

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



XXXVII. STUDENT SCIENTIFIC CONFERENCE

PROGRAM and ABSTRACTS

April 27, 2016

Martin, SLOVAK REPUBLIC

XXXVII. Student Scientific Conference
Jessenius Faculty of Medicine in Martin
Comenius University in Bratislava

The conference is supported by Dr. Jozef Lettrich Foundation

Dr. Jozef Lettrich Foundation

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MARTIN

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PROGRAM AT GLANCE

Date: April 27, 2016

Place: Aula A Novomeského 9, Martin
Aula B Novomeského 9, Martin

Registration:

April 27, 2016 - 7.30
 - before the beginning of your section

Opening ceremony (Aula A – Novomeského 9) **08.00 – 08.10**

Aula A

A1: Section of Preclinical Disciplines 08.20 – 10.10

- coffee break

A2: Section of Clinical Disciplines 10.45 – 12.45

Aula B

B1: Section of Theoretical Disciplines 08.15 – 10.15

- coffee break

B2: Section of Non-Medical Study Programmes 10.25 – 11.50

- coffee break

B3: Section of Nursing 12.00 – 13.15

Closing ceremony (Aula A – Novomeského 9) **13.30**

Duration of lectures: 8 minutes, discussion – 4 minutes

Language: Slovak, Czech or English

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Pavol Mikolka²**

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Department of Pathophysiology, JFM CU, Martin

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AWARDING AND CLOSING CEREMONY

(13:30)

NOVOMESKÉHO 9, AULA A

ABSTRACTS

All abstracts are available in English at <http://www.jfmed.uniba.sk/veda/svoc/>

EFFECT OF EXPERIMENTAL ACUTE LUNG INJURY ON SYSTEMIC OXIDATIVE MODIFICATION

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Introduction: Experimental acute lung injury (ALI) is serious disorder characterized by diffuse alveolar injury, surfactant dysfunction and triggering of inflammatory and oxidative pathways especially in the lungs. At present, mechanisms of distant organ-systems injury during ALI are not fully understood. Thereby, the study focuses on heart, liver and plasma oxidative modifications in experimental ALI models.

Material and methods: The experiments were performed on adult rabbits (New Zealand white) in two models of acute lung injury (ALI): 1) ALI elicited by repeated lung lavage (Lav-ALI) with saline (volume dose of 30 ml/kg) with healthy non-lavaged and non-ventilated animals serving as controls (C-NoLavNoVent); 2) meconium-induced lung injury (Mec-ALI) with intratracheal (*i.t.*) instillation of neonatal meconium (25 mg/ml, 4 ml/kg) with ventilated animals instilled with saline *i.t.* serving as controls (C-NoMec). All ventilated groups received conventional ventilation with oxygen for additional 5 hours. After sacrificing animals, levels of 3-nitrotyrosine (3-NT) and thiobarbituric acid reactive substances (TBARS) in heart, liver tissue and plasma were measured. Statistical analysis was performed using Mann-Whitney U test.

Results: In animals within Lav-ALI group, values of 3-NT in heart ($p=0.0286$) and plasma ($p=0.0033$), and TBARS in heart ($p=0.0381$) and plasma ($p=0.0081$) were significantly higher compared to C-NoLavNoVent group. In Mec-ALI model, levels of 3-NT in liver ($p=0.0286$) and plasma ($p=0.0159$), and TBARS in liver ($p=0.0286$), heart ($p=0.0317$) and plasma ($p=0.0087$) significantly increased in comparison to ventilated animals without meconium (C-NoMec group).

Conclusion: Increasing levels of 3-NT and TBARS in organs other than lungs demonstrate that systemic oxidative damage occurs in acute lung injury. Oxidative stress seems to be one of the pathways involving in the remote organ damage.

Supported by: Vega 1/0305/14; APVV-0435-11.

CILIA MOTION ANALYSIS IN THE AIRWAYS

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Introduction

Bronchoscopy is a key investigation in the assessment of children with chronic respiratory disorders. It also allows evaluation of the tracheobronchial anatomy and dynamics. In children, usually bronchoscopy needs to be performed under intravenous anesthesia and assisted ventilation.

The aim of our study is to verify whether anesthetic drugs stimulate or inhibit the movement frequency of respiratory cilia in guinea pigs. A potential confirmation would suggest increased likelihood of false positive or negative diagnostic results caused by the anesthetic drugs used in the respiratory sampling process.

Material and Methods

The number of animals used in the experiment was 80. Animals were randomly divided in 20 groups, from which 10 were pretreated intraperitoneally with midazolam 0.1 mg/kg. The following drugs were used with different concentrations (Propofol 1% - 10^{-2} – 10^{-6} M; Sufentanyl – 10^{-5} - 10^{-8} M). They were added directly to the biological material obtained from a cytological brush. The movement of cilia were examined by microscope equipped with high speed camera and special software developed for measurement of ciliary movement frequency.

Results

The results show that the beat frequency of cilia is unaffected by Propofol alone, even when Propofol is combined with Midazolam. Sufentanyl has similar effect with no significant decrease in the ciliary beat frequency when administered alone. Acute pretreatment with Midazolam caused significant reduction in the ciliary beat frequency when Sufentanyl was added.

Conclusion

Our results may suggest that the anesthesia for bronchoscopy with combination Midazolam/Sufentanyl therapy may significantly decrease the ciliary beating frequency with false positive diagnosis of primary ciliary dyskinesia.

Further experiments are necessary to determine optimal drug combination without cilio-depressive response to anesthetics.

Acknowledgement: This research was supported by the grant projects VEGA 1/0165/14; „Measurement of Respiratory Epithelium Cilium Kinematics“, BioMed Martin (ITMS 26220220187).

HOUSE DUST MITES INDUCED MODEL OF ALLERGIC AIRWAY INFLAMMATION

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Department of Pathophysiology finalized a project devoted to development and validation of new model suitable for cough research using the most common, perennial indoor allergen, which is house dust mite (HDM). In this abstract we present the most reliable trial.

10 male guinea pigs (Dunkin-Hartley) were used as controls, 10 guinea pigs were sensitized by i.p. administration of ovalbumin and 10 guinea pigs were sensitized by inhalation of 0.25% of HDM aerosol in 7 days followed by inhalation of 0.5% of HDM in same protocol. Sensitization was confirmed by prick tests. In sensitized animals, nasal symptoms, cough response and airway resistance were measured. Inflammatory status was confirmed by measurement of IL-4 and IL-5 levels in lung tissue homogenate and in blood and differential count of leukocytes was assessed in BALF and blood, samples were taken from euthanized subjects.

Score of nasal symptoms was higher in OVA than in HDM group (control vs OVA vs HDM: 0 vs 5 ± 1 vs 4 ± 1 $P < 0.05$ vs control). Number of coughs were significantly higher in both experimental groups (control vs OVA vs HDM 9 ± 2 vs 16 ± 3 vs 15 ± 2 $P < 0.05$ vs control), similar tendency was observed in cough latency. In HDM group, no significant changes were observed in airway resistance. IL-4 levels in lung homogenate were increased in both experimental groups (control vs OVA vs HDM 33.9 ± 1.14 vs 46.6 ± 2.69 [$P < 0.001$] vs 43.17 ± 1.55 [$P < 0.01$]). Similar pattern was seen in IL-5 levels (control vs OVA vs HDM 203.3 ± 15.52 vs 305.1 ± 10.94 vs 477.8 ± 7.07 [$P < 0.0001$]). Differences in plasma levels of aforementioned IIs were not significant.

HDM model can be used in the basic cough research and it is comparable with the OVA model, the disadvantage is the price of the antigen and possible risk of sensitization of the laboratory staff during manipulation with HDM.

This work was supported by the project „Biomedical Center Martin“ ITMS code: 26220220187, the project is co-financed from EU sources.

INFLUENCE OF VOLTAGE-GATED SODIUM CHANNELS BLOCKERS ON AIRWAYS DEFENCE MECHANISMS

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Introduction: The parasympathetic nervous system carries out its function through the vagal nerves, providing innervation of the lungs and the airways. Na_v channels, presented in nine different isoforms according to their Na_v alpha subunits (Na_v 1.1-1.9), play an essential role in the rising phase of the action potential regulation. Airways sensory neurons express Na_vs 1.7, 1.8, 1.9, and these are not expressed to any great extent in either part of the CNS, cardiac or skeletal muscles. Presented project focuses on the intriguing and markedly divergent isoform Na_vs 1.8 because of its more depolarized voltage dependence of activation and fast inactivation, allowing a continuous-action potential discharge during periods of sustained depolarization, and focuses on A803467, a small inhibitory molecule with high selectivity for Na_v 1.8. Material and methods: Current study evaluated therapeutic potency of Na_v 1.8 selective blocker, substance A803467, acute administration in healthy animals and in experimental animal asthma model. Airways allergic asthma was induced by ovalbumin in guinea pigs for 21 days. Influence of acute administration was observed on airway smooth muscle reactivity changes *in vivo*, number of cough efforts and cilia beat frequency (CBF). Number of coughs and values of specific airways resistance (sRaw) were measured before and 1, 2, 5 hours after drugs administration. For *in vitro* CBF measurement was tested substance applied directly on biological samples in concentrations 10⁻⁷, 10⁻⁶, 10⁻⁵ M/L. Conclusion: Blocker of Na_v 1.8 significantly reduced number of coughs and sRaw values in group of animals with allergic inflammation, otherwise did not influence observed parameters during physiological conditions. A803467 did not significantly reduce CBF values during physiological as well as pathological conditions. Presented data confirmed that although selective blockers of Na_v 1.8 would alleviate cough and bronchial hyperreactivity in asthmatic patients with no effect on CBF.

