

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



**XXXIV. STUDENT SCIENTIFIC CONFERENCE**

**PROGRAM and ABSTRACTS**



**April 23, 2013  
Martin, SLOVAK REPUBLIC**

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

Modern education for modern society



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## PROGRAM AT GLANCE

**Date:** April 23, 2013

**Place:** Aula A Novomeskeho 7 (next to the student hostel)  
Aula B Novomeskeho 7 (next to the student hostel)  
Aula C Mala hora 5, Stefanik Institute

**Registration:**

April 22 (13.00 – 17.00) Simulation Education Centre, Novomeskeho 7A  
April 23 (before the beginning of your section)

**Opening ceremony** (Aula A – Novomeskeho 7) **8.00 – 8.10**

**Aula A**

A1: Section of Theoretical Disciplines 8.15 – 9.45  
- coffee break  
A2: Section of Preclinical Disciplines 10.00 – 12.00  
- lunch  
A3: Section of Guests 13.00 – 14.00  
A4: Closing ceremony 14.00

**Aula B**

B1: Section of Clinical Disciplines 8.15 – 10.15  
- coffee break  
B2: Molecular and Oncological Section 10.30 – 12.15

**Aula C**

C1: Section of Nursing 9.00 – 10.00  
- coffee break  
C2: Section of Non-Medical Study Programmes 10.30 – 11.30

**Closing ceremony** (Aula A – Novomeskeho 7) **14.00**

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**Duration of lectures** (Slovak or English language):

**8 minutes, discussion – 4 minutes**

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## PROGRAM IN DETAILS

„Aula A“

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- Milan Čipkala:** The effect of combined therapy on inflammation in meconium aspiration syndrome 12
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„Aula A“

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**Coffee break**

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„Aula A“

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## **ABSTRACTS**

All abstracts are available in English at [www.jfmed.uniba.sk](http://www.jfmed.uniba.sk) – ŠVOČ

## **COUGH REFLEX REDUCTION DURING UNILATERAL COLD BLOCK OF VAGUS NERVE IN CATS**

**Lucia Babálová**

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Unilateral cooling of the vagus nerve at a cervical level was performed on 7 (and control cooling of the sympathetic part of vagosympathetic trunk on 5) pentobarbitone anesthetized spontaneously breathing cats. Inhibition of cough induced mechanically from the tracheobronchial region during the nerve cooling below 6 °C, that presumably blocks conduction in myelinated nerve fibers, was studied. EMGs of the diaphragm (DIA) and the abdominal muscles (ABD) together with blood and esophageal pressures (EP) were recorded. EMGs were normalized to the average values of control pre-cooling coughs. ANOVA analysis was employed in statistical evaluation. The unilateral cold block of the vagus nerve reduced the number of coughs induced by 10 s stimulation from  $14.2 \pm 4.8$  to  $2.7 \pm 1.8$  ( $p < 0.001$  vs. control,  $p < 0.01$  vs. re-warming value of  $9.7 \pm 4.0$  coughs). Cough related ABD EMG amplitudes decreased to  $7 \pm 2$  % during “vagus cooling” coughs ( $p < 0.001$ ;  $p < 0.01$  vs. re-warming value of  $54 \pm 19$  %). Similarly, the cough expiratory amplitudes of EP lowered from  $2.13 \pm 0.50$  kPa to  $0.30 \pm 0.09$  kPa ( $p < 0.01$ ;  $p < 0.05$  vs. re-warming values of  $1.30 \pm 0.47$  kPa). Inspiratory cough efforts were reduced by the unilateral cold block of the vagus nerve just nonsignificantly. Control cooling of the sympathetic part of the trunk had no effect on tracheobronchial cough (all  $p > 0.4$ ). Surprisingly, intense cough inhibition induced by the unilateral vagus nerve cold block (at a high cough excitability level – see the number of coughs) suggests a complex central processing of afferent signals from the cough receptors in order to induce cough response.

## THE EFFECT OF METHANOL AND ITS METABOLITES ON THE SURVIVAL OF NEURAL CELLS

**Oliver Causov, Alexander Schubert, HenrietaŠkovierová, Radovan Murín**

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Methanol is commonly used solvent, occasionally present in low quality beverages. The intoxication with methanol is associated with early development of neurological symptoms followed by neurodegeneration.

To evaluate the effect of methanol and its metabolites – formaldehyde and formate – on the survival of neural cells, we used glioblastoma (C6 and T98G) and neuroblastoma (NS-20 and SH-SY5Y) cell lines of murine and human origin as a study model. The culture media were supplemented with methanol or formaldehyde or sodium formate up to concentration 25 mM. After 72 h incubation, the viability of cells was tested by determining the total enzymatic activity of lactate dehydrogenase in cell lysates and visualization of cell nuclei. The both methods revealed that methanol up to 25 mM concentration had no toxic effect on all tested types of cell lines. In contrast, sodium formate or formaldehyde present in culture media in concentrations above 2.5 mM or 2.5  $\mu$ M, respectively, induced cell death among all tested cell lines.

Our results indicate that methanol in comparison to formaldehyde and formate exerts no toxic effect on cultured neural cells. Therefore, we may hypothesize, that neurological symptom typical for methanol intoxication may be a result of the methanol metabolism in non-neural tissues linked with generation and subsequent release of both, formaldehyde and formate, into the blood stream. The successive uptake of these two compounds into the brain parenchyma may be essential for exertion of their neurotoxic effects.

