contusion and/or ICH, obliterated perimesencephalic cisterns and midline shift above 16mm. This conclusion is limited by the number of patients. Further observation of more patients is recommended.

INDIVIDUALIZED FORTIFICATION OF MATERNAL MILK IN PRETERM INFANTS

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There has been used individualized fortification of maternal milk for preterm infants within the last several years at the Neonatal Intensive Care Unit of Neonatology Department at the University Hospital in Martin. AIM of the study was to establish an overview of the effect of fortification of maternal milk based on individual needs of preterm infants, and to assess its effect on their growth and nutrition. METHOD: The study was designed as a pilot study with a group consisting of ten preterm infants. Data such as gestational age, anthropometric parameters, individual growth over time, duration of fortification, volume of fortification and biochemical indices (serum proteins, urea, creatinine, ALP) were assessed. RESULTS: The infant's weight increased with values between 15g/kg/day to 47,85g/kg/day with fortification. The length and head circumference increased slightly with fortification. The serum levels of urea, total protein and creatinine were in normal range during fortification. However, the serum levels of ALP were higher with fortification then without it. DISCUSSION: Preterm infants fed with fortified maternal milk receive higher intake of nutrients such as proteins, energy and minerals, leading to a greater gain of weight and linear growth. It is assumed that maternal milk with fortification provides an appropriate amount of nutrients and energy, since the infants' growth significantly improved. CONCLUSION: Individual fortification of maternal milk reduces the variability in nutritional intake and optimizes protein and energy intake. It causes adequate growth in premature infants and satisfies the specific nutritional requirements.

CARDIOPULMONARY RESUSCITATION (CPR)- ANALYSIS OF RESUSCITATION SKILLS IN SIMULATED CONDITIONS

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The aim of our work was to study the effectivity of providing CPR in simulated conditions in different age brackets of students, different genders, even correlation between CPR success rate and weight of rescuer. We perform our study on sample of 160 people (80 men, 80 women) in age from 12 to 26, where methodics of resuscitation was 5 minutes of CPR without any theoretical or practical prepare. To detect our results we used manikin Laerdal Q-CPR, in cooperation with ResusciAnne Wireless SkillReporter software and SimPad SkillReporter device.

In our results we focused on hand position accuracy during resuscitation, number and depth of compression, sufficiency of decompression, number of breaths and average tidal volume and total score of CPR. Mentioned parameters were applied also on age groups: group A (12-15y.), group B (15-19y.), group C (19-22y.) and group D (22-26y.). Study showed, that total CPR success rate in sample of 160 people was only 46,40% - in women 38,88% and in men 53,93%. The major problems were to maintain the proper position of hands during CPR-84,20%, decompressions-77,17% and depth of compressions- 43,68 mm (average 50-60mm). Surprisingly, 12,50% of rescuers did not perform a head tilt. The group results were as follows: in group A- 18,39% (women:8,53%, men:28,24%), group B 45,30% (women:42,40%, men:48,20%), group C 58,18% (women:49,25%, men:67,10%) and group D 56,70% (women:55,35%, men:58,05%). Men over 60kg had higher percentage (55,21%), than men under 60kg, except the frequency and decompressions. Both groups had similar hands position. Women over 60kg had, except decompressions, higher success rate (50,61%), than women under 60kg. The frequency of compressions was almost equal in both categories. Generally women under 60kg had lower CPR success rate.

Results of study are significant indicator of the real knowledge situation of providing CPR and reflect necessity of education in this area.

COMPLEX MANAGEMENT USING INSULIN PUMP, BOLUS ADVISOR (WIZARD) AND COMPETITION IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

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Aim: The aim of study is to prove the effect of complex management using insulin pump (IP), bolus advisor and competition on metabolic control (HbA1c) of children with type 1 diabetes mellitus (T1DM).

Material and methods: 100 children with T1DM (6-18 years) examined at Paediatric Diabetologic Centre of Martin University Hospital were enrolled to prospective 6 month study and divided to three subgroups. All subgroups were motivated by competition in two categories: the winner of the best average of HbA1c and the winner of the best improvement in HbA1c, both without severe hypoglycaemia. The first subgroup was treated by insulin pens (n=33). The setting of bolus advisor (Wizard) parameters was done in 67 children on IP, the second subgroup started bolus advisor at the time of initiation of IP therapy (n=36). In the third group the bolus advisor was set up with delay, in paediatric patients using IP previously without bolus advisor (n=31). The evaluation of HbA1c (% , DCCT) was done at beginning of study and after 6 month observation.

Results: All subgroups showed decrement in HbA1c during the study, improvement in HbA1c was observed in 79,7 % of patients. T1DM children treated with IP were able to achieve greater improvement in HbA1c (δ HbA1c -1,23 %, $p < 0,001$) comparing with insulin pen subgroup (δ HbA1c -0,9 %, $p < 0,05$). The setting of bolus advisor at the time of IP initiation was non-significantly more effective than setting with delay, both insulin pump subgroups showed significant decrement in HbA1c (δ HbA1c -1,4 %, $p < 0,001$ vs. δ HbA1c -1,2 %, $p < 0,001$). **Conclusion:** Complex management using insulin pump, bolus advisor and competition was effective in improvement of metabolic control of children with T1DM. IP children on bolus advisor were able to achieve deeper decrement in HbA1c comparing to insulin pen group. The setting of bolus advisor at the time of insulin pump initiation was slightly more effective in aspect to metabolic control of T1DM children.