Affiliations: Presented work was supported by the project "Centre of Experimental and Clinical Respiriology II" co-financed from EC sources, Gran MZ 2012/35-UKMA-12, APVV-0305-12, Measurement of Respiratory Epithelium Cilium Kinematics, BioMed Martin (ITMS 26220220187) and Grants VEGA No 1/0165/14. Authors want to thank Ms Katarina Jesenska for technical support.

TRPV4 AND ITS ROLE IN THE TUSSIVE POTENTIAL OF OSMOTIC STIMULI

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Changes of the osmotic force of the airway superficial fluids is associated with cough – both hypoosmotic and hyperosmotic aerosols are potent tussive stimuli used in experimental and clinical studies, however, the response is less intense than response to capsaicin or citric acid. While the citric acid, and capsaicin induced cough pathways are well described, little is known about the activation of airway afferents by osmotic changes. TRPV4 is calcium channel, which was initially described as osmolarity-sensitive channel and in the airways, it can play indirect role in increasing cough reflex sensitivity via regulation of endothelial permeability, airway muscle contraction and mucocilliary transport. The aim of our study was to test the hypothesis that cough to osmotic stimuli is mediated via TRPV4 channel.

Cough response was measured in 12 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; positive control) for 10 minutes in whole body plethysmograph. Simultaneous recording of airflow and audio from head chamber was performed to quantify the cough response. Data obtained in naïve animals, and later in animals pre-treated with selective TRPV4 antagonist GSK 2193874 300 µg/kg (GSK300) and 900 µg/kg of body weight (GSK900).

Cough response in animals to hypotonic and hypertonic solution and to citric acid was significantly higher than to saline (3 ± 2 vs 5 ± 2 vs 9 ± 3 vs 27 ± 5 ; $p < 0.05$). Similar pattern was observed for cough latencies. Pre-treatment with GSK300 did not influenced the response to osmotic stimulus, it only slightly reduced the cough to citric acid (7 ± 2 vs 8.5 ± 3.5 vs 19.5 ± 5 , $p > 0.05$).

Changes of the osmolarity in the airways fluids are potent tussive stimuli, however TRPV4 mediated activation of airway afferents does not seem to be the exclusive mechanism.

This work was supported by the project „Biomedical Center Martin“ ITMS code: 26220220187, the project is co-financed from EU sources.

GENDER DOES MATTER IN COUGH STUDIES

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The main goal of biomedical research is to provide data which could be successfully translated and applied into healthcare. Lack of successful translational studies, production and marketing of compounds which work less in females and more side effects of treatment in females is serious problem. Exclusion of females from experimental studies lead to gender bias in biomedical research. Chronic cough affects mainly postmenopausal females and in their case the researchers fail to provide an explanation why. Therefore, an optimal treatment is missing. What is important – only male guinea pigs are used in cough research empirically. The aim of our study is to identify the reason of exclusion of female guinea pigs from cough research by database search and to assess the female guinea pigs as a variable, comparing the reproducibility and variability within the group and between the female versus male groups. Cough response to inhalation of citric acid (0.4M) was obtained in female and male guinea pigs (12 each). The cough response was tested several times, min 2 days apart to avoid tachyphylaxis. The cough was detected from the airflow traces, cough sound analysis and the presence of cough motor pattern. The cough was investigated in naïve animals, after ACEi and danazol treatment. Cough response to citric acid in females and males is similar in number of cough bursts (9 ± 2 vs 7 ± 4) and cough latencies (180 ± 10 vs 150 ± 20 s), female groups showed also similar reproducibility (7 ± 4 vs 9 ± 5) and the response to short-lasting ACEi medication than males. Both groups have high variability of the cough response with the similar distribution of hypo/hyper and normoreactors. Further investigations are necessary to characterize the cough response in female guinea pigs in large number of animals and cough response of mixed groups to see their potential in cough research.

This work was supported by the project „Biomedical Center Martin“ ITMS code: 26220220187, the project is co-financed from EU sources.

ELECTROPHYSIOLOGICAL ANALYSIS OF SENSITIZATION MECHANISMS IN VISCERAL NOCICEPTORS BY ADENOSINE

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Introduction

Adenosine is an important pronociceptive and inflammatory mediator. Clinical studies suggest that endogenous adenosine can contribute to non-cardiac chest pain and mechanical hypersensitivity in the esophagus by activation of esophageal nociceptive pathways. Esophageal vagal nodose nociceptors, as we have previously found, express the adenosine A_{2A} receptors. We hypothesized that the activation of A_{2A} adenosine receptors can induce mechanical sensitization in esophageal C-fibers that is mediated by TRPA1 channel.

Material and Methods

The activity of vagal nodose afferent C-fibres was recorded extracellularly in the isolated guinea pig esophagus preparation *ex vivo*. We evaluated the effect of pharmacological activation and inhibition of A_{2A} receptor and potential downstream effector ion channel TRPA1 on mechanical responsiveness of esophageal nodose C-fibers.

Results

The selective adenosine A_{2A} receptor agonist CGS21680 induced reversible mechanical sensitization of the response to esophageal distention (10-60 mmHg) in vagal nodose C-fibres. Without causing an overt activation, CGS21680 induced (2.4±0.3)-fold increase in the mechanical response (to 30mmHg) at the half maximally effective concentration (EC₅₀) 3nM. The selective A_{2A} antagonist SCH58261 abolished this sensitization. The activator of adenylate cyclase forskolin (1-10µM) caused a (1.9±0.3)-fold increase in mechanical response, which was comparable to that of GCS21680. The nonselective protein kinase inhibitor A H89 partially inhibited the GSC21680-induced mechanical sensitization. Finally, the selective TRPA1 receptor antagonists AP18 and HC030031 prevented mechanical sensitization of nodose C-fibres evoked by GSC21680.

Conclusion

We show that the activation of adenosine A_{2A} receptor induces mechanical sensitization of vagal nodose C-fibres. Our results indicate that the signalling may involve Gs-adenylate cyclase-PKA pathway and that the downstream effector ion channel is the TRPA1.

SKIN INTERSTITIAL GLYCOSAMINOGLYCANS – SODIUM HOMEOSTASIS

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Introduction : According to the literature in case of Na⁺ overload reversible changes in the ratio of free/bound Na⁺ in subcutaneous tissue serve as a volume buffer and protect against volume expansion and the rise of blood pressure. The system consists of the negatively charged glycosaminoglycan (GAG) molecules of the skin, the macrophages sensing hypertonicity and the VEGFC protein secreted by macrophages. VEGFC secretion results in hyperplasia of the existing lymph capillary system draining liberated Na⁺ back to circulation. The weakness of the theory is that it does not explain the mechanism of release of the bound Na⁺. To address this question we measured the changes of VEGFA-levels in the skin, a signaling protein that might decrease charge density of interstitial GAGs thus playing a key role in the release of bound Na⁺.

Materials and methods : Normotensive female Wistar rats aged 8 weeks were assigned to three groups (n=8) each receiving either high salt diet (HS, NaCl=8% m/m), low salt diet (LS, NaCl<0.1% m/m) for 4 weeks or high salt diet followed by low salt diet for 8 weeks (HS/LS). Na⁺ content of the skin was measured by flame photometry, skin hyaluronic acid and chondroitin 4,6 sulphate content was measured by high performance liquid chromatography, skin VEGFA and VEGFC mRNA levels were measured by PCR.

Results : Our results confirm that the changes in dietary sodium intake have a strong influence on skin GAG levels. Significant correlation has been found between skin sodium and GAG content.

Conclusions : We have demonstrated that in the skin of salt resistant rats the accumulation of Na⁺ on skin GAGs correlates with proportionate amount of water, the blood pressure remaining unchanged. The decrease of skin Na⁺ and GAGs in the HS/LS group supports the hypothesis of the regulated degradation of skin GAGs.

(The research was supported by the the Hungarian National Scientific Research Foundation OTKA K-108688)

MOLECULAR MECHANISM OF CANCER CELL DEATH INDUCED BY MODULATED ELECTROHYPERHERMIA

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Modulated electro-hyperthermia (mEHT) using capacitive impedance-coupled radiofrequency and the concomitant heat (<42°C), can selectively target malignancies due to their elevated glycolysis, ion concentration and conductivity compared to normal tissues. The mEHT has been used as a complementary to radio- or chemotherapy of human cancer.

In colorectal cancer xenografts of athymic mice mEHT provoked programmed cell death through apoptosis inducing factor (AIF) activation and damage associated molecular pattern (DAMP) signals compatible with a potential immunogenic cell death (ICD).

Aims: Here we tested the mEHT related tumor damage and immune response in colon-26 (C26) colorectal cancer allografts in immunocompetent (Balb/C) mice.

Results: mEHT induced significant and progressive cell damage in the treated right-leg tumors of the animals compared to their symmetrical but untreated left-leg tumors. The programmed cell death response proved to be caspase-dependent causing significant increase in cleaved/activated caspase-3 levels, besides elevated cytochrome-c release from the mitochondria but without AIF activation, or major mitochondrial accumulation of Bcl-2-associated X protein (BAX). Significant increase in TUNEL positive cell nuclei also indicated apoptosis.

Elevated release of stress-associated Hsp70, calreticulin and HMGB1 proteins was also observed in mEHT treated tumors related to DAMP signaling and for an ICD response. In line with this, the number of S100 positive dendritic cells and CD3 positive T-cells was significantly elevated in the treated tumors, at low number of FoxP3 positive regulatory T cells. In addition, mEHT supplemented with a flavonoid rich CD8+ T-cell promoting agent induced cell death also in the untreated left-leg tumors indicating a systemic anti-tumor effect.