This work was supported by VEGA 1/0242/13 and project "Center of excellency for research in personalized therapy (Cevypet)", code: 26-220120053.

## THE EFFECT OF COMBINED THERAPY ON INFLAMMATION IN MECONIUM ASPIRATION SYNDROME

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**Introduction:** Meconium aspiration syndrome (MAS) is a serious disorder of new-borns with respiratory failure and inflammatory processes. This inflammation may aggravate therapy of exogenous surfactant, which is commonly used in clinical praxis. Therapeutic effect of surfactant could be improved with anti-inflammatory agent - budesonide.

**Material and methods:** Experimental animals (White New Zealand rabbits) were divided into four groups: control group with saline i.t. (Sals), meconium i.t. without therapy (Mec), meconium with surfactant therapy (Surf), meconium with combined therapy (Surf+Bud). Respiratory parameters (PaO<sub>2</sub>/FiO<sub>2</sub> and hemoglobin saturation) were measured at hourly intervals throughout the experiment. Biomarkers of oxidative damage and inflammation as thiobarbituric acid reactive substances (TBARS) and cytokines (IL-1 $\beta$ , IL-8) were determined in final plasma. Lung edema represented as wet/dry weight ratio (W/D) and TBARS was evaluated in lung tissue after sacrifice the animal.

**Results:** Surf+Bud therapy improved PaO<sub>2</sub>/FiO<sub>2</sub> compared to other groups ( $p < 0,05$ ), decreased W/D ( $p < 0,005$  vs. Mec), TBARS in plasma (0,001 vs. Mec), TBARS in tissue (0,005 vs. Mec) and level of IL-1 $\beta$  (0,02 vs. Mec), IL-8 (0,06 vs. Mec).

**Conclusion:** Combined surfactant and anti-inflammatory therapy improved lung functions, reduced lung edema, oxidative damage and levels of cytokines and had better effect compared to surfactant monotherapy.

**Supported by:** APVV-0435-11, VEGA 1/0057/11, VEGA 1/0291/12

## PARTICIPATION OF SINGLE NUCLEOTIDE POLYMORPHISMS OF INTERLEUKINS IN PATHOPHYSIOLOGY OF CHILDREN WITH BRONCHIAL ASTHMA

Gabriela Decká

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**Introduction:** In recent years cytokines and their receptors have been shown to be highly polymorphic. It has also been established that cytokines play key roles in the regulation of immune responses. Interleukin 10 (IL-10), the main anti-inflammatory and immunoregulatory cytokine, is known to play various roles in immune-regulating and anti-inflammatory responses, especially in the prevention of hypersensitivity and autoimmunity development. Several polymorphic sites within the promoter region of the IL-10 gene have been described. In our research, we decided to focus mainly on the polymorphism at position -592. The -592 polymorphism is a C to A substitution and lies within a region with a negative regulatory function. We studied the possible involvement of IL-10 polymorphism in the susceptibility for childhood asthma.

**Patients and methods:** Our study group consisted of 248 patients (aged  $12.28 \pm 0.24$  years). The control group was composed of 249 patients of comparable age ( $13.14 \pm 0.48$  years). All the children underwent blood sampling for genetic analysis.

**Results:** The allele frequency of A in healthy subjects was 18.7 % and in asthmatic patients 28.8 % ( $p < 0.0001$ ). The CC genotype was found in 68.7 % of healthy subjects, CA in 26.5 % and AA genotype in 4.8 %. In asthmatics, the CC genotype was present in only 53.6 %, remarkably less than in healthy subjects. CA was found in 35.1 % of the patients and AA genotype in 11.3 % ( $p = 0.01$ ).

**Conclusion:** The polymorphism within the IL-10 promoter at position -592 C/A may have a disease-modifying effect in the asthmatic airway. It may lead to decreased IL-10 expression by immune cells, what can be thought to favor asthma susceptibility.

**Acknowledgement:** This work was supported by VEGA 1/0071/11.

## **MODULATION OF COUGH BY GABA-ERGIC INHIBITION WITHIN THE MEDULLARY RAPHE NUCLEI IN CATS.**

**Boris Dobroľubov**

Institute of Medical Biophysics, JFM CU, Martin

Tutors: Doc. RNDr. Ivan Poliaček, PhD; Prof. MUDr. Ján Jakuš, DrSc; RNDr. Michal Šimera, PhD; Ing. Marcel Veterník

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Ten pentobarbitone anesthetized spontaneously breathing cats (n=10) were used to study effects of GABA microinjections in the medullary raphe on cough. GABA is the principal inhibitory neurotransmitter in the mammalian CNS. Six microinjections of 1 mM GABA in artificial cerebrospinal fluid were made during one protocol (206±15 nl per all 6 microinjections) at the depths (2 injections at each) of approximately 1.1, 2.2, and 3.3 mm below the dorsal medullary surface at four separated rostro-caudal positions: approximately 1 mm caudal (n=5), 1 mm (n=6), 2.5 mm (n=5), and 4 mm (n=4) rostral to the obex. Cough was induced mechanically by a soft catheter in the tracheobronchial area. Blood pressure, esophageal pressure (EP) and EMGs of the diaphragm (DIA) and the abdominal muscles (ABD) were recorded. DIA and ABD EMGs and EP were normalized to their mean amplitudes during control pre-microinjection coughs. ANOVA and paired t-test were employed in statistical analysis. GABA microinjections 4 mm rostral to the obex reduced the number of coughs during 10s stimulation from 4.7±0.5 to 3.4±0.3 (p<0,05; 4.4±0.7 coughs in recovery 6-60 min after microinjections, p<0,05 vs. post-microinjection value) and amplitudes of cough ABD EMG to 41±3% (p<0.01; recovery 58±10%, p<0.01 vs. control) and of cough expiratory EP to 57±14% (p<0.05; recovery 80±13%, p>0.05). Cough expiratory efforts were also reduced by GABA microinjections 1 mm rostral to the obex: amplitudes of ABD EMG to 48±10% (p<0.001) with recovery 74±5% (p<0.01 vs. both post-microinjections and control values) and of cough expiratory EP to 62±12% (p<0.05 vs. both control and recovery of 99±14%). Other parameters were not significantly altered by GABA microinjections. Our data confirmed a contribution of active GABA-related inhibition in medullary raphe in generation (and inhibition) of cough reflex, however, with markedly different efficiency and diversity of effects at different rostro-caudal raphe positions.