ACID INFUSION INTO THE STOMACH DOES NOT AFFECT THE NUMBER OF MEAL-INDUCED TRANSIENT LOWER ESOPHAGEAL SPHINCTER RELAXATIONS (TLESR)

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TLESRs are considered to be the most important mechanism of gastroesophageal reflux (GER). However, the regulation of TLESRs by acid is incompletely understood. We have recently reported that acid in the esophagus enhanced TLESR (Neurogastroenterol Motil. 2014, 26(10):1469-76.). Specifically, we found that the acid infusion into the esophagus increased the number of meal-induced TLESRs by 60% compared to control infusion. In the present study we evaluated the effect of acid infusion into the stomach on the meal-induced TLESRs. The study was carried out in healthy subjects (age 23 ± 0.3 years). None of the subjects had any esophageal motility abnormality as defined by Chicago criteria. TLESRs were evaluated by using high resolution manometry (HRM). The study was performed in sitting position. For infusions a tube (O.D. 1mm) was attached to the HRM catheter with the opening positioned in the stomach at least 5 cm below the manometrically identified lower esophageal sphincter. Each subject was studied at two occasions (control or acid infusion) separated by at least 7 days. Following a standard meal (chicken sandwich and soda drink), acid (0.15 M HCl) or water was infused into the stomach (8ml/min, 20 min) by using a perfusion pump. TLESRs were counted during 2h following the completion of the infusion. In some subjects TLESRs were also counted during 20 min of acid infusion. The study conformed to Declaration of Helsinki. All subjects gave informed consent. 10 subjects (7M/3F) completed the study. We found that acid infusion into the stomach did not affect the number of meal-induced TLESRs. The number of TLESRs (median[interquartile range]) during 2h following the control vs. acid infusion was 17[13-17] and 14[13-16], $n=10$, $p=NS$, Wilcoxon Signed-Rank Test). The average duration of TLESRs was not changed ($16.9 \pm 0.5s$ and 16.6 ± 0.4 , $P > 0.2$, unpaired T-test). The number of TLESRs during the acid infusion was also not affected (quantified in 6 subjects, 4M/2F). The number of TLESRs during the 20 min of control vs. acid infusion was 5[5-5] vs. 4[3.25-4], $n=6$, $p=NS$, Wilcoxon Signed-Rank Test). We conclude that the acid infusion into the stomach does not affect the meal-induced TLESR. These results are consistent with the notion that the direct effects of acid in the stomach has limited role in the regulation of TLESR. Our results also indicate that the substantial enhancement of TLESR by acid infusion into the esophagus observed in our previous study was not due to acid effect in the stomach.

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POSTPARTUM BONDING

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Postpartum bonding can be defined as a direct and uninterrupted connection of the mother and the baby arising from skin-to-skin contact immediately following the delivery.

AIM: The aim of this study was to find out whether women are familiar with postpartum bonding, what their perceptions during bonding were and what experience they have with its performing in the Slovak maternity hospitals.

METHODS: The number of women who took part in the study was 2001. All the partaking women (age $29,96 \pm 5,34$) had a spontaneous delivery in the time period of the last three years. To collect data, a questionnaire created for the purposes of this study was used. The data were evaluated using descriptive statistics and the Chi-square test of independence. RESULTS: It was discovered that the majority of women had been informed about bonding (48,43 % sufficiently and 41,88 % partially). Most of the women desired bonding (93,90 %) and had sufficient strength (82,86 %) to be in contact with their baby immediately after delivery. It was discovered that postpartum bonding is not promoted sufficiently in Slovak maternity hospitals. The influence on the women's awareness of bonding before delivery was deemed statistically significant in the case of age ($p < 0.002$), education ($p < 0.000005$), and the fact whether the child was planned ($p < 0.002$). The presence of a close person exercised a statistically significant influence on the performance of postpartum bonding ($p < 0.000005$). CONCLUSION: Willingness and understanding of postpartum bonding on the part of midwives is important for the promotion of bonding, so that it becomes one of the priorities in the care for the mother and the baby. By promoting bonding, the midwife facilitates physiological, immunological and psychological adaptation of the baby and the mother and lays the foundations of their attachment.

EFFECT OF ANTENATAL EDUCATION ON COURSE OF LABOR

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Introduction: Several studies, carried out in recent years, have identified positive links between antenatal education and higher rate of vaginal birth, shorter duration of the first and second stage of labour, identification of active labour as well as decreased anxiety. Aim of our study was to determine the effectiveness of antenatal education to influence duration and course of the first stage of labour as well as pain management of women.

Materials and Methods: 83 low risk primiparas recruited to the study were assigned into two groups: 27 women attending antenatal education sessions and 56 with no antenatal education. Data were obtained through observation sheet containing basic information of the mother, the course of pregnancy and childbirth.