In conclusion, mEHT can induce caspase-dependent programmed cell death in CT26 colorectal cancer allografts and the release of stress associated DAMP proteins calreticulin, Hsp70 and HMGB1, which may support dendritic cell activation and T cell mediated tumor immunity for a potential ICD response.

ROLE OF RADIOGRAPHIC PREDICTIVE FACTORS IN PROGNOSIS OF PATIENTS AFTER TRAUMATIC BRAIN INJURY

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INTRODUCTION: Computed tomography (CT) is widely used in diagnostics of patients after traumatic brain injury with specific findings described as negative prognostic factors. Therefore our aim was quantification and detailed analysis of these factors in order to link specific CT-findings with unfavourable outcome.

MATERIAL AND METHODS: A group of 82 patients was analysed in prospective study since November 2014 to February 2016. Severity of their injury was determined using GCS (Glasgow Coma Scale). Using CT, type of the injury with use of Marshall score, volume of the lesion, possible presence of midline shift, perimesencephalic cisterns compression and subarachnoid or intraventricular haemorrhage was assessed. These findings were compared to GOS (Glasgow Outcome Scale) in time of patients' discharge.

RESULTS: In patients with subdural haematoma (SDH), we observed a negative correlation between the volume lesion and patients' outcome (for acute SDH: $p < 0,001$; for chronic SDH: $p = 0,012$). We didn't observe this correlation in brain contusion or intracerebral bleeding.

Combined intracranial lesions which consist of intracerebral and extracerebral bleeding are connected with the worst outcome ($p = 0,033$) and mortality of 20,8%. Increased volume of these lesions is associated with worse outcome ($p = 0,025$) and our findings suggest that extracerebral bleeding plays a dominant role in this process. Furthermore, narrowing or complete obliteration of perimesencephalic cisterns and midline shift above 5 mm is associated with worse outcome ($p < 0,0001$). Using the Marshall score, we confirmed that type VI lesions have an unfavourable outcome ($p < 0,0001$).

CONCLUSION: Combination of intracerebral and extracerebral lesion and Marshall score VI are associated with worst outcome. Upsurge of lesion volume predicts unfavourable outcome in subdural haematoma and combined lesion. Midline shift above 5 mm and narrowing of perimesencephalic cisterns are linked with worse outcome.

GENETIC BACKGROUND OF STICKY PLATELET SYNDROME IN PATIENTS WITH FETAL LOSS

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INTRODUCTION: 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, venous thromboembolism and pregnancy complications. 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 or intracellular signal pathways involved in platelet activation and aggregation are responsible for the disorder. **MATERIAL AND METHODS:** We examined 23 female patients with SPS and history of spontaneous abortion, and 42 healthy women. SPS is diagnosed by aggregometry and it is classified as type I (hyperaggregation after both ADP and EPI), type II (hyperaggregation after EPI alone), and type III (hyperaggregation after ADP alone). Then we were interested in four single nucleotide polymorphisms (SNPs) of GAS6 gene (rs7400002, rs1803628, rs8191974, rs9550270), two SNPs of PEAR1 gene (rs12041331, rs12566888), two SNPs of MRV11 gene (rs7940646, rs1874445). **RESULTS:** We identified two SNPs in PEAR1 gene (rs12041331; rs12566888) and one SNP in GAS6 gene (rs9550270), both with higher occurrence in patients with SPS and abortion. **CONCLUSION:** Our results suggest that variability of GAS6 and PEAR1 genes (as well as variability of GP6 gene from previous study) can be associated with platelet hyperaggregability in patients with SPS and fetal loss. The study also suggests a possible polygenic type of SPS heredity. These results have to be confirmed by further research.

CD30 ANTIGEN IN DIFFUSE LARGE B-CELL LYMPHOMAS

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Introduction: CD30 antigen, originally a marker of tumor cells of classical Hodgkin lymphomas, is recently known to be expressed in various non-Hodgkin lymphomas, especially in diffuse large B-cell lymphomas (DLBCL). Although its biological function is not yet fully understood. The CD30 expression is highly restricted that makes it ideal as a diagnostic and prognostic marker and therapeutic target as well. However, the incidence and intensity of CD30 expression in DLBCL is not exactly known.

Material and methods: Consecutive biopsies of 145 patients with DLBCL expressing immunohistochemically CD30 positivity registered in the Lymphoma Registry were analyzed retrospectively to distinguish cases with a) < 5%, b) 5-50% and c) >50% positive tumor cells. All these cases were divided into types and variants according to WHO classification and all the data were processed and analysed.

Results: In the group of 47 patients with primary mediastinal B-cell lymphoma (PMBL), the CD30 expression in >50% positive tumor cells was identified in 62% of the cases. In the group of 98 patients with DLBCL, NOS, the anaplastic morphological variants of non-GCB phenotype expressed CD30 positivity most frequently (63%) and most extensively, 61% of cases showed >50% positive tumor cells. In contrast, anaplastic variants with GCB phenotype and other variants irrespectively of the GCB or non-GCB phenotype did not show substantial differences neither in frequency nor in the extensivity of the CD30 positivity.

Conclusion: Generally, the expression of CD30 positivity in DLBCL seems to be higher than previously expected. In particular, as documented in our analysis, it is true for PMBL and anaplastic DLBCL variants, especially of non-GCB phenotype. Identification of CD30 expression in biopsies of DLBCL patients represents a significant contribution to the diagnosis of these lymphomas and to the prognosis and treatment of the patients.

THE INFLUENCE OF SHORT-TERM AND LONG-TERM GLUCOSE CONTROL ON HEART RATE VARIABILITY IN CHILDREN AND YOUNG ADULTS WITH TYPE 1 DIABETES

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Introduction: Decreased heart rate variability (HRV) is considered as a gold standard in diagnosis of diabetic autonomic neuropathy (DAN). In literature, non-optimal long-term metabolic control (increased HbA1c) and longer duration of Type 1 Diabetes (T1D) are the risk factors for development of DAN. Data concerning short-term glucose control and HRV are limited.

Goal: The aim of the study is to determine the relationship between HRV and short-term and long-term glucose control, duration and onset of T1D.

Methods and materials: 32 children and young adults with T1D (aged 10-21 ys, 14 boys) were examined at Clinic of Children and Adolescents of Martin University Hospital. Following parameters were observed: I/E, CVrr, HbA1c, average sensor glucose (48 hs), actual glucose at the time of HRV examination, sensor glucose variability (SD), duration and onset of T1D. 7 children exhibited hypoglycemia ($\leq 3,9$ mmol/l) before HRV examination.

Results: We found positive correlation between sensor glucose variability (SD) and I/E ($r = 0,499$). We did not observe relationship between other short-term parameters (average sensor glucose, actual glucose). Surprisingly, HbA1c did not show correlation with parameters of HRV (I/E, CVrr). Negative correlation was observed between CVrr and duration of T1D ($r = -0,437$), positive mild correlation was found between onset of T1D and I/E ($r = 0,307$), and CVrr ($r = 0,398$). Comparing cohort with and without hypoglycemia, we did not reveal significant changes in HRV parameters.

Conclusion: In children and young adults, early onset and longer duration of T1D was associated with autonomic dysfunction. Glucose variability can influence parameters of HRV. Previous hypoglycemia and other parameters of short-term and long-term glucose control did not lead to changes in HRV parameters.

Key words: Type 1 Diabetes, heart rate variability, glucose control, glucose variability.

BONE MARROW STROMAL REACTION IN PRIMARY AND MYELOYDYSPLASIA-RELATED ACUTE MYELOBLASTIC LEUKAEMIAS

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Introduction: Acute myeloblastic leukaemias (AML) represent a heterogenous category of precursor myeloid neoplasia, usually with very bad clinical outcome, which tends to be worse in AML arising from previous myelodysplastic syndrome (MDS). Majority of AML studies point their interest on analysis of tumour cells, but there are only limited data about the role of bone marrow (BM) microenvironment and supporting stroma in these disorders.

Material and methods: We analysed 84 patients with AML diagnosed and treated on our departments in years 2009-2015, including 47 cases of primary AML without history of myelodysplastic syndrome (MDS) or treatment (group 1), 13 cases of secondary AML evolving from previously confirmed MDS (group 2) and 24 cases of AML with myelodysplastic features verified in the time of AML diagnosis (group 3). BM stromal macrophages were visualised immunohistochemically using anti-CD68 antibody (clone PG-M1) and their proportion was estimated semiquantitatively and histomorphometrically.

Results: Overall survival of patients in analysed cohort was short (median 142 days) with only insignificant differences between primary and MDS-related AML (166 versus 119 days), when cases of group 3 were not included. Cases of AML with myelodysplastic changes (group 2 and 3) showed more frequently increased proportion of stromal macrophages (45% cases in primary versus 91% cases in myelodysplasia-related AML), but intensity of macrophage stromal reaction did not have significant influence on survival.

Conclusion: In our small cohort we did not confirm significant differences in survival of patients with primary and myelodysplasia-related AML. We found that activation of BM stromal macrophages is more frequent, but not specific for secondary myelodysplasia-related AML, what may be helpful for diagnostics, but depending on many influencing factors, prognostic relevance of this finding in AML is disputable.

Acknowledgements: Supported by the Grant VEGA Nr. 1/0268/2015.

SEASONALITY AND OBSTRUCTIVE SLEEP APNOEA SYNDROME IN CHILDREN

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Introduction. During the past decades obstructive sleep apnea syndrome in children has become widely recognized as a frequent disorder associated with potentially serious clinical morbidities as neurobehavioral consequences, cardiovascular and metabolic morbidity.

Goal. The aim of the study was to determine the distribution of obstructive apnoe/hypopnoe index during year seasons in children with obstructive sleep syndrome.