## STRESS: IMPACT ON THE SELECTED PHYSIOLOGICAL VARIABLES IN THE INTERACTION WITH SUBJECTIVE CHARACTERISTICS

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**Introduction:** Pavlov's psychophysiological concept of higher nervous activity typology, based on experiments with conditioned reflexes, consisted of four types of temperaments characterized by the strength of excitation and inhibition, the equilibrium and mobility between these processes. Recent sophisticated theory of personality, Cloninger's model, is focused on the inherited *temperament* dimensions [novelty seeking (NS) indicating behavioural activation system (BAS), harm avoidance (HA) indicating behavioural inhibition system (BIS), reward dependence (RD) and persistence (P)] and *character components* [self-directedness (SD), cooperativeness (CO) and self-transcendence (ST)]. The aim was to study the cardiac autonomic control in the interaction with personal traits at rest and in response to stress (cardiovascular reactivity).

**Methods:** The continuous ECG signal was monitored in the group of fifty students (age:  $22.9 \pm 0.1$  yr., BMI:  $22.0 \pm 0.4$ ; 25 women) at rest, and during mental stressors. Subjective characteristics were evaluated using Temperament and Character Inventory. Heart rate variability (HRV) was analyzed in time and frequency domains: RR interval, SD, rMSSD, pNN50, low frequency and high frequency band. Symbolic dynamics parameters - 0V%, 1V%, 2UV%, 2LV%, NCI, NUPI - were evaluated.

**Results:** Significant positive correlation was found between HA and 1V% at rest ( $r=0.422$ ;  $p=0.008$ ); however, the correlation between HA and 0V% was negative during arithmetic test ( $r=-0.356$ ;  $p=0.028$ ). Temperament trait P was inversely correlated with 1V% ( $r=-0.455$ ,  $p=0.004$ ). Significant positive correlation was found between resting complexity parameters (NCI, NUPI) and CO ( $r=0.391$ ,  $p=0.015$ ;  $r=0.383$ ,  $p=0.018$ , respectively).

**Conclusion:** Our study revealed several associations between subjective characteristics and HRV parameters, which have not been described until today. These findings could improve towards the individual therapeutic approach in the diseases associated with stress.

**Acknowledgement:** This study was supported by European Centre of Excellence for Perinatalogical Research no. 26220120036, by European Centre of Experimental and Clinical Respiriology no. 26220120034, grant VEGA 1/0059/13, grant UK no. 459/2012.



## TUMOR-SUPPRESSIVE EFFECTS OF *CHLORELLA PYRENOIDOSA* IN THE PREVENTION OF EXPERIMENTAL MAMMARY CARCINOGENESIS

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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. This study is the first report on the preventive effects of *Chlorella pyrenoidosa* in experimental mammary carcinogenesis *in vivo*.

In this experiment, the antineoplastic effects of *Chlorella pyrenoidosa* in the chemoprevention of N-methyl-N-nitrosourea induced mammary carcinogenesis in female rats were evaluated. Chlorella powder was dietary administered at two concentrations of 0.3 % and 3 %. The experiment was terminated 14 weeks after carcinogen administration; mammary tumors were removed and prepared for histomorphological and immunohistochemical analysis.

Basic parameters of experimental carcinogenesis, chosen metabolic variables and side effects after long-term chlorella treatment in animals were assessed. Chlorella at higher concentration suppressed tumor frequency by 61 % ( $P < 0.02$ ) and lengthened tumor latency by 12.5 days ( $P < 0.02$ ) in comparison with the controls. A histopathological analysis of mammary tumors has revealed a shift in the rate of poorly differentiated (high grade, HG) and well differentiated (low grade, LG) carcinomas to higher representation of LG lesions after treatment with chlorella. The effects of chlorella on selected parameters of apoptosis, proliferation, and angiogenesis in mammary tumor cells will be in a short time determined. Phytopharmacum in higher dose significantly decreased serum LDL-cholesterol and significantly increased body weight in animals.

Our results suggest apparent tumor-protective effects of *Chlorella pyrenoidosa* in rat mammary carcinogenesis.

This work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic under contract no. VEGA 1/0071/13 and VEGA 1/0043/12.