Results: The study indicates positive impact of antenatal education on the length of the first stage of labour, which is in case of women who passed it shortening. Pain intensity during childbirth expressed by primiparas who were attending antenatal education was 2 degrees lower than the pain intensity expressed by the ones who did not attend it. Primiparas without antenatal education needed to be educated in 69,6% of cases, whilst women with antenatal education only in 40,7%. Right usage of abdominal press during childbirth was 70,4% of cases for women without antenatal education and 81,5% for the women who passed antenatal education. Similar results appeared in the study of proper breathing during a childbirth, showing that 96,3% of antenatally educated women and only 73,2% of women without antenatal education breathed correctly.

Conclusion: This study identified positive effect of antenatal education on duration and course of the first stage of labour as well as pain management of women. However, further research is required to explore the impact of antenatal education of women regarding outcomes and course of labour.

ATTITUDES AND KNOWLEDGE OF STUDENTS OF DIFFERENT STUDY PROGRAMS IN JLM CU ABOUT HAND HYGIENE

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INTRODUCTION: Aim of this study was to evaluate the perception of hand hygiene in students of JLF UK and the comparison of results influenced by their knowledge of the way of hygienical hand washing technique. This method is used in medical professions. We were interested in differences in knowledge and perception between students of four study programs in JLF UK. **METHODS:** We used the questionnaire with 10 questions dealing with topic of interest (knowledge of hand washing, technique of hand washing and their influence to transmit bacteria). Sample consisted of 70 students of the 3rd year's of study programs and included: 24 students of general medicine program, 24 students of nursing, 10 students of midwifery and 12 students of public health. **RESULTS:** According to practical knowledge of hygienical hand washing technique the students formed two groups: better knowledge (67%) and worse knowledge (33%). Practical knowledge of hygienical hand washing did not significantly correlate with the perception of hand washing and its influence to transmit bacteria in the majority of cases. According to study programs the biggest differences were in questions about the common use of alcohol-based hand washing products (nursing vs. public health; 54,2% vs. 16,7% resp.). Approximately one out of five students admitted that they do not perform hand hygiene in the cases they think they should. **CONSLUSION:** The perception of hand hygiene does not have relation with practical hand washing technique. Even when hand hygiene is one of the most important topic of education in all study programs at JLF UK, we found differences in attitudes to the topic between them.

VALIDATION OF NURSING DIAGNOSIS ACTIVITY INTOLERANCE

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The name of the nursing diagnosis of Activity Intolerance may suggest problems of the musculoskeletal system and can thus easily be confused with the diagnosis Impaired Physical Mobility. Though both diagnoses are characterized by a reduction in physical activity by exercise-induced dyspnea, the clinical findings can be very similar, etiology and pathogenesis leading to this defining characteristic are different. The key factor in distinguishing of these diagnoses is identifying defining characteristics, based on specific symptoms of the cardiac diseases and justify its inclusion into class Cardio-Pulmonary Responses. AIM: Content validation of nursing diagnosis Activity Intolerance in patients with cardiac disease and the identification of the defining characteristics in the conditions of Slovak clinical practice. METHODS: For content validation, we used Fehring's Diagnostic Content Validity Model. Worksheet contained 8 defining characteristics of nursing diagnosis according to NANDA-I Taxonomy II, 5 symptoms of NYHA-FC and 2 neutral characteristics. The sample of respondents was 53 nurses working in 8 clinical departments at the Martin University Hospital. Conditions for selection of nurses-experts according to modified Fehring's criteria (min. 4 pts, max. 17 pts) met 47 nurses (average score was 6.49). RESULTS: The main defining characteristics achieved value of weighted score (WS) more than 0.75. Experts thus identified only one item - stenocardia (WS 0.77), which is symptom of NYHA. Secondary defining characteristics achieved WS value 0.5-0.75 and experts thus identified 12 of 15 items. 2 items with value of WS less than 0.5 were discarded. CONCLUSION: Experts did not consider any item of NANDA-I Taxonomy II belonging to the defining characteristics of the diagnosis to be very important. This can be influenced by an incompatibility of NANDA-I Taxonomy II with a List of nursing diagnoses, absence of standardized nursing terminology and low average experts criteria level.

NURSING CARE OF PATIENTS WITH CUTANEOUS STOMA COMPLICATION

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Stoma complications can be described as an undesirable condition or circumstances that make it difficult to adapt to the new conditions of life with a stoma, whether in early or late season. The problematic of stoma complications strikes physical as well as psychosocial area of Stomics life. GOAL: The goal of our study was to assess, on the basis of subjective assessment of the respondents, which interventions the nurse realizes in case of occurrence of cutaneous stoma complications, whether the nurses in the clinical practice are using DET score evaluation during healing of skin complications and the realization of which nursing interventions for patients with stoma skin complications is the most problematic in practice. METHODOLOGY: For obtaining empiric data was used method of non-standardized self-made questionnaire. The questionnaire was divided into subscales, depending on the type of skin stoma complications. Individual items were evaluated on Likert scale from 1 to 5 (1-never, 2-very few, 3-sometimes, 4-almost always, 5-always). The respondents were nurses working in surgical fields to standard treatment unit and ICU septic surgery at UNM in Martin, UVN SNP Ruzomberok and in FNTN in Trencin (n = 70). RESULTS: Based on the analysis of the obtained data, we found that nurses in practice are not using DET score evaluation during the healing of skin complications, or any other referral tools; the most frequent skin complication is irritant dermatitis and maceration; further we have found that the type of skin complications influences the selection of stoma aids; and that the most independent nursing interventions is implemented by nurse during the occurrence of folliculitis. CONCLUSION: The outlet of stoma is radical surgery, which brings many problems. Therefore, it is more necessary to pay attention to the treatment of cutaneous stoma complications. Nurses in the practice should use evaluation tools and realize nursing interventions recommended in guidelines based on EBN and EBP.