Material and methods. Basic antropometric parameters, sleep child questionnaire (Sleep Clinical Record) and specific polysomnographic breathing parameters (obstructive apnoe/27anuary2727 index) were analysed retrospectively and separated depending on year season in 4 groups. Qualitative and quantitative parametric statistical analyses were used.

Results. 112 children (69 boys, 43 girls) with clinical symptoms of obstructive sleep apnea were included. Average age was 7,37 years, BMI 18,329, obstructive apnoe/hypopnoe index 4,885. There were no significant differences in age, BMI, allergy between year seasons.

There was established significant difference in obstructive apnea/hypo between spring and summer (8,1/hour vs 1,9/hour, $p<0.05$), spring and autumn (8,1/hour vs 2,3/hour, $p<0.05$), spring and winter (8,1/hour vs 2/hour, $p<0.05$).

Conclusion. The study showed higher obstructive apnoe/hypopnoe index during spring.

IBRUTINIB AND PLATELET AGGREGATION

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Introduction. Ibrutinib is an inhibitor of Burton's tyrosine kinase (BTK). Ibrutinib is generally well tolerated but is associated with an increased risk of bleeding.

Material and methods. The local Ethical Committee approved this study. Light transmission aggregometry (LTA) was performed using the international protocol for the laboratory investigation of platelet function. The antecubital venous blood was collected into tubes containing 3.2% buffered sodium citrate (anticoagulant-blood ratio 1:9) to assess platelet aggregation. Platelet aggregability was tested with platelet-rich plasma using platelet aggregometry (PACKS-4 aggregometer, Helena Laboratories, USA). Each sample was tested with adenosine diphosphate (ADP) (2.5 $\mu\text{mol/L}$), epinephrine (EPI) (1.2 $\mu\text{mol/L}$), arachidonic acid (AA) (1.0 mmol/L), and collagen (1.25 $\mu\text{g/mL}$).

Results. We tested 7 relapsed/refractory CLL patients (5 males) with median age 69 years (range 52 – 80 years); 5 of patients were aged 65 years or older. 5 patients received ≥ 2 previous lines of treatment. All patients received 420 mg oral ibrutinib daily. Median time from ibrutinib initiation to platelet aggregometry was 171 days (range 80 – 272 days). Median platelet count at aggregometry was $117 \times 10^9/\text{L}$ (range $103 - 195 \times 10^9/\text{L}$). None of these patients received antiplatelet or anticoagulation treatment. As expected, patients on ibrutinib showed impairment of responses to collagen. All patients showed reduce platelet aggregation after AA. In addition, majority of patients had also reduced responses to EPI and ADP.

Conclusion. We showed an effect on collagen and AA-induced platelet aggregation, and some evidence on ADP and EPI-induced aggregation. Our findings could have some important clinical implications because platelet activation and aggregation is affect in many levels.

LIVER INJURY IN TREATED INFLAMMATORY BOWEL DISEASE PATIENTS

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Introduction: Drug induced liver injury (DILI) is the most common cause of acute liver injury. IBD patients might have an increased risk of DILI due to long – term treatment, multiple therapies and altered immune and nutritional status.

Materials and methods: 1. Retrospectively analyse the prevalence of hepatotoxicity in IBD patients during a one-year period. 2. Evaluate the risk factors of liver injury. 3. Evaluate the risk of liver injury for every drug used. All IBD cases in our center during a 9-month period were included. We recorded demographic and anthropometrics, localization, activity and treatment of IBD, levels of hepatic enzymes and bilirubin. These parameters were measured at 5 time-points 3 months apart (0, 3., 6., 9., 12. Month). Hepatotoxicity was defined according to CTCAEv.4.03 consensus.

Results: 251 IBD patients were included, median age was 41 years, 51,4% were women, Crohn's/ UC ratio was 61,4% / 38,6%. One patient was diagnosed with PSC. IBD was treated with mesalamine in 173 cases, 66 had no immunomodulatory therapy, solo azathioprine had 47 patients, 74 were on solo anti-TNF, 64 took combination therapy. Grade 1 ALT and GGT elevation was transient in 21,5% and 10,8%, and persistent in 4,78% and 5.18% of cases. In multivariate analysis only BMI and solo anti-TNF therapy were predictors of grade 1 ALT elevation. Grade 2. ALT elevation, was more common among solo infliximab treated patients (7,3 vs. 0,95%).

Conclusion: IBD therapy often leads to abnormalities in hepatic tests, but these are mainly transient. BMI and solo anti-TNF therapy seem to be the risk factors for the grade 1 ALT elevation. Grade 2 elevation was found more often among patients using solo infliximab therapy.

PANCREATIC CANCER: EVALUATION OF FORECAST OF SURVIVAL

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Background: Pancreatic Cancer is the malign tumor, originates from exocrine part of 30 January 2015, represented most often by pancreatic duct carcinoma. Standardized incidence in population of Czech Republic (12,09/100 000 men and 8,17/100 000 women) increases and is accompanied by high mortality rate (8,72/100 000). Pancreatic Cancer is the 4th most often cause of death from oncologic disease in Czech Republic. The symptoms of early stages are nonspecific.

Purpose: The study follows up a group of patients operated on Pancreatic Cancer in period 2006 – 2010 in FN, Olomouc. The purpose of the study is to specify expectation of 5 years survival rate, average survival rate and median of survival in group of patients and to evaluate prognosis of survival rate according to defined factors.

Methods: The data were processed by programme IBM SPSS Statistics version 22. Appraisal of relevancy in defined factors was verified by Kaplan-Meier analysis with Long-rank test and Cox regressive analysis. Test were executed 0,05 stage of significance.

Results: In group of 60 patients (41 men and 19 women) was average age of patients 62,7 years (63,1 in men, 61,8 in women). Expectation of 5 years survival of whole group was 20,0% (22,0% in men, 15,8% in women), average survival rate of patients was 32 months and median of survival 13,9 months. Significantly approved defined factors were: factor of severe complications, angioinvasion and chemotherapy. Patients suffered from severe complications CD IIIA-V had significantly shorter survival rate (19,4 months) compared to patients without complications CD 0, or with complications CD I-II (35,2 months). In group of patients with angioinvasion was detected significantly shorter survival rate (16 months) compared to patients without angioinvasion (38,8%). Patients treated by chemotherapy had survival rate significantly longer (39,7 months) than patients without chemotherapy (12,2 months). Other factors were not significantly relevant.

Conclusion: In our group of patients was 5-years survival rate 20%. The average survival rate was 32 months with median of survival 13,9 months. Significantly relevant factors founded in our study were factor of severe complications, angioinvasion and chemotherapy.

Grant support: IGA UP 2015-010

ASSESSMENT OF METABOLIC TUMOR PARAMETERS USING PET/CT IN PATIENTS WITH HODGKIN LYMPHOMA

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Introduction: Among patients with HL, PET has been used for qualitative assessment of the stage of disease and the treatment response. However, this imaging technique offers continuous “quantitative” data (QT-PET) that might unlock new possibilities in prognosis prediction. Our goal was to perform a retrospective analysis of QT-PET at the time of the diagnosis. Materials and Methods: We analyzed PET scans of 32 patients using an imaging software Singo.via. Regions of interest (ROIs) were defined as voxels with the activity 41% of the Maximal Standardized Uptake Volume (SUVmax) and more. We calculated these quantitative values: maximum and average Standardized Uptake Volume (SUVmax, SUVmean), Metabolic Tumor Volume (MTV) and Total Lesion Glycolysis (TLG). In order to become more accurate we re-counted results using patient’s height (SULmax, SULmean) and average activity around one voxel (highest peak). Results: Correlation between QT-PET and the stage of HL was not found for TLG ($p=0.60$). Advanced stages of HL usually had higher MTV ($p=0.18$). The presence of a bulk correlated with higher MTV and TLG ($p=0.036$). Treatment response did not overall correlate with MTV or TLG ($p=0.29$). Nevertheless, 3 out of 4 patients without complete remission had higher MTV as well as TLG. Relapsed patients and those with progression usually had higher MTV and TLG at time of diagnosis ($p=0.047$). Patients with higher MTV and TLG usually had worse 5-year Progression-free survival (66.7% versus 87.5%, $p=0.11$). Conclusion: Patients diagnosed with HL have very variable metabolic tumor parameters, which only partially correlate with the established methods for assessing the extent of HL. Our pilot study indicates a possible QT-PET capability of predicting the prognosis. We will continue to assess interim and final PET scans of these patients with the same method.

CHANGES OF THE COUGH PATTERN DURING INSPIRATORY AND EXPIRATORY OCCLUSION

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The role of feedback mechanisms modulating coughing, primarily those related to lung volume, pressures and airflows in the respiratory tract are not fully understood. Our previous data have shown increased cough motor drives and concomitant phase duration changes when inspiratory cough volume was limited (inspiratory resistance).

Present study tested the cough motor pattern during inspiratory (IO) and expiratory occlusions (EO) on 8 pentobarbitone-anesthetized spontaneously breathing cats (3.74 ± 0.40 kg). Cough reflex was induced by mechanical stimulation in the intrathoracic trachea. Electromyograms of the diaphragm, and abdominal muscles together with esophageal pressures (Epr) were used to evaluate the cough efforts and phase durations.

The number of coughs was moderately reduced ($p < 0.05$) by permanent IO and EO (one way valve attached to the airways during the whole cough trial). Cough peak inspiratory Epr were increased by permanent IO ($p < 0.001$) and by both IO and EO ($p < 0.01$) applied temporarily (only during cough inspirations and expirations, respectively). Temporary EO resulted in increased peak expiratory Epr. Occlusions had no significant effect on maximum cough motor drive measured as peak activations of the diaphragm and abdominal muscles. However, temporal changes in cough motor pattern were similar to those observed during manipulated cough volumes and applied airflow resistance.