## **ANTITUSSIVE EFFECTS OF NASAL THYMOL AND EUCALYPTOL CHALLENGES IN HEALTHY VOLUNTEERS**

**Tomáš Buday, Eva Lichnerová**

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Cough, the most important airways defensive mechanism is modulated by many afferent inputs either from respiratory tussigenic areas, but also by afferent drive from other organs. Modulation of cough by nasal afferent inputs could either facilitate cough response or inhibit it in animal models, depending on the type of trigeminal afferents which are stimulated. In recent study we addressed the question of possible modulation of cough response in human healthy volunteers by nasal challenges with thymol and eucalyptol as agonists. After nasal challenges using thymol and eucalyptol (both 0,025 ml,  $10^{-3}$  M, both nostrils) nasal symptom score, cough threshold (C2), urge to cough (Cu) and cumulative cough response had been tested.

Nasal challenges using both agonists induced pleasant olfactic sensations and in 6 out of 18 subjects also mild cooling sensation. Cough threshold was not influenced when compared with saline and vehicle intranasal challenge, but the total cough after nasal thymol challenge was significantly lower than that obtained after saline or vehicle. Importantly, subjects did not report the urge to cough, which appeared to correspond to C2.

We conclude that modulation of cough by thymol and eucalyptol is probably combined of trigeminal and olfactory origin.

Acknowledgement: Supported by VEGA 1/0031/11

## POTASSIUM ION CHANNELS IN MYOMETRIAL SMOOTH MUSCLE OF UTERUS

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Introduction: Myometrial smooth muscle of uterus is characterized by the presence of various ion channels that activity can be modulated pharmacologically using the specific agonists or antagonists. The aim of our experimental study was to clarify some mechanisms in human pregnant myometrium which could be responsible for the uterine activity during pregnancy and by using the selected substances to modify this activity. Material and methods: Samples of myometrium were taken from term pregnant women in which the pregnancy had to be terminated by Caesarean section. Subsequently the samples were processed in myometrial strips and placed in organ bath with Krebs-Henseleit solution. Myometrial contraction activity was evoked by the application of oxytocin used in clinical practice ( $10^{-6}$  mol.l<sup>-1</sup>). The amplitude of myometrial contractions was assessed after administration of agonist and antagonist K<sub>ATP</sub> potassium ion channels - *pinacidil* ( $10^{-5}$  mol.l<sup>-1</sup>) and *glybenclamide* ( $10^{-6}$  mol.l<sup>-1</sup>); agonist and antagonist BK<sub>Ca2+</sub> potassium ion channels - *NS1619* ( $10^{-6}$  mol.l<sup>-1</sup>) and *tetraethylammonium* ( $10^{-4}$  mol.l<sup>-1</sup>). The study was approved by the Ethical Committee of JFM CU. All women were instructed and signed informed consent. Results: K<sub>ATP</sub> ion channel agonist - *pinacidil* significantly decreased the contractile myometrial activity of human term pregnant myometrium induced by oxytocin in *in vitro* conditions. Inhibitory effect of pinacidil on the amplitude of myometrial contractions was significantly blocked by its antagonist of K<sub>ATP</sub> ion channels - *glybenclamide* which resulted in significant increase of contractile myometrial activity. In contrast, BK<sub>Ca2+</sub> ion channel agonist - *NS1619* and its antagonist of BK<sub>Ca2+</sub> ion channels - *tetraethylammonium* did not affect significantly the contractile myometrial activity of human term pregnant myometrium induced by oxytocin in *in vitro* conditions. Conclusion: Our findings could contribute to clarify the participation of some mechanisms that affect the contractile myometrial activity and thereby to reduce the risk of preterm labour.

Acknowledgements: VEGA 1/0062/11

## **CINEOLE, THYMOL AND CAMPHOR NASAL CHALLENGES AND THEIR EFFECT ON NASAL SYMPTOMS AND COUGH IN AN ANIMAL MODEL**

**Tomáš Dolák, Hana Lichá, Silvia Krížová**

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Inhalation of aromatic vapors in animals suppressed cough induced by citric acid (CA), however, new data based on rtPCR and experiments on animals with separated airways suggested that this effect is mediated via nasal afferents.

The aim of our study was to ascertain the efficacy of nasal application of cineole, thymol and camphor on nasal symptoms and CA induced cough in a model of airway hyperresponsiveness. Guinea pigs (n=13) were sensitized by intraperitoneal administration of ovalbumin, 21 days later sensitization was confirmed by skin tests.

Cough was induced by CA in plethysmograph for 10 minutes after nasal pretreatments with mentioned substances in rhinitis model (all 10-3M). Cough was recognized from record of sudden airflow changes interrupting breathing pattern and cough sound. Data were recorded via data acquisition system by Biopack and processed by ACQ Knowledge Software. Final count of coughs was established by blind analysis using SonicVisualiser. Dose responses curves, total cough count and cough latency were analyzed.

Repeated intranasal challenge with ovalbumin induces reproducible gradually worsening symptoms, and cough induced by CA during acute phase of allergic rhinitis was enhanced. Nasal pretreatment with cineole, thymol and camphor did not prevent onset of nasal symptoms, and the magnitude of symptoms was comparable to those without pretreatment. The most potent antitussive effect had camphor ( $25 \pm 3$  vs  $7 \pm 2$ ,  $p < 0.05$ ) and thymol ( $25 \pm 3$  vs  $14 \pm 2$ ,  $p < 0.05$ ). The data for nasal eucalyptol challenge did not reach significance. Cough latency followed this trend.

Our data are in agreement with already published results that aromatic substances can only minimize subjective symptoms perception, however, objective symptom scale remain unchanged. The trends for nasal challenge of these substances follow the trends for an inhalation of vapors published by Morice in 1998. Local nasal application in a form of sprays/drops is equally effective when comparing to inhalation technique.