MAP OF NURSING CARE (PATIENT AFTER RENAL TRANSPLANTATION)

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Transplantation is the process by which an organ is transferred from the body of a donor to the body of a recipient for the purpose of restoring certain functions of the human body. The basic goal is to save life or substantial improvement in the quality of life of the patient. In order to raise the quality of nursing care were maps of nursing care implemented into clinical practice. Maps are prepared and scheduled for each day of care, and are intended for precisely defined group of patients in our case for renal transplant patients. AIM: To find out which specific categories and nursing interventions should be included in nursing care map for patient after renal transplantation. METHODS: We have worked out the map of nursing care for 5 postoperative days, in which the data were collected from the scientific literature. Map of nursing care included nursing interventions, diagnostic tests (laboratory tests), diagnostic, treatments, procedures and interventions/ procedures, panels, consulting, medical therapy, exercise regime, diet, education of patient/ family, planned release, expected results of care. Then the report was given to 5 doctors and 15 nurses from the Department of Surgery and Transplant center involved in providing of care to patients after renal transplantation. RESULTS: We have found that the largest deviations were interventions in the category of "state of consciousness", "breathing" namely, monitoring of the renewal of cough reflex and the amount and character of expectorated sputum and dependent interventions, especially in the category of diagnostic tests (laboratory tests) and medical therapy. CONCLUSION: Map of nursing care was adjusted according to the observations of doctors and nurses from the Department of Surgery and Transplant center involved in providing of care to patients after renal transplantation and was subsequently allowed for the workplace. Map of nursing care is proposed to provide information to new nurses and as a teaching material for students.

ROLE OF TRPV4 CHANNEL IN PATHOGENESIS OF COUGH IN HDM SENSITIZED GUINEA PIGS

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Background: TRPV4 is calcium channel, which was initially described as osmolarity-sensitive channel. In the airways, it can play indirect role in increasing cough reflex sensitivity via regulation of endothelial permeability, airway muscle contraction and mucocilliary transport.

Methods: Cough response was measured in 10 guinea pigs (Dunkin-Hartley, males) by inhalation of saline, hypotonic solution (distilled water), hypertonic solution (1100 mOsmol/L NaCl) and citric acid (0.4 M) for 10 minutes in whole body plethysmograph. Simultaneous recording of airflow and audio from head chamber was performed. These guinea pigs were sensitized by inhalation of 0.5% aerosol of house dust mites (Greer Labs, USA) for 5 minutes per day for 7 days. Successful sensitization was confirmed by skin prick test (intradermal application of 15 μ L of 0.5% HDM). Cough responses in animals with positive prick test were measured in the same way as previously described 15 minutes after intranasal application of 15 μ L of 0.5% HDM.

Results: Cough response in healthy animals to hypotonic solution was significantly higher than to saline (1.7 ± 0.56 vs 4.50 ± 1.12 ; $P=0.003$) and no significant changes were observed when using hypertonic solution (1.7 ± 0.56 vs 1.4 ± 0.60 ; $P=0.171$). There were no significant changes observed in cough latency. In HDM-sensitized animals, cough responses were significantly higher both to hypotonic (1.9 ± 0.69 vs 5.4 ± 0.83 ; $P<0.001$) and hypertonic (1.9 ± 0.69 vs 5 ± 0.93 ; $P=0.015$) solutions when compared to saline. Similar changes were observed in cough latency (saline vs hypotonic vs hypertonic solution 377.8 ± 69.92 s vs 60.3 ± 16.88 s vs 167.6 ± 47.6 s).

Conclusion: Results show at least indirect involvement of osmolarity of pericilliary fluid in increased sensitivity of cough reflex, however, exact mechanisms remain to be elucidated – next step will be repeating these challenges with pre-treatment by TRPV4 antagonist GSK2193874 in near future.

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MOVEMENT OF TRACHEAL CILIA IN AN ANIMAL EXPERIMENT

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Background: Research suggests that some inflammatory stimuli may alter airway ion transport and thus mucociliary clearance in which cilia represent a driving force.

Aim: The aim of our study was to verify whether potassium ion channels involve in controlling of movement frequency of respiratory cilia (CBF) in guinea pigs, which are experimentally made hyperresponsive by the allergen, ovalbumine. The potential increase of ciliary movement by drugs would make life easier for asthmatic patients.

Methods: Allergen sensitization was performed using standardized procedures. Agonists/antagonists of either KATP - ATP-sensitive K⁺ channels or BKCa²⁺-calcium activated potassium channels were used with three different concentrations (10⁻⁵, 10⁻⁶ and 10⁻⁷ M). They were added directly to the biological material obtained from a cytological brush. The ATP-sensitive agonist used was PINACIDIL, and the antagonist was Glibenclamide. The calcium sensitive agonist used was NS1619, and the antagonist was TEA (tetraethylammonium chloride). The movement of cilia were examine by microscope equipped with high speed camera and special software developed for measurement of CBF.