Experimental data confirmed the significance of airway mechanics in the formation of cough motor pattern and the differences in feedback modulation of cough motor drives when resistance vs. occlusions act on respiratory system during coughing.

This work was supported by the VEGA 1/0126/12 and 1/0253/15. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0189-11".

MODULATION OF COUGH AND BREATHING BY GABA-ERGIC INHIBITION WITHIN THE MEDULLARY RAPHE IN CATS

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Effects of GABA and baclofen (GABA_B receptors agonist) microinjections in the medullary raphe on cough and breathing were studied on 25 pentobarbitone anesthetized spontaneously breathing cats. Cough was induced mechanically in the tracheobronchial area. Blood pressure, esophageal pressure (EP) and EMGs of the diaphragm (DIA) and the abdominal muscles (ABD) were recorded.

GABA microinjections (6 injections, 3 depths, all approximately 200nl) 4 mm rostral to the obex reduced the cough number related to 10s stimulation from 4.48 ± 0.30 to 3.17 ± 0.24 ($p < 0.01$; 4.20 ± 0.45 in recovery > 7 min post-microinjections, $p < 0.01$), cough ABD EMG amplitudes to $50 \pm 10\%$ ($p < 0.05$; recovery $85 \pm 24\%$), and cough expiratory EP to $61 \pm 9\%$ ($p < 0.01$; recovery $83 \pm 9\%$, $p < 0.05$) and prolonged cough DIA activity by 23% ($p < 0.05$) and cough inspiratory phase by 22% ($p < 0.05$). GABA microinjections 1 mm rostral to the obex reduced cough ABD EMG amplitudes to $49 \pm 9\%$ ($p < 0.001$; recovery $82 \pm 9\%$, $p < 0.01$) and cough expiratory EP to $64 \pm 11\%$ ($p < 0.05$; recovery $102 \pm 13\%$, $p < 0.01$). Baclofen 4 mm rostral to the obex (2 microinjections in the ventral raphe, all approximately 70nl) reduced cough ABD EMG amplitudes to $63 \pm 11\%$ ($p < 0.05$; recovery $89 \pm 16\%$) and cough expiratory EP to $57 \pm 13\%$ ($p < 0.05$; recovery $72 \pm 18\%$). Baclofen 1 mm rostral to the obex (2 injections to the dorsal raphe) reduced the cough number to $74 \pm 7\%$ ($p < 0.01$; recovery $93 \pm 7\%$, $p < 0.05$). No significant changes of heart and respiratory rate and mean arterial blood pressure were found.

GABA-related neurotransmission employing GABA_B and likely also GABA_A receptors contribute to control of coughing by medullary raphe with limited effects on cardiorespiratory performance. The distribution of cough related neurons and/or their GABA-ergic modulation vary depending on the rostro-caudal positions and likely the particular structures of raphe.

This work was supported by the VEGA 1/0126/12 and 1/0253/15. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0189-11".

SERUM LEVEL OF URIC ACID IN PATIENTS WITH MULTIPLE SCLEROSIS

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Background: Uric acid (UA) is a waste product of metabolic breakdown of purine nucleotides. It can also have an antioxidant activity, which can play a role in the etiopathogenesis of multiple sclerosis (MS). A few studies have proven discrepancy in serum level of uric acid between group of MS patients and control subjects. Therefore, we decided to try to study the potential role of uric acid in MS patients in Slovak population.

Material and methods: In our study we have examined sex matched groups of 120 clinically diagnosed MS patients and 120 controls. The clinical disability of all patients was measured by the Extended Disability Status Scale (EDSS) with mean value of $4,0 \pm 1,1$ in men and $3,5 \pm 1,3$ in women patients. The serum level of uric acid was assessed by using the uric acid kit OSR 6298 ® (BeckmanCoulter, USA) on Olympus AU 640/680 analyser with analysis based on spectrophotometric principle.

Results: We found the mean value of serum uric acid level of $339 \pm 69,37 \mu\text{mol/l}$ in group of male MS patients compared to $342 \pm 93,15 \mu\text{mol/l}$ in group of male controls. In female group of MS patients, the serum UA level was $255 \pm 53,42 \mu\text{mol/l}$ in comparison to $285 \pm 74,32 \mu\text{mol/l}$ in female controls

Conclusion: In our study we observed lower serum levels of uric acid in female MS patients when compared to healthy female controls. However, we did not observe a significant difference in serum levels of uric acid between male groups of MS patients and controls. Our findings support the hypothesis of possible role of UA in MS pathogenesis.

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

EVOLUTION IN UNDERSTANDING HUMAN MICROBIOME

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Introduction: We have known for decades that microorganisms on human body (i.e. in gut or vagina) are important for proper body functions. We knew microbes are important for roles such as vitamin production and competing with pathogen and thus keeping them from overgrowth. The last decade have been however revolutionary in understanding human microbiome since technological advancement allowed projects as Human Microbiome Project to be economically possible. Today study of human microbiome is popular area, generating new data every year.

Materials and methods: We reviewed 17 studies and compared latest and older editions of microbial textbooks dealing with this human physiological flora. We also reviewed PubMed database for additional information about the topic.

Results: The aim of this work is to search and analyze new research regarding human microbiome and present the literature review about new theories. In this work we also present hypotheses, future challenges and possible clinical applications of this new information.

Conclusion: The study of human microbiome constantly reveals new information. Human microbiome is part of immune system and its composition influences the development of immune reaction. Changes in human microbiome are associated with wide range of diseases. It is important to further investigate in which cases this changes are the cause or effect of the disease and in the former case develop methods to alter microbiome composition to positively affect patient.

PLANT FUNCTIONAL FOODS IN THE PREVENTION AND TREATMENT OF EXPERIMENTAL BREAST CARCINOMA

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Previously, isolated phytochemical administered alone did not show any anti-cancer effects in rat mammary carcinogenesis in our laboratory. However, it is supposed that the mixture of phytochemicals with plethora biological activities, present in whole foods, may have additive or synergistic effects against carcinogenesis.

The tumour-suppressive effects of dried oregano were evaluated in the preventive/curative model of N-methyl-N-nitrosourea-induced mammary carcinogenesis in female rats. 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.

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 did not improve results from low-dose group. Analysis of rat tumour cells showed Ki67, VEGFR-2, CD24, and EpCAM expression decrease and caspase-3 expression increase after low-dose ORE treatment. High-dose ORE lengthened tumour latency by 12.5 days; moreover, Bcl-2, VEGFR-2, CD24, and EpCAM expression decrease and caspase-3 expression increase in carcinoma cells were observed. Histopathological analysis revealed a decrease in the ratio of high-/low-grade carcinomas in both treated groups.

Our results pointed to substantial tumour-suppressive effect of the phytochemicals' mixture from oregano in the breast cancer model. This is the first study to confirm the inhibitory effect of oregano against cancer stem cells in vivo.

This work was supported by the grant VEGA 1/0108/16.

HOMOCYSTEINE ALTERS MOLECULAR PROCESSES IN HUMAN GLIAL CELLS WITH IMPACT ON CELLULAR METABOLISM AND THEIR SURVIVAL

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The essential amino acid methionine plays an indispensable role as a methyl group donor and a cysteine precursor by entering the S-adenosylmethionine (SAM) pathway. Disturbances in the SAM pathway may lead to the accumulation of its intermediate – homocysteine (Hcy). On a molecular level, Hcy is able to inhibit methylation reactions by negative feedback. The methylation of proteins and nucleic acids may participate directly in the regulation of enzymatic activities and conveys important epigenetic information able to control gene expression. An increased level of Hcy exerts neurotoxic and gliotoxic effects and is considered to be involved in the etiopathogenesis of several cardiovascular and neurodegenerative diseases, as well as neoplastic transformation and formation of malignancies. To investigate the impact of Hcy on human glial cells we used cultured T98G glioblastoma cells as a study model. The cells were incubated in a medium supplemented with Hcy for 24 hours. We used biochemical methods to estimate the metabolic rate of glucose, lactate and glutamate as markers of cellular metabolism. Immunoblotting methods were used to evaluate the putative alterations in the expression of Bax, p53 and caspase 3, as well as the methylation status of histone H3. Hcy negatively affected the glucose uptake by glial cells and simultaneously stimulated the lactate release, which points to the suppression of oxidative metabolism. In addition, hypomethylation of histone H3 and changes in the levels of tested pro-apoptotic proteins were revealed. Our results indicate that Hcy potently alters the energy metabolism of human glial cells; affects the expression of pro-apoptotic proteins and induces hypomethylation of H3. Therefore we can hypothesize, that increased concentrations of Hcy can compromise the cellular metabolism of human glial cells by altering the status of the epigenome. Such molecular changes could contribute to the etiopathogenesis of neurodegeneration linked with hyperhomocysteinemia.

This work was supported by the project VEGA 1/0242/13.

MISMATCH REPAIR PROTEINS AND COLON CARCIN

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Background: Mismatch repair proteins (MMRP) and survivin represent diametrically opposed signals which may control apoptotic pathways. Aim of our work was to analyse immunohistochemical expression pattern of MLH1, MSH2, MSH6 and PMS2 mismatch repair proteins and survivin in group of 124 tubular adenomatous colon polyps and their relationship with dysplastic abnormalities. In addition, we studied their relation to clinicomorphological parameters such as age of patients, size of adenoma, degree of dysplastic changes and localization of lesion.

Methods: Our adenoma group included 87 males and 37 females (average age for males was 60.9 ± 10.1 years and for females 68.7 ± 9.1 years). Location of the polyps was in the right colon (the cecum, ascending and transverse colon) in 33/13 (male/female) and in left colon (the descending colon, sigmoid and rectum) in 54/24 (male/female) cases. Pathology reports contained information about age, sex, localization and size of polyps. The samples were formalin-fixed and paraffin-embedded. Immunohistochemical reactions for mismatch repair proteins were performed using Flex monoclonal mouse anti-human MLH1 and MSH2 antibodies. Flex monoclonal rabbit anti-human PMS2 and MSH6 antibodies and anti-survivin antibody for survivin.