## THE EFFECT OF ALCOHOL INTAKE ON RECOVERY TIME AFTER HEAVY RESISTANCE EXERCISE

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Alcohol is regularly consumed by many athletes and people that train for recreation. As large doses and misuse of alcohol are known to have several adverse effects on the human body, alcohol is considered to be detrimental for recovery from and adaptation to exercise (3). Indeed, alcohol consumption has recently been demonstrated to hamper recovery from muscle-damaging eccentric exercise (1). Less is, however, known about the effects of alcohol consumption after traditional resistance exercise in trained individuals.

The aim of this study was to investigate the effects of alcohol consumption on the recovery of muscle function after resistance exercise.

Nine recreationally trained volunteers (8♂, 1♀, 26±4 yrs., 81±4 kg) conducted four resistance exercise sessions and consumed a moderate (0.6 [females] and 0.7 [males] g/body weight) or a high (1.2-1.4 g/kg) dose of alcohol after exercise at two occasions. The first session was considered as familiarization to tests and exercise, and without alcohol. One session was followed with alcohol-free drinks (control). The sequence of sessions followed by alcohol consumption (moderate/high dose) and alcohol-free drinks was randomized. Recovery of maximal voluntary contraction (MVC; knee-extension), counter movement jump (CMJ) and grip-strength were assessed before, after and 12 and 24 hours after alcohol consumption. The exercise program consisted of 3x8RM in squats, leg-press and knee-extensions.

MVC was reduced by 13-15% and CMJ by 7-10% 10-15 min after the exercise sessions. Muscle function was still reduced at 12 hours, but generally recovered after 24 hours after alcohol consumption. Grip-strength (non-exercised muscles) was reduced after 12 hours (in the morning). No alcohol interactions were found.

Alcohol may adversely affect many physiological systems, including hormones and metabolism, as well as cell signaling (mTOR inhibition; (2). Thus, it is reasonable to anticipate a negative effect of alcohol on recovery from exercise, as seen after muscle-damaging eccentric exercise (1). Considering that we tested both a moderate and a high dose of alcohol, it was interesting that no effects were observed. This indicates that that recovery processes after resistance exercise are at least not highly sensitive to alcohol. Importantly, this does not mean that alcohol consumption after exercise cannot have adverse effects on muscle growth and strength increase over a training period.

In conclusion, neither a moderate (0.6-0.7 g/kg) nor a high (1.2-1.4 g/kg) dose of alcohol affected recovery of muscle function after a session of resistance exercise in recreationally trained participants.

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## QUESTIONNAIRE BASED STUDY OF THE COMPLIANCE OF MENTHOL CONTAINING MEDICATION IN CHILDREN

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Menthol is frequently used in over-the-counter medications, reduces dyspnoea and nasal obstruction and it has antitussive and calming effect. However, mentholated over-the-counter medications can also induce adverse reactions such as airway irritation, dyspnoea, chest tightness and respiratory failure. The mechanisms responsible for these are not completely known and mothers are probably not familiar with potential side effects this medication may have.

We developed a questionnaire focusing on the use and safety of mentholated products and treatment compliance in paediatric population. Mothers participating in the study were given questions with multiple choice answers divided into categories, mapping overall respiratory morbidity, antibiotics and over-the-counter remedies use. The second part focused on appearance of adverse effects and the third was addressed to ascertain the level of education of mothers with respect to patient's safety.

Results showed that children have an airway infection once in the three months, majority of children takes antibiotics once in the six months, and there are children at the age older than 6 who never had antibiotics. From over-the-counters, the most frequently used are herbal teas, decongestives, antitussives and hypertonic saline preparations. The suggestion to use over-the-counter meds comes equally from the paediatrician, pharmacist and mothers. The use lasts until the symptoms relieve and majority of mothers are more or less satisfied with efficacy. The use of the meds is not explained satisfactorily by the health care professionals, only on request. Given info is only more or less satisfying. 3/75 children had side effects after menthol/eucalyptol meds. Mothers are not aware of side effects of nasal drops, night management of antitussives/expectorants. Vast majority of caregivers would appreciate lectures with health care professionals to educate them in the cold and cough medications.

Menthol is effective in relieving respiratory symptoms, but cough and cold medications should be used with caution.

**ATP SENSITIVE POTASSIUM ( $K^+_{ATP}$ ) CHANNEL AND ALLERGIC ASTHMA****Maroš Oravec**

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Introduction:  $K^+_{ATP}$ , localized on plasma membrane of respiratory epithelial cells, on smooth muscle, on sensory and autonomous airways neurons, play an important role in airway physiology as well as pathophysiology of airways diseases. Asthma is an inflammatory disorder of airways characterized by bronchial hyperresponsiveness, by repeated attacks of breathlessness and cough and by variable airways obstruction.  $K^+_{ATP}$  ion channels agonists cause hyperpolarisation of cell membrane what leads to bronchorelaxation and goblet cells secretion decrease. This effect is considered as base for its therapeutic application in asthma treatment. This work presents study results in which we verified the influence of long term activation of  $K^+_{ATP}$  ion channels on animal experimental asthma model.