Results: The results show that the CBF is significantly increased by PINACIDIL in all three concentrations, and reversed in the presence of Glibenclamide, when administered during natural non allergic condition. In allergic condition, the agonist of KATP channel, PINACIDIL, has no positive influence on ciliary beating because of CBF suppression.

Presented data confirmed that the agonist and antagonist of BKCa²⁺ channels have no significant influence in natural condition, but the agonist NS1619 produces significant increase in allergic condition when administered with the highest concentration (10⁻⁵ M).

Conclusion: The results presented here suggest that the BKCa²⁺ channel agonist NS1619 has the potential to have a therapeutic effect on asthmatic patients in the future.

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INFLUENCE OF ROFLUMILAST AND TADALAFIL ON APOPTOSIS IN OVALBUMIN - SENSITIZED GUINEA PIGS

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Bronchial asthma (BA) is a chronic inflammatory disease of the airways characterized by bronchial hyperresponsiveness and a variable degree of airway obstruction. Increased persistence of eosinophils in the lungs of patients with BA is a consequence of inhibition and defects in the apoptotic process. Inhibition of phosphodiesterases (PDE) leads to bronchodilating and anti-inflammatory effects and also influences apoptosis of immune cells. In chronic obstructive pulmonary disease, roflumilast, a selective PDE4 inhibitor, has been recently approved for pharmacotherapy. Highly selective PDE5 inhibitor tadalafil was used to compare ability to induce the apoptotic process in inflammatory cells. AIM: To evaluate the effects of long-term administration of roflumilast on in vivo and in vitro airway reactivity and on apoptosis of white blood cells (WBC) in experimental allergic inflammation. METHODS: We used male adult guinea pigs and divided them into 4 experimental groups and sensitized with ovalbumin for 14 days and thereafter treated with roflumilast, tadalafil or vehicle with the same dose 1.0 mg/kg for 7 days. Then we assessed in vivo airway reactivity using whole-body plethysmograph and in vitro airway reactivity using organ baths. We also estimated differential counts of WBC in blood and bronchoalveolar lavage fluid (BALF). ELISA assay was used for evaluation of interleukin (IL)-4, IL-5 and platelet activating factor (PAF) levels in blood and lung homogenate (LH) and activity of caspase-3 and annexin-5 in LH. An automatic cell counter was used to estimate the cell viability and the total cell count in BALF. RESULTS: Tadalafil led to the most significant suppression of in vivo and in vitro airway reactivity, with viability reduction of WBC in blood as well as in apoptosis induction. Roflumilast was more effective in decreasing inflammation markers in LH. CONCLUSION: Selective inhibition of PDE4 and PDE5 seems to be potentially useful in BA therapy.

EFFECT OF INHALATION β 2-SYMPATHOMIMETICS AND GLUCOCORTICIDS ON DEFENCE AIRWAYS MECHANISMS

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Combination of inhalation glucocorticoids and β 2-sympathomimetics is considered to be the most effective therapy of moderate and severe asthma. We studied the effect of combined therapy on defence airways mechanisms (cough, bronchoconstriction, mucociliary clearance) during the single and long-term (21-days) administration of substances. Defence mechanisms were evaluated in conditions of experimentally induced allergic asthma on the model of guinea pig airways hyperreactivity induced by 21-days ovalbumin administration. The experimental animals were treated with a single inhalation of budesonide ($7 \times 10^{-3}M$), salbutamol ($4 \times 10^{-3}M$) and half-dose combination of these substances; or with a 21-days therapy by budesonide ($10^{-3}M$), salmeterol ($1,7 \times 10^{-4}M$) or their half-dose combination. Antitussive and bronchodilatory effects were measured by in vivo method in double chambered body-plethysmograph. Mucociliary clearance was studied by in vitro brushing method. Our results showed that single administration of substances did not have antitussive effect, bronchodilatory effect was proven only after salbutamol inhalation. Salbutamol increased the ciliary movement frequency while budesonide and its combination with salbutamol lead to diminution of ciliary movement frequency. The results of long-term administration of all 3 therapeutic substances demonstrated diminution of the airways smooth muscle reactivity, decreased the parameters of chemically induced cough reflex and increased ciliary movement frequency. We can summarize that acute combined therapy did not increase antitussive and bronchodilatory activity of substances administered separately and did not reverse the negative impact of budesonide on ciliary movement frequency. In contrast, long-term combined therapy was more effective than monotherapies and had additive and in some cases even synergic effect.

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THE EFFECT OF FEMALE SEXUAL HORMONES ON COUGH REFLEX SENSITIVITY

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Background: From the literature it is known that females cough more than males (Fujimura, 1990) and have heightened cough reflex sensitivity (Dicpinigaitis & Rauf, 1998). The basis of these gender differences has not been elucidated yet, although they are believed to be caused by hormones as they occur at pubertal age and are highlighted in late adolescence (Varechova et al., 2006). Chronic cough patients are also more often females, usually experiencing premenstrual worsening.