Results: Survivin was expressed in 97 cases (78,2%), MLH1 was found in 111 cases (89,5%), MSH2 in 115 cases (92,7%), MSH6 in 118 cases (95,2%), PMS2 in 105 cases (84,7%). Majority of absent MMRP cases were detected in adenoma size less than 10mm and with low grade dysplasia. Survivin expression significantly correlated with adenoma size and dysplasia grade. Subcellular survivin compartmentalization was statistically associated with adenoma size, dysplasia grade and adenoma localization. Furthermore, we confirmed significant relation between survivin expression and MMRP, and intensity of immunohistochemical reaction was stronger in MMRP in comparison with survivin intensity.

Conclusion: Based on our recent results and literature review, we think that MMRP may suppress antiapoptotic activity of survivin in low grade dysplasia and high grade dysplasia colon adenomas.

HISTOPATHOLOGICAL CHANGES IN THE RAT BRAIN FOLLOWING EXPOSURE TO FRACTIONATED IRRADIATION

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Introduction In the present study we investigated whether short-term and long-term survival period following irradiation affected the neuronal and glial cell population in the rat brain. **Material and methods** Adult male Wistar rats were randomized into control (0 Gy) and 2 irradiated groups (survived 30 and 100 days after treatment). The rats received fractionated whole-brain irradiation (a dose of 5Gy per fraction/week for 4 weeks) with a total dose of 20Gy of ionizing radiation. Histochemical, immunohistochemical staining and confocal microscopy were used to determine the radiation-induced alteration of neurogenesis, astrocytic response and activation of microglia in the neurogenic region of the rat forebrain, the subventricular zone-olfactory bulb axis (SVZ-OB axis). Quantitative image analysis of tissue slides was performed in the selected regions along the SVZ-OB axis represented by the anterior SVZ, vertical arm, elbow and horizontal arm. **Results** Neurodegenerative changes were characterized by a significant increase of Fluoro-Jade-positive cells 30 days after irradiation. The SVZ-OB axis displayed a steep decrease of neuroblasts and less obvious decline of stem/precursor cells until 100 days after treatment. Strong decline or re-expression of astrocytes was seen in the SVZ-OB axis until 100 days after irradiation. A decrease and subsequent re-expression of the activated microglia was seen during the experiment. **Conclusion** This study showed, that fractionated irradiation led to initiation of neuronal cell death, inhibition of neurogenesis, activation of astrocytes and microglia indicated an early delayed radiation-induced changes.

ON THE SEARCH FOR A RELIABLE SYMPATHETIC ACTIVITY INDEX

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INTRODUCTION: Preejection period (PEP), Heather index (HI, index of cardiac contractility), and total peripheral resistance (TPR) are regarded as indices of the sympathetic activity. The aim of this study was to evaluate and compare their behaviour during orthostatic and mental stress. We hypothesized that during both manoeuvres, sympathetic activation should increase HI and TPR and decrease PEP. **METHODS:** 31 healthy young adult volunteers (19 female), mean age 20.17 years (SD=1.82) participated in this study. Using noninvasive continuous finger blood pressure measurement (Finometer Pro, FMS, Netherlands) we recorded the mean arterial pressure. Other cardiovascular parameters (PEP, HI, and cardiac output) were measured by the impedance cardiography (CardioScreen® 2000, Medis, Germany). TPR was calculated as a ratio of mean arterial pressure and cardiac output. **RESULTS:** During orthostasis (HUT, head-up tilt), mean PEP was significantly higher than during supine rest ($P<0.0001$) possibly caused by a decrease of cardiac preload. HUT evoked an increase of mean TPR ($P=0.0048$) and a decrease of mean HI ($P<0.0001$). During mental stress (mental arithmetic, MA), mean PEP was significantly lower than during preceding supine recovery ($P=0.0008$), indicating an increased sympathetic activity, but we also found a paradoxical decrease in HI ($P=0.0010$) and no significant change in mean TPR ($P=0.1156$). Despite expected negative correlation between PEP (cardiac sympathetic activity index) and TPR (vascular sympathetic activity index) a positive correlation ($P<0.05$) was observed in supine position and during MA. During all phases, a significant negative correlation ($P\leq 0.023$) between PEP and HI unless during MA ($P=0.06$) was found. **CONCLUSION:** The behaviour of sympathetic indices during stress challenges is very complex and possibly influenced by changes of other factors requiring further study. The study was supported by grants APVV-0235-12, VEGA 1/0059/13 and ITMS project "Biomedical Center Martin" no. 26220220187, this project is co-financed from EU sources.

VIDEOPROJECT FyzióHD – PRACTICALS FOR PHYSIOLOGY

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Abstract

The aim of project FyzióHD is to help students to prepare for their future as physicians and also for the physiology exam. Knowledge required to pass the exam consists of theoretical and practical section. The practical part includes 55 practical labs. The only source of instructions for students has been a text book “Laboratory Guide to Medical Physiology”. It is often difficult for students to perform examinations following only written instructions from a book. Therefore we decided to prepare a single video for each examination question to demonstrate the same conditions as during lessons.

At first, we have chosen the most difficult practicals requiring visualization due to lack of images and schemes in the written book. Then we wrote a double script for each one – one for the scene and one for the speech about the theoretical background of the examination. Following our written scripts we taped every video with camera, artificial lighting and the exact same equipment we usually use during the lessons. Filmed material was cut, subtitles and graphic content were added. Theoretical speech and instructions were recorded separately to accurately match the scene.

During the time of five months our team of 21 students, tutors and professional supervisor filmed 27 videos. For our videos we created the web page www.fyziohd.com where the videos are available for any student of medicine and are also provided in other languages. There is a possibility to examine acquired knowledge by the test. Every video has a generated QR code that will be included in the next version of written practicals.

Project FyzióHD improves the educational system of future physicians and helps students and teachers during the lessons of medical study. Videos with explanatory and understandable graphic on the web page www.fyziohd.com can have a great influence on perception, remembering and engagement of our medical students.

WOMEN'S SEXUALITY AWARENESS DURING PREGNANCY

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Introduction: The public often lives under influence of widespread sexual myths, half-truths and prejudices that strongly influence stance on sexuality. The aim of the study was to ascertain women's level of awareness, relevance, accessibility and interest in the sexuality issue during pregnancy.

Material and method: The study was implemented on 107 pregnant women (age 28 ± ...). A self-made questionnaire was used as the data collection method and descriptive statistics was employed at evaluation process.

Results: The survey found out that at most women (71%), there came certain changes in sexuality during their pregnancy. Problems occurred at 39,3% of respondents. Some 55,1% revealed concerns about sexuality in pregnancy while most of these concerns were connected with fear of hurting the baby. Although most women (64,4%) were informed about sexuality during pregnancy, only 33,6% of them stated they have sufficient knowledge. At inquiring their level of awareness we also find out that some of the women had wrong or distorted information. Some 65,4% of respondents expressed some interest about sexuality awareness in this issue and they mainly got information from their gynaecologist or midwife. The most common source of information was the Internet (62,6%), but these information were not relevant. We found lack of high-quality awareness from experts. Only 32,7% of women were informed by their gynaecologist or midwife.

Conclusion: Midwives, as primary health-care providers together with other members of health-care professions bear collective responsibility for education in sexuality, especially during pregnancy; mainly because of increased level of need for information and support caused by physical and mental changes that among others influence not only sexuality but also women's interest in being educated. As our study proves, many of these women have been given insufficient or distorted information.

SEXUAL BEHAVIOR AMONG ADOLESCENTS: KNOWLEDGE AND USE OF CONTRACEPTION

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Introduction: In adolescence period reproductive capacity and even physical growth are gradually reaches. In terms of health, it is important to pay attention to the sexual 43anuary4343 of adolescents, their health education within the meaning of minimizing the possible risks and incidence of sexually transmitted diseases.

Materials and methods: The aim of this study was to determine the level of social 43anuary4343 of adolescents in relation to knowledge and use of contraception. The data collection was used by method of an exploratory questionnaire of our own design. We use non-standardized questionnaire designed for students aged 15 – 21 years. It contained 26 questions conceived into 2 areas – knowledge and behavioral. Within the frame of answers, we used a 5 point Likert scale. Researched group consisted of 381 respondents. The average age was 17.75 (± 1.28). We achieved 95% responsiveness.

Results: From the analysis of the results, we found that the behavioral field (bO) of a questionnaire over more than half of adolescents 61.35% (2, 49 \pm 1, 91) already had sexual intercourse. In the 16th – 17th years, the majority of respondents 58, 01% (3, 43 \pm 1, 92) has more than two sexual partners. We found out differences in using contraceptive methods for various reasons (39.37% vs. 7.63%). Analysis of knowledge areas (vO) points to the fact that only 24, 41% (2, 68 \pm 1, 26) know that contraception prevents STD transmission. 71, 65% (3, 91 \pm 1, 10) of adolescents agree that it is important to know the health status of the partner before the beginning of sexual activity.

Conclusion: Our findings are comparable with similar studies (Dobáková, 2014. Kalina, 2006, HBSC, 2010) and the established facts shows that adolescents currently beginning to live an active sex life earlier (16th-17th year) as in the past. Identified sexual 43anuary4343 can be considered as a risk. Evidence of significant differences in terms of gender and age could be important argument for more effective education of the adolescents by midwives, and for strengthening compliance for safe sexual behavior.