Material and Methods: We administered  $K^+_{ATP}$  selective opener pinacidil to guinea pigs with experimental allergic airways inflammation in the dose of 1mg/kg s.c. daily for the period of 14 days. We followed its influence on airway smooth muscle (ASM) reactivity and the inflammatory reaction itself. The airway reactivity (basal and provoked by mediators) was expressed by the value of specific airway resistance (sRaw) *in vivo* conditions and by the contraction amplitude of isolated ASM *in vitro* by tissue bath method. Airway reactivity was compared with salbutamol. The influence of pinacidil on airways allergic inflammation was evaluated by setting levels of exhaled NO ( $E_{NO}$ ) and by immunohistochemical staining of tryptase positivity in comparison to budesonid.

Results: The results of our experiments confirmed that the long term application of pinacidil leads to a significant decrease of the basal value sRaw *in vivo* conditions; it also leads to decreased response of ASM on some bronchoprovoking substances and decreases airway smooth muscle reactivity *in vitro*. Measured  $E_{NO}$  values were not influenced by pinacidil what corresponds with results of immunohistochemical staining.

Conclusion:  $K^+_{ATP}$  agonists can suppress contractile response of ASM and thereby influence airway hyperresponsiveness and prevent provoked bronchoconstriction. Long term activation of  $K^+_{ATP}$  by pinacidil leads to stable bronchodilator effect but it doesn't influence the intensity of allergic airway inflammatory response itself.

Key words: asthma,  $K^+_{ATP}$  ion channels, hyperresponsiveness, pinacidil.

## MOLECULAR MARKERS FOR POSITIVE IDENTIFICATION OF VAGAL LOW THRESHOLD A-FIBER MECHANOSENSORS BY SINGLE CELL RT-PCR

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*Introduction:* Afferent regulation of the esophageal motor reflexes is mediated by the vagal low threshold A-fiber mechanosensors. Clinical studies demonstrated that in esophageal diseases the reflex regulation of esophagus undergoes neuroplastic changes leading to reflex derangement and symptoms. However, the nature of neuroplastic changes in the vagal A -fibre mechanosensors is unknown. One difficulty in addressing this question experimentally is the lack of suitable molecular markers for positive identification of vagal A-fibres. Our aim is to find molecular markers that can be selectively detected in vagal nodose A-fibres by single cell RT-PCR. We hypothesize that certain markers functionally linked to A-fiber phenotypes such as medium- and high-molecular weight neurofilaments (NEFM and NEFH), glutamate transporter VGLUT1, mechanosensitive channel PIEZO2 or transcriptional factor RUNX3 are selectively expressed in nodose A-fibers.

*Materials and methods:* Single cell RT-PCR was performed on individual neurons isolated from the guinea pig vagal nodose ganglia. In some experiments nodose neurons innervating the esophagus were labeled by injection of retrograde tracer Dil into the esophagus.

*Results:* The neurons were considered as A-fiber and C-fiber neurons based on the absence and presence of the C-fiber marker TRPV, respectively. NEFM and NEFH were detected in 5/8 and 4/8 of TRPV1-negative neurons, respectively. However, NEFM and NEFH were detected in nearly all (11/11 and 10/11, respectively) of TRPV1-positive neurons. PIEZO2 was also detected in the majority of TRPV1-negative (17/27) esophageal neurons, but also in many TRPV1-positive (11/25) esophageal neurons. RUNX3 was virtually absent in TRPV1-negative (1/13) and TRPV1-positive (0/10) neurons. VGLUT1 was found in many TRPV1-positive neurons (5/11).

*Conclusion:* The medium- and high-molecular weight neurofilaments, mechanotransducer PIEZO2, transcriptional factor RUNX3, and glutamate transporter VGLUT1 are not suitable markers for positive molecular identification of nodose A-fibers by single cell RT-PCR. A marker for vagal A-fibers remains elusive.



## INFLUENCE OF ROFLUMILAST ON *IN VIVO* AND *IN VITRO* AIRWAY REACTIVITY IN OVALBUMIN-SENSITIZED GUINEA PIGS

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**Introduction:** Chronic inflammatory diseases, associated with airway obstruction and cough, are usually treated with bronchodilating and anti-inflammatory drugs. Inhibition of phosphodiesterases (PDE) leads to both of these effects. However, presence of special isoforms of PDE influences the desired effect of selective PDE inhibitors. In chronic obstructive pulmonary disease, roflumilast, selective PDE4 inhibitor, has been recently approved for the pharmacotherapy. The aim of this study was to evaluate the effect of long-term administration of roflumilast in experimentally induced allergic inflammation (model of allergic asthma) in guinea pigs.

**Material and methods:** 24 male adult guinea pigs, divided into 3 groups, have been used in the study. Control group has been left without sensitization. The latter two groups have been sensitized with ovalbumin over two weeks and thereafter treated perorally for 7 days with roflumilast at the daily dose of 0.5 mg/kg b.w., or with vehiculum, respectively. Specific airway resistance measured in whole-body double-chamber plethysmograph has been used as a marker of *in vivo* airway reactivity. The *in vitro* reactivity of tracheal and lung smooth muscle has been tested using organ bath method.

**Results:** Sensitization with ovalbumin has led to significant increase in *in vivo* and *in vitro* airway reactivity. Roflumilast reduced both specific airway resistance after nebulisation of histamine, and *in vitro* airway reactivity to cumulative doses of acetylcholine in tracheal and lung tissue strips. These changes have been associated with suppression of haematological markers of inflammation in animals treated with roflumilast.

**Conclusion:** The results of this study demonstrate suppressive effect on airway responsiveness in ovalbumin-sensitized guinea pigs, suggesting potential anti-inflammatory use in conditions associated with allergic inflammation.