Aim: The aim of our study was to determine the effect of female sexual hormones on cough reflex sensitivity, urge to cough, sound of voluntary and induced coughs, laryngeal sensitivity and other parameters in healthy volunteers with normal menstrual cycle (MC) and females taking oral contraceptives (OC).

Methods: 11 healthy females with normal MC and 5 females taking OC, who consented to participate in our study, underwent ENT exam and lung function test to meet the inclusion criteria. Cough sensitivity and other parameters were examined in follicular and luteal phase of MC according to ERS Guidelines (2007). Level of oestrogen, progesterone, testosterone, prolactin, LH and FSH were determined from blood samples and the phase of MC was confirmed by USG.

Results: Cough sensitivity expressed as C2 and C5 increased significantly in luteal phase from 91.2 ± 1.75 to 58.7 ± 1.59 (GM and 95%CI) for C2 and 364.9 ± 1.90 to 250.0 ± 2.12 (GM and 95%CI) for C5, $p < 0.05$ for both. FeNO levels and FEV1/FVC also follow the cyclic pattern in normal menstrual cycle group. Correlation analysis showed negative correlation of cough threshold with oestrogen levels in follicular phase ($r = -0.44$) and also negative correlation between cough threshold and progesterone in luteal phase ($r = -0.19$).

Conclusion: Our data indicate that premenstrual worsening of chronic cough could be caused by hormonally influenced rise of TRP channels activity.

VAGAL AFFERENT NEURONS INNERVATING THE GUINEA PIG STOMACH

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Gastric afferent nerves regulate the function and mediate perceptions from the stomach. Perturbation of these nerves is thought to contribute to pathophysiology of functional disorders of the gut. Previously, we found that the vagal afferent innervation of stomach in the rat is exclusively derived from the nodose portion of vagal ganglia. Here we addressed the hypothesis based on preliminary data that in the guinea pig both nodose and jugular ganglia neurons innervate the stomach. METHODS: Vagal afferent neurons innervating the stomach were retrogradely traced by using Dil injections (3-5) into the ventral wall of the stomach (n=7). Vagal jugular and nodose ganglia from both sides were isolated and fixed. The whole mounts of ganglia were evaluated for Dil-positive neuronal profiles under fluorescent microscope. In 4 animals cryostat sections (12 μ m thick) of vagal ganglia were obtained and the Dil-positive neuronal profiles were counted in sections. RESULTS: Dil in all animals was restricted to the stomach indicating selective labeling of gastric neurons. The evaluation of whole mount ganglia suggested that jugular ganglia contribute little to vagal gastric innervation (~17%). However, the evaluation of sections from vagal ganglia revealed larger proportion. We focused on the left vagal ganglia known to innervate ventral part of the stomach in many species. We found 106 ± 25 and 236 ± 29 Dil-positive neuronal profiles in the left jugular and nodose ganglia, respectively (n=4), showing that $30 \pm 5\%$ of vagal gastric innervation originates from jugular ganglia. We also noted a tendency for more labeled jugular neurons when the Dil injections were located more proximally. CONCLUSIONS: In contrast to the rat, jugular neurons substantially (~30%) contribute to vagal afferent innervation of the stomach in the guinea pig. The species differences need to be considered when selecting appropriate model for the study of specific questions pertinent to gastric innervation.

NATURAL SUBSTANCES IN THE PREVENTION OF EXPERIMENTAL BREAST CANCER

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In our previous study, resveratrol administered alone did not show any tumour-suppressive activities in rat mammary carcinogenesis. However, it is supposed that mixture of phytochemicals with plethora biological activities, present in whole foods, may have additive or synergistic effects against carcinogenesis. The anti-tumour effects of fruit peel polyphenols (Flavin7) in the chemoprevention of N-methyl-N-nitrosourea induced mammary carcinogenesis in female rats were evaluated in this study. Lyophilized flavin was dietary administered at two concentrations of 0.3 % and 3 %. The experiment was terminated 14 weeks after carcinogen administration; mammary tumours were removed and prepared for histopathological and immunohistochemical analysis.

Flavin at higher dose significantly suppressed tumour frequency by 58 % and tumour incidence by 24 %, and lengthened latency by 8 days in comparison with the control rats. Histopathological analysis of rat tumours showed significant decrease in the ratio of high/low grade carcinomas after flavin treatment. Immunohistochemical analysis of rat carcinoma cells in vivo found significant pro-apoptotic, antiproliferative, and anti-angiogenic changes in the group with higher dose of flavin. Flavin in both doses demonstrated significant positive effects on plasma lipid metabolism in rats. In parallel in vitro study, flavin significantly decreased survival of MCF-7 cells in MTT assay by dose- and time-dependent manner compared with the control.

Our results pointed to substantial tumour-suppressive effect of the fruit polyphenols` mixture from Flavin7 in the breast cancer model. We suppose that additive or synergistic effects of phytochemicals present in fruits were responsible for observed potent anti-cancer activities.