Keywords: knowledge, contraception, sexual behavior, adolescence

EFFECT OF ANTENATAL EDUCATION ON FIRST STAGE OF LABOUR

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Introduction: In the last couple of years, many studies were realized, and they pointed out a positive influence of antenatal education (AE). Midwives are responsible for leading an antenatal education and it should be an integral part of prenatal care.

Goal: The main goal of our study, was to ascertain effectivity of an AE during the first stage of labor in regard to psychic needs, fear and perception of a pain of woman in labor. We focused on comparisons between women with and without antenatal education.

Methodics: 253 primiparas, all with a low risk during the labor were included in the research. This sample included 111 respondents, who underwent AE during pregnancy and 142 respondents who did not. Datas were obtained by using an observation sheet. Research took place from November of 2015 to February of 2016. The results were processed by simple descriptive statistics.

Results: Results pointed out positive influence of AE during the labor of primiparas. Female respondents, who underwent an AE, indicated lower numbers on the pain scale (on average 6) than primiparas, who did not undergo AE and whose average on the pain scale was 8. 78,37% (N 87) of respondents, who underwent AE and 64,08% (N 91) respondents without AE, did not have to be educated during the labor. Up to 96,30% (N 107) of respondents with AE were breathing properly unlike 83,80% (N 119) without AE.

Conclusion: This study, points out the positive influence of AE during the labor for a sample of respondents with AE, especially in the field of perception of the pain and in the field of using the proper breathing techniques. On the other side, this study did not prove strong influence of AE on the duration of the first stage of labour.

THE KNOWLEDGE, ATTITUDES AND SELF-EVALUATION OF YOUNG WOMEN ABOUT THE PREGNANCY

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Introduction: The pregnancy is an important period of women life, but this part can be connected with many complications. The knowledge, attitude and self-evaluation of women about the pregnancy can affect lifestyle of woman during the pregnancy and hence prevent complications.

Methods: The aim of this study was to assess self-evaluation, attitudes and knowledge in relation to pregnancy. In this study was used questionnaire method – questionnaire of our own design. It contains 11 demographic and categorization of questions, 41 questions of this three studied areas (self-evaluation, attitudes and knowledge). The studied group consisted of $n=402$ women in age 20-40 years. The mean used age was 27.82 years (SD 4.85).

Results: In the sub-group SelfAssessment (DSA) 79.1% (4.08 ± 0.85) woman to evaluate their knowledge about pregnancy very high. There was evidence of statistical significance of the effect of age in the sub-group questionnaire DSA. We found differences in a positive self-evaluation of readiness for birth versus assessing the readiness of their friends (63.18% vs 49%). Even 75.37% (5.36 ± 1.58) of women think that we will be able to cooperate during the birth. In the subgroup focused on attitudes (DSP) to 99.51% (4.88 ± 0.37) women perceive as the most important their psychological well-being. Even 98.26% (4.90 ± 0.45) women perceive the very important visit gynecological counseling during the pregnancy. There was evidence of statistical significance of the effect of education related to pregnancy throughout DSP sub-group ($p = 0.006$). In the knowledge part of the questionnaire, 72.81% of women showed proper knowledge about their lifestyle and the pregnancy.

Conclusion: The results point to a high degree of self-evaluation of knowledge of young women in relation to the pregnancy and lifestyle. The knowledge level of young women about pregnancy in our research group can be considered satisfactory at 72.81%, while high selfassessment knowledge was shown in more respondents (79.1%). The results are comparable to similar studies (Kamenská, 2014; Gruberová, 2007). Evidence of significant differences in selfassessment and attitudes of women could be beneficial to argue concerning the requirements to streamline the educative role of midwives in relation to the prevention of complications during pregnancy and childbirth.

Keywords: knowledge, attitudes, self-evaluation, pregnancy, childbirth and lifestyle.

KNOWLEDGE AND ATTITUDES OF WOMEN RELATED TO PREVENTIVE GYNAECOLOGICAL EXAMINATIONS

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Introduction: Knowledge and attitudes of women related to preventive gynaecological examinations we consider as important in terms of their significance in relation to the early detection of gynaecological malignancies.

Material and methods: The aim of this study was to find out what is the level of knowledge and attitude of women to preventive gynaecological examinations (PGP). To determine the knowledge and attitudes of women, we used a questionnaire of an own construction. The questionnaire was divided into 3 areas – behavioral (dB), knowledge (dV) and the attitudes and factors that influence women to visit PGP (dP), contained 5 categorization of items and 48 questions. Researched group consisted of 550 respondents. The average age of respondents was 25.85 (± 7.68). Statistical data and hypothesis testing were processed by using methods of descriptive statistics and using tests – Kruskal-Wallis test, Mann-Whitney test and Spearman correlation coefficient $r(S)$.

Results: In the behavioral area (dB) more than half of the respondents, 61.09% (4.09 ± 1.41) visit a PGP annually. Statistically significant differences were shown in all three study areas (dB, dV, dP) in terms of age, education, geographical breakdown and sex of gynaecologist. In the area (dV) women achieved underaverage level of knowledge (52.11%). Area of attitude (dP) and the factors that influence women to visit PGP showed that most women 76.18% (4.65 ± 0.76) consider gynaecological examination as important.

Conclusion: The results point to the fact that the women's level of knowledge on the subject is below average. The majority of respondents have a positive attitude to preventive gynaecological examinations. Our results are comparable with similar studies (Borovský, 2015; Uričková, 2014; National Health Information Center, 2014). Due to the ascertained facts it is important to strengthen the educative role of health workers and increase compliance of women with PGP.

Keywords: knowledge, attitudes, gynaecological examination, prevention

FEAR OF PREGNANT WOMEN AND WAYS OF COPING

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Introduction: Fear is a natural emotion of pregnant women. Sometimes not communicated it may intensify and then it has a negative influence on a woman, a child and a birth. The aim of the study was to find out whether pregnant women experience fear, its contents, coping with it, the intensity of the fear of the birth and its connections with selected variables (age, birth history and stage of pregnancy).

Material and methods: 156 pregnant women were interviewed (29.66 ± 4.73 years). The method of data collection was a questionnaire. The first part of the questionnaire questions was focused on the findings from what women feel fear during the pregnancy and their support systems in coping with fear. The second part was constituted by the standardized questionnaire W-DEQ, A version that deals with the detection of intensity of experiencing fear of childbirth. For evaluation we used descriptive statistics, Spearman's rank correlation coefficient, ANOVA, Student's t-test.

Results: We have found that pregnant women experience the greatest fear of the health of the child, and the fear of the childbirth. The most common support person in coping with their fear was a partner / husband (51%) and most women felt help in coping with fear in the conversation with a support person (47.12%) and in the interview and support of a doctor / midwife (21.58%). The average intensity of the fear of childbirth in our group was 68.74. The strong fear of childbirth (W-DEQ ≥ 85) was identified in 9 women (5.73%). We have found the statistical significance between the intensity of the fear of birth and the age ($R = -0.16$, $p = 0.05$). The differences in experiencing fear between the primiparas and women who already gave birth in the past were on the border of the statistical significance ($t = 1.819$, $p = 0.071$). The statistical significance was also shown between the intensity of experienced fear and the previous complicated birth ($F = 4.387$, $p = 0.005$). The differences in experiencing the intensity of the fear according to the trimester of women's pregnancy were not statistically significant ($F = 2.01$, $p = 0.137$).

Conclusion: Our research has shown some facts that may help to improve primary nursing care for pregnant women within preventing and coping with the fear in pregnancy and during the birth and that midwives may have a significant share with positive accompanying women during pregnancy and childbirth.

Keywords

Fear of a pregnant woman, fear of childbirth, coping with fear, fear prevention, midwife

ACCOMPANYING PERSON DURING CHILDBIRTH FROM THE PERSPECTIVE OF THE LABORING WOMAN

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Introduction: Recently, greater emphasis is put on survival positive birth experience. Women in labor can easily handle such a difficult situation as childbirth, when they feel continuous support from a close person. The aim of the article was to focus on women's expectations on accompanying person before the childbirth and to learn, in which field the accompanying person played the most important role during labor.

Material and methods: The research was based on a questionnaire. The questionnaire was distributed via 22 maternal centres in the Slovak republic during December 2015 and February 2016. The sample consisted of 166 respondents (n=166) with average age of 30.4 years. All of them had experience with accompanying person during labor. Data were processed using simple descriptive statistics and for question No.17 chi-squared test was used.

Results: The most accompanying person was a partner of woman in labor (93.9%, n=156). Before childbirth women expected help especially in the field of psychological area – elimination of loneliness (83.7%, n=139); in the field of emotional area – greater feeling of safety (83.1%, n=138), expression of support by embracing, holding hands (76.5%, n=127); in the field of physical support – help during walking and showering (60.8%, n=101); and finally in the field of information support – obtaining information from medical staff (65.7%, n=109). Due to the accompanying person 96.4% (n=160) of women felt less stress and 82.5% (n=137) of respondents experienced greater pleasure of childbirth. According to 53.6% (n=89) respondents the presence of accompanying person positively influenced the staffs' behavior.

Conclusion: The results indicate the validity of theoretical knowledge that the presence of a close person for woman in labor has a great importance especially in the field of psychological and emotional support and positively affects the survival of childbirth.

FACTORS THAT AFFECT THE USE OF NURSING DIAGNOSES IN PRACTICE

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INTRODUCTION: The main aim of the study was to identify which factors influenced the use of nursing diagnoses in clinical practice.

MATERIAL AND METHODS: A non-standardized questionnaire (authors Macková, Zeleníková, 2012) on factors influencing the use of nursing diagnoses was administered to a sample consisting 57 nurses from Hospital with out-patient clinic in Levoča. Using Likert's 5 point scale, the nurses rated the importance of several factors in influencing their use of nursing diagnoses in practice.