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## VISUALIZATION OF VAGAL VS. SPINAL NOCICEPTORS IN THE ESOPHAGUS BY TRANSGENE EXPRESSION

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*Introduction.* Treatment-resistant pain from internal organs (visceral pain) is a prevalent grave clinical problem. The development of novel therapies is hampered by poor understanding of pain-mediating visceral nerves (visceral nociceptors). In particular, the locations and innervation patterns of nociceptive nerve terminals are essentially unknown. Novel methods to visualize visceral nociceptive terminals are necessary for progress in this area. We hypothesized that the transgene expression of green fluorescent protein (GFP) in nociceptive nerves will reveal the structure of vagal and spinal nerve terminals. We used adeno-associated virus vector encoding GFP (AAV-GFP) to induce GFP expression selectively in neurons of vagal nodose ganglia and spinal dorsal root ganglia (DRG) to address the hypothesis that nodose and DRG ganglia innervate distinct compartments of the esophagus. *Materials & Methods.* AAV-GFP was microinjected into surgically exposed left vagal nodose or left thoracic (T2) DRG. The esophagus, nerve ganglia and adjacent tissue were harvested 6-8 weeks later, stained for GFP in wholmount preparations of esophageal mucosa/submucosa and muscle, and evaluated by fluorescent microscopy. *Results.* AAV-GFP injection resulted in expression of GFP in the afferent neurons of the injected ganglia. The GFP-positive DRG fibers in the myenteric layer formed areas of dense network of fine branching fibers often forming intraganglionic structures in myenteric ganglia that were often apposed to fiber networks in the mucosa. This indicates that the fibers branch to innervate both muscle and mucosa. In contrast, vagal nodose nociceptive terminals formed localized intramuscular arrays limited to the luminal aspect of the innermost circular muscle layer. *Conclusions.* The spinal DRG fibers that are developmentally derived from neural crest innervate both the myenteric layer between circular and longitudinal muscle and mucosa, and have relatively large terminal fields. In contrast, the vagal nodose nociceptive fibers that are derived from placodes innervate the innermost circular muscle apposed to mucosa and have relatively small receptive fields. Our results indicate that the developmentally distinct nociceptor innervate distinct separate compartments of the esophagus and therefore provide the CNS with distinct information and may regulate different reflexes.

## **MONITORING OF ANTIPLATELET TREATMENT EFFECTIVITY USING OPTIC AGGREGOMETRY IN PATIENTS WITH ACUTE STEMI**

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Dual antiplatelet therapy is the keystone of acute ST elevation myocardial infarction (STEMI) pharmacotherapy. Variability of antiplatelet response/resistance to this therapy may lead to insufficient effectivity and risk of thrombotic complications. Monitoring of antiplatelet treatment effectivity using laboratory methods may identify patients with uncomplete antiplatelet response. The aim of this study was to determine whether optic aggregometry represents a clinically applicable method for antiplatelet therapy monitoring and whether this therapy given in standard doses is always sufficient. Then to determine whether there is a difference in antiplatelet therapy response according to different ADP receptor antagonists.

The study group included 37 patients with acute STEMI and subsequent urgent coronarography (24 men, 13 women). All patients were treated with aspirin loading dose (400 mg) and ADP receptor antagonist loading dose: in 23 patients clopidogrel (600mg) and in 14 patients prasugrel (60mg) was used. Optic aggregometry with specific inducers (arachidonic acid and ADP) was used for antiplatelet therapy effectivity assessment. Samples were taken prior to coronarography as well as on following day after this procedure.

First examination showed ineffective antiaggregation after arachidonic acid (AA) in 22 patients and after ADP in 20 patients. Second examination demonstrated ineffective drug activity after AA in 9 patients and in 2 patients after ADP. More patients with insufficient antiplatelet response after ADP was seen in clopidogrel group in first (16 vs. 4) as well as in second (2 vs. 0) sample. Patients treated with prasugrel had significantly lower aggregability after ADP in first ( $35.2 \pm 17.2\%$  vs.  $53.4 \pm 11.7\%$ ;  $p < 0.01$ ) and also in second examination ( $18.1 \pm 10.3\%$  vs.  $41.9 \pm 16.2\%$ ;  $p < 0.001$ ).

Optic aggregometry seems to be suitable and clinically applicable method for antiplatelet therapy effectivity monitoring. However, antiaggregant effectivity of this treatment is not always sufficient. Prasugrel treatment seems to be more effective than clopidogrel in patients with STEMI.

## MANDIBULAR FRACTURES IN CHILDREN

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**Objective:** Analysis of patients aged 0 – 18 years with a diagnosis of mandibular fracture for a period of five years.

**Methods:** Data from the medical records of patients recorded at the Department of Stomatology and Maxillofacial Surgery, JFM CO and University Hospital in Martin for the period 2010 – 2006 were evaluated in absolute numbers, percentages and processed in graphs and tables.

**Results:** The object of observation consists of a set of 50 hospitalized patients Department of Stomatology and Maxillofacial Surgery, JFM CO and University Hospital in Martin aged 0 to 18 years with a diagnosis of mandibular fracture. Boys were 42 (84%) and girls 8 (16%). At the age of 15 – 18 were 48 patients (96%), aged 6 – 14 years were 2 patients (4%). Simple fractures were 27 (54%), duplex fractures were 18 (36%). Triplex mandibular fractures were 4 (18%), multiple fractures mandibular maxillary had 1 patient (2%). Conservative therapy was used in 39 patients (78%), surgical therapy in 11 patients (22%). The most common cause of fractures of mandible in infants was of brachial violence 32 (64%), fall from bicycle 5 (10%), fall from height 5 (10%), sport accident 4 (8%), car accident 4 (8%).