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THE EVALUATION OF ANTI-TUMOUR ACTIVITY OF OREGANO IN THE MODEL OF BREAST CANCER

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In clinical and preclinical research, there has been considerable interest about the role of phytochemicals in the risk reduction of cancer disease in humans. In this experiment, the antineoplastic effects of *Origanum vulgare* L., phylloma in the prevention of N-methyl-N-nitrosourea-induced mammary carcinogenesis in female rats were evaluated. Dried oregano leaves were dietary administered at two concentrations of 0.3 % and 3 %. The experiment was terminated 13 weeks after carcinogen administration. At autopsy, mammary tumours were removed and prepared for histopathological and immunohistochemical analysis. Basic parameters of experimental carcinogenesis, parameters of oxidative damage, and chosen metabolic variables, after long-term chlorella treatment in animals were assessed. The mechanism of action (biomarkers of apoptosis, proliferation, and angiogenesis) is under evaluation. Oregano at lower concentration significantly suppressed tumour frequency by 56 %, tumour incidence by 44 %, and decreased tumour volume by 45 % in comparison with the controls. Oregano at higher dose significantly shortened the latency period by 12.5 days compared to controls. Histopathological analysis of rat tumours showed significant decrease in the ratio of high/low grade carcinomas in both treated groups. The lower and higher dose of oregano in the diet yielded the tendency of decreased dityrosine levels (by 10 and 11 %) when compared to control. Oregano supplementation in diet had no effect on level of conjugates of lysine with the products of lipid peroxidation. This study is the first report on the antineoplastic effects of oregano in experimental breast cancer in vivo.

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PRIMARY NODAL “DOUBLE HIT” DIFFUSE LARGE B- CELL LYMPHOMA (DH DLBCL) IMITATING PRIMARY DLBCL of CNS (PCNSL) – A CASE REPORT

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DLBCL represents a potentially curable neoplasia, therefore it is important to identify its subtypes showing worse prognosis, incl. DH DLBCL (characterized by simultaneous MYC and either BCL2 or BCL6 translocations) and PCNSL. AIM of the presented case is to demonstrate the complexity of these analyses, which necessarily have to be supplemented by sufficient clinical data. METHODS and RESULTS: case report of two biopsies of a CNS lymphoma patient. The first one, in 2009, was referred to as intraspinal tumor causing lumbar vertebrae fracture. Large neoplastic blasts showed immunohistochemically s.c. non-GCB DLBCL phenotype with very high proliferation index, which allowed the diagnosis of spinal cord DLBCL. In 2011, the second biopsy of a cerebral basal ganglia tumor showed identical morphology and similar phenotype, with acquisition of bcl2 protein positivity – the tumor represented a relapse of CNS DLBCL phenotypically resembling PCNSL. Retrospectively obtained informations showed, that the disease was disseminated (IV. stage) at 1st biopsy, the patient absolved intensive chemotherapy and in 2010 autologous stem cell transplantation leading to complete remission up until relapse in 2011 and patient's death 2 months following the relaps. The biopsies were retrospectively analyzed by fluorescence in-situ hybridization (FISH) techniques. The first one showed break of MYC and BCL6 5' locus deletion and IGH/MYC gene fusion, the second one an identical BCL6 deletion and IGH rearrangement, without rearrangements of BCL2 and MYC loci. CONCLUSIONS: The tumor originally evaluated as a PCNSL represented a disseminated DH DLBCL (associated usually with resistance to standard chemotherapy) with CNS involvement and associated with a poor prognosis in spite of an intensive therapy and bone marrow transplantation. The final diagnosis represents a puzzle requiring combination of all available data.

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DO FOLLICULAR LYMPHOMAS OF THE LARGE SALIVARY GLANDS ARISE IN THE GLANDULAR TISSUE?

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Primary malignant lymphomas (ML) of salivary glands are rare; they include most common ML of MALT type, followed by follicular (FL) and diffuse large B-cell lymphomas (DLBCL). While MALT-type ML emerge from lymphocytes accumulated due to a chronic salivary gland tissue inflammation, the pathogenesis of others remains obscure. For FL, a possibility of their origin in the intrasalivary gland lymph nodes (ISLN) was discussed, but a secondary gland's infiltration by systemic FL has to be considered. AIM: We focused on salivary gland FL to analyze their infiltrates in ISLN versus salivary gland compartments together with signs of its inflammatory changes. METHODS: From 105 salivary glands ML diagnosed during Jan 2004 - Feb 2015 period, we selected a series of FL and re-evaluated them according to the WHO classification 2008. For analyses, we used a standard panel of histological and immunohistochemical stainings. In some of cases, also the results of BCL2 and IGH gene rearrangements from FISH examinations were available. In all the cases: a) the presence of FL infiltrates in the glandular versus intraglandular lymphonodal tissue together with signs of glandular diffuse chronic inflammation was recorded, and b) all available data were evaluated to distinguish systemic versus primary salivary FL. RESULTS: While 4 cases of our series represent secondary involvement, 16 fulfill criterias of a primary salivary FL; at least in 7/16 the lymphoma appeared only in ISLN. In none of the cases diffuse chronic inflammatory changes could be recognized. CONCLUSION: FL arises from the germinal center cells of lymphoid follicles. As the lymphoid tissue is ubiquitous, the follicles might arise by chronic inflammation also extranodally. However, dominant features of FL cases of our series offer more pros than cons for the hypothesis, that a primary salivary gland FL arises in the ISLN and not in the glandular tissue.