RESULTS: Based on our sample, there are several factors which influence the use of nursing diagnoses in practice. Some of them are: number of patients per nurse, administrative strain, lack of staff. The less influencing factors are differences in education of nurses, the opportunity to educate in this issue and diagnostic terminology.

The results show that nurses working in standard nursing units considered to be the most influencing factor the number of patients per nurse and nurses working on the intensive care wards considered to be the most influencing factor the lack of staff. There are also differences in terms of education of nurses. Nurses with bachelor's and master's degree considered a factor most influenced the number of patients per nurse and Registered Nurses administrative strain and lack of staff.

CONCLUSION: Recognizing the factors which influence the use of nursing diagnoses and enhancing institutional support can facilitate the implementation of nursing diagnostics in practice.

PAIN OF PATIENT WITH SYNDROM OF DIABETIC FOOT

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Introduction: The pain of diabetic foot syndrome affects physical, mental and social aspects of patient's life. Pain worsens the overall comfort and mobility, lead to loss of independence motivation and more patients to depression, which has an impact on patient management.

Materials and methods: The research sample consisted of 50 patients with diabetic foot syndrome of diabetes care and hospitalization for vascular Division Department of Surgery, University Hospital in Martin from January to April 2016. For clear examination of pain and its impact on life, we used protocol for nursing assessment of pain in diabetic patients with diabetic foot. The research was approved by the ethics committee of the University Hospital in Martin.

Results: The collected data were evaluated using descriptive statistics. Our sample consisted of 31 male patients and 19 female patients, with average duration of diabetes 14 years and the average age was 67 years. The pain occurred in 86% (n=43) patients with diabetic foot syndrome and 63% were male patients. Pain was most commonly reported by respondents as burning and stabbing. Intensity designed on the numeric scale of pain was evaluated from 0 to 10. Most often patients reported problems in physical area such as: a significant limitation of walking, physical activity and sleep related frequent waking and tingling in the legs. Pain in the psychosocial sciences patients especially to the problems of powerlessness and social isolation.

Discussion and Conclusion: Respect the impact of pain on all areas of life of patients with diabetic foot syndrome could help professionals to its comprehensive resolution. Documenting and pain therapy should be multidisciplinary in nature.

NURSING DOCUMENTATION FOR PATIENT WITH STOMA

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Introduction: Stoma is an artificial opening of a hollow organ to the body surface, which is created by surgeon in order to ensure the excretion of stool or urine. We have same types of nursing documentation, designed for specifically defined group of patients on particular wards. They dedicate in detail particular problems and help to adapt nursing care to requirements of particular disease.

Materials and methods: On the grounds of nursing documentation standards commonly used in Slovak Republic and also abroad we drew up a file of stoma card. It consists of a form of perioperative and post-operation assessment (14 days). We focused on general assessment of patient's health, assessment of skin in area of planned stoma opening, selection of appropriate place for stoma, perioperative medical treatment preparation, education and post-operation 14days long assessment of stoma, peristomal area, and surgical wound. Further, we consider dietary intake and food tolerance, possible complications, mental condition and his capability of self-dependant treatment of stoma. The proposed file of stoma patient is analyzed by 20 nurses, working on septic nursing unit of Surgical clinic and transplantation centre in UNM in Martin and septic Intensive Care Unit and also 20 graduated nurses of a certificated course Nursing of patients with stoma from the whole territory of Slovak Republic.

Results: Our conclusion is that the biggest cons are within the scope of documentation (time-consuming by documentation), we would welcome its integration to the hospital information system.

Discussion and conclusion: The proposed file of stoma patient was after examination adjusted to the suggestions of nurses in order to serve for practical purposes in clinical practise and education of students.

EVALUATION OF CLINICAL PRACTICE FROM THE STUDENT PERSPECTIVE

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Introduction. The professional training of nursing students is preferred such a style of teaching, which mainly supports the activity of a student, their autonomy and creativity in clinical practice. The emphasis on linking theory with practice is increasing in order to keep leading nursing students to the acquisition of their competencies in the profile of the graduate program of a nursing study.

Material and methods. We used the method of standardized questionnaire Clinical Learning Environment and Supervision evaluation scale (CLES) constructed for nursing students to evaluate their clinical placements. The questionnaire was completed by 34 final year nursing students. They evaluated clinical practice to 4 workplaces after their clinical practice by the mentors.

Results. The students were generally satisfied with the conduct of clinical practice through mentoring. In all subscales of the questionnaire they evaluated the clinical practice better and more positive in a psychiatric placements in comparison with surgical placements, internal medicine placements and pediatric placements. In the subscale evaluating Atmosphere on the Ward most of the students rated the Psychiatry ward (4.28) and the lowest average scores were in internal placements (3.68). Subscale of the Teaching Style Head Nurse was the best evaluated in a psychiatry ward (4.08) and the lowest average scores were achieved in wards of surgical disciplines (3.60). Subscale of Nursing Care on the Wards was the best in pediatric placements (4.39) and the lowest average scores were achieved in internal medicine placements (4.04). Learning on the Ward subscale was the best evaluated in a psychiatric placements (4.01) and the lowest average scores were in pediatric placements (3.62). Subscale Supervisory Relationship got the best results in internal medicine placements (4.33) and the lowest average scores were achieved in pediatric placements (3.96).

Conclusion. The score in each subscale ranged from 3.60 to 4.39, which can be interpreted as a relatively high satisfaction. Due to the effective assessment of the overall student's satisfaction with the conduct of clinical practice. It remains essential to ensure such an approach in teaching of other nursing students.

REALIZATION OF THE NURSING PROCESS METHOD IN CLINICAL PRACTICE

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Introduction: Nursing process is a systematic, rational and cyclic method of providing of nursing care. Legislation of SR regulates its realization. Nurses are required to write medical records at range of nursing care to documented realization of nursing process.

The goal of study was to find out realization of nursing process at selected department in University Hospital in Martin.

Material and methods: We included 102 medical records of patients, which were hospitalized at Intermediate Care Unit from november 2015 to january 2016. The selection of medical records was deliberated. Data were gained by content analysis of medical records at range of nursing care based on prepared form. We issued from valid legislation of SR.

Results: Results of study indicated several shortages. We found out, that assessment of patients is incomplete. At 99 medical records we identified absence of record about 1 or more required data concerning to patient`s needs. Diagnosing is regulated by Decree of Ministry of health of the SR 306/2005 including List of nursing diagnosis. The most frequent mistake was incomplete record of actual nursing diagnosis at structure P-E-S (absence of symptoms).

Realization of planning nurses documented through elaborated plans of nursing care. Professional guide of Ministry of health of the SR about writing of medical records doesn`t determinate it. This plans didn`t provide data about individualized nursing care. Realization of nursing interventions was documented. In 102 medical records we found out incompleteness of data about realization of nursing interventions.

Evaluation was documented by record at Nursing transmitted form. In 7 cases this form was incomplete, in 4 cases this form was missed.

Conclusion: Results of study indicated, that nurses don`t respect consistently requirements about nursing process in clinical practice. Barriers for implementation of nursing process are different and they can be object of other study.

NURSING STUDENTS' ATTITUDES TOWARDS EUTHANASIA AND ASSISTED SUICIDE

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Introduction. Nursing students' attitudes toward euthanasia (EUT) and assisted suicide (ASIST) are of particular importance because of their future involvement in the care of the dying patient. The aim of the study was to identify nursing students' attitudes toward the EUT and ASIST arguments and to determine differences between respondent's groups.

Material and methods. Research was realized by the questionnaire created by ourselves. The questionnaire included two scales (EUT; ASIST) based on arguments about EUT and ASIST. The Likert's scale 1-5 was adopted. The Mann-Whitney Test was used to find out the differences between the groups. There were 41 students of nursing at JFM CU and 199 at CU in Ružomberok as participants.

Results. Student's attitudes were slightly negative (EUT: $2,44 \pm 0,79$; ASIST: $2,55 \pm 0,81$). Argument: „It will be good for the patients, if there is an opportunity for euthanasia and/or assisted suicide even if they don't used it. Because they know, if their health is very bad, this opportunity is still here” achieved the highest score as a pro argument (EUT: $3,07 \pm 1,584$; ASIST: $3,00 \pm 1,609$). On the other hand, the argument against with the highest score was: “If euthanasia and assisted suicide were legalized, there is a chance, it will be abused” (EUT: $3,98 \pm 1,267$; ASIST $3,97 \pm 1,301$). There were no differences between student's attitudes between the universities. Statistical differences were found between students with ($2,50 \pm 0,80$) and without ($2,79 \pm 0,79$) experience with dying patients on the scale ASIST. There were statistical differences between religious students (EUT: $2,38 \pm 0,75$; ASIST: $2,49 \pm 0,77$) and nonreligious students (EUT: $3,22 \pm 0,87$; ASIST: $3,36 \pm 0,86$) on both scales.

Conclusion. Our results indicated the connection between respondent's religion, student's experience with dying patient and attitudes to EUT and ASIST. For the verification of this other research on representative sample is needed. Questionnaire can be used in education to reflection EUT and ASIST argumentation.

Acknowledgements. The abstract was written as a part of grant KEGA 006UK-4/2014: *Human dignity in the context of death and dying.*

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The conference is supported by



Dr. Jozef Lettrich Foundation
Nadácia Dr. Jozefa Lettricha
Benkova 10
036 01 Martin
www.nadacialettricha.sk

MARTIN

Authors are responsible for content of abstracts.

Abstracts from XXXVII. Student Scientific Conference JFM CU, Martin

Created by: RNDr. Veronika Čapláková and RNDr. Eva Blahovcová, PhD.

Comenius University in Bratislava

Jessenius Faculty of Medicine in Martin

Number of pages: 60

2016