EVALUATION OF HUMAN PAPILLOMAVIRUS GENOTYPING USING INTERNET DATABASES

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Cervical cancer is caused by persistent high-risk human papillomavirus (HPV) infection that represents an increased risk for the development of cervical lesions and carcinoma. HPV infection can be easily detected in cytological specimens, therefore, more frequent monitoring of suspicious cervical lesions in compulsory intervals and HPV DNA test are a part of a screening test. AIM: To detect HPV infection in cervical specimens; identify HPV genotypes by dideoxysequencing and analyze sequences in internet databases. METHODS: The presence of HPV genotypes was investigated in 60 cervical specimens, including 27 LSIL (Low-grade Squamous Intraepithelial Lesions), 21 HSIL (High-grade Squamous Intraepithelial Lesions), 4 cervical carcinomas and 8 samples without cervical lesion. HPV L1 specific DNA sequence was amplified in PCR and analysed by dideoxysequencing. The sequences were evaluated in internet databases. RESULTS: HPV infection was present in 63.5% (33/52) of women with cervical lesion and in 12.5% (1/8) of normal cervical epithelium. We detected HPV in 59.3% (16/27) of LSIL, 71.4% (15/21) of HSIL and 100% (4/4) cervical carcinomas. Dideoxysequencing was shown to be an appropriate tool for distinguishing HPV genotypes. Majority of HPV positive cervical lesions were infected by HPV16 (in 78.8% (26/33) cases); other high-risk HPV genotypes 18, 31, 39, 52, 58 or 66 were present and co-infection with HPV16 and other subtypes (HPV39, 6, 45) were detected in 3 cases. One case of infection with HPV83 was detected in normal epithelium, representing an intermediate risk for the development of cancer which may be cleared by the immune system. CONCLUSION: Infection by high-risk HPV genotypes produces significantly increased risk for the development of cervical lesions or cancer and women at greater risk of lesion progression could be also identified by the detection and genotypisation of HPV infection by dideoxysequencing.

SELECTIVE PROGESTERONE RECEPTOR MODULATORS (SPRMs) AND CONSERVATIVE MANAGEMENT OF UTERINE FIBROIDS IN PERIMENOPAUSAL WOMEN

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Uterine fibroids are hormone dependent tumors, with over-expression of estrogen and progesterone receptors as compared to normal myometrium. Estrogens stimulate the proliferation activity of myometrial cells by activation of DNA and protein synthesis, progesterone initiates the growth of fibroids by "up" regulation of epidermal growth factor (EGF) and the Bcl-2 gene and "down" regulation of the gene for tumor necrosis factor (TNF). Selective progesterone receptor modulators are a new group of PR ligands. The effect depends on the abundance (expression) of co-activators and co-repressors in the target tissue (tissue-specific) and stimulate apoptosis in myoma cells but not in cells of the myometrium (cell-specific). AIM: compare the effect of treatment with ulipristal acetate to reduce the size of myoma, hemoglobin levels and frequency of adverse events compared to treatment with GnRH analogues. METHODS: The study included 352 women with symptomatic fibroids in the perimenopausal age (45-50 years), randomized into two subgroups. The first group takes three months ulipristal acetate daily at dose of 5 mg, the second one received leuprorelin acetate 3.75 mg monthly intramuscularly in three portions. Onset of amenorrhoe, Hb levels, myoma size and frequency of side effects before have been evaluated before and after week 13th of therapy. RESULTS: Median times to amenorrhoea were 5 days for patients receiving ulipristal acetate, and 20 days for those receiving leuprorelin acetate. Moderate-to-severe adverse events reported for 5 % of patients receiving ulipristal acetate, and for 37% of those receiving leuprolide acetate The median changes in total fibroid volume were -27% versus -14% CONCLUSION: Ulipristal acetate treatment for 13 weeks effectively controlled bleeding due to uterine fibroids, significantly reduced the size of the fibroids and was allocated with less adverse effects in comparison with GnRH therapy.

ACUTE LEUKAEMIA IN CHILDREN

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Introduction: 26000 new cancer cases are registered in Norway each year, and 150 of this are affecting children (0,6%) <15 years old. Acute leukaemia is the most common of all childhood cancer and accounts for 31% of all cancers in children.

We have two main types of acute leukaemia in children. The most common is acute lymphoblastic leukaemia (ALL) affecting the lymphoid lineage, and more rare acute myeloid leukaemia (AML) affecting the myeloid lineage.

Material and methods: study of literature and my own case record from the period when I was treated for acute lymphoblastic leukaemia. The aim is to see how the course of treatment is from the admission to the hospital until the last check up. And also see how a child will react to the treatment and also side effects later in life.

Results: From the literature and my case record we can see that children tolerate though treatment quiet well. Due to rapid diagnostic and the possibility to give high doses chemotherapy the overall prognosis is very good.

Conclusion: Today Acute leukaemias of paediatric patients have really favourable prognosis. The overall survival rate for ALL is >80% and for AML 65%. So the results are good, but there is still a long way to go before we can be satisfied. Up to date we do not have follow up program for children treated for acute leukaemia after the age of 18 years (neither in Norway, nor in Slovakia). So maybe this is the future – follow up patients also as adults to be more aware of late effects that can arise many years after treatment.

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