**Conclusion:** In childhood are often condylar fractures, but in adolescence, the location of mandibular fractures is similar to the localization of mandibular fractures in adults (corpus mandibulae, angulus mandibulae), the most common cause of mandibular fractures in this age was of brachial violence 32 (64%). Atraumatically conservative therapy is always preferred to surgical therapy. That is chosen to prevent possible post-traumatic complications (open bite, the complications of teeth in fracture lines, jaw contractures, ankylosis).

**TOBACCO SMOKING IN PREGNANCY – MATERNAL AND NEONATAL OUTCOME****Lenka Krišková, Veronika Spišská**

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Introduction: Tobacco smoking has a negative impact on the course of pregnancy, labour, foetal and postnatal development. The aim of our work is to determine and compare the differences between non-smoking and smoking pregnant women.

Material and methods: We retrospectively assessed a total cohort of 305 women delivered their babies at Dpt. of Gynaecology and Obstetrics, JFM in Martin in the years 2011–12. We used a questionnaire focusing on active and passive maternal tobacco smoking, and medical records to determine maternal and neonatal outcome. We compared 3 groups of patients: A – non-smokers (n=237; 77.71%), B – active smokers (n=27; 8.85%), and C – passive smokers (n=41; 13.44%). A specific group was created from patients with a history of paternal smoking (n=108; 35.41%). Statistics: histograms, T-test, Mann-Whitney test.

Results: The only significant difference (p=0.001) among studied groups was found in the maternal age. We found a tendency of tobacco smoking in younger age groups (A – 30.00 years, B – 26.11years, and C – 27.88 years, resp.). An average number of cigarettes per day was 7.81 in group B, and an average time spent in a smoky environment was 5.95 hours daily in group C. We found no significant differences in pregnancy complications, maternal outcome, gestational week in time of delivery, birth weight (BW) and length (BL), Apgar score, and neonatal outcome among the groups. Although, we found the tendency for more frequent hypertensive disorders in pregnancy in group A (A – 5.06%, B – 0.00%, and C – 2.44%, resp.).

Conclusion: We were unable to prove a significant effect of tobacco smoking in pregnancy on maternal and neonatal outcome.

## COMPLICATIONS OF GASTRODUODENAL ULCERS TREATED SURGICALLY

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The work in a retrospective analysis deals with comparison of ulcer's disease complications occurrence solved surgically in patients treated using H2 blockers in 1990-1993 and in patients treated with proton pump inhibitors in 2009-2012.

In the first part, we compared the age structure of the files. The average age of hospitalized patients in the second set was 7,04 years higher than in the first set. In the second set was decrease in needs of surgical hospitalization for ulcer's complications 57.21%.

In the second part, we compared the need for a surgical treatment which in the second monitored period decreased by 5.38% as a result of increased success of endoscopic treatment by 19.44%. In the second set was decrease in needs of conservative treatment by 14,05% as result of success of endoscopic treatment. In the second set decreased the planned operations by 28.64%, but increase of the acute operations proportion (77.78%).

In the third part, we compared the types of surgical procedures. In the first set dominated perforation (30.17%) and pyloric stenosis (24.19%), in the second set the local performance occurred most frequently in perforation (51.11%).

In the fourth part, we compared the overall and the local complications. In acute operations was a higher incidence of complications in the second set by 22.63%. In the planned operations were lower complications in the second set.

In the last section, we compared mortality. Despite the much higher representation of acute operations and higher average of patient's age in the second set, there was 13.95% mortality, compared to the first set (4.48%). At a higher mortality rate in the second set involved particularly patients underwent acute surgery and polymorbid older patients.

## MEASUREMENT OF BONE DENSITY IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

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**Aim:** Diabetes mellitus type 1 (DM1) as chronic disease influences all metabolic pathways including calcium-phosphate metabolism resulting in alteration of bone density. The aim of this study was to evaluate if environmental and life style factors contributed to decreased bone density in children with DM1.

**Material and methods:** 30 children (19 males and 11 females) at the age 10 – 18 years (14,27±2,63 average) with DM1 either hospitalized at Department of Pediatrics JFM CU or examined at Endocrinology ambulatory care were enrolled to this prospective study. Children underwent densitometry examination by Hologic Discovery Bone Densitometer. All subjects filled in the questionnaire about lifestyle and dietary habits related to calcium metabolism. Data about diabetes duration and biochemical values (glycosylated hemoglobin, vitamin D, calciuria) were achieved from the documentation. Results were statistically processed; Student's t test and Pearson correlation test were used.

**Results:** Patients with lower physical activity, lower exposure to sunshine and lower intake of vitamin D supplements did not significantly differ in densitometry parameters compared to the patients with better life style. Z score of lumbar densitometry mildly positively correlated with concentration of vitamin D ( $r=0,232$ ) and mildly negatively with calciuria ( $r=-0,369$ ). Subjects with lower Z score of lumbar densitometry ( $Z<-1,0$ ) had significantly longer diabetes duration ( $p<0,001$ ), significantly lower concentration of vitamin D ( $p<0,05$ ) and higher calciuria ( $p=0,01$ ) compared to subjects with normal Z score, while no significant difference was found in dietary habits or sunshine exposure.

**Conclusion:** In our study, environmental factors had no influence on bone density in subjects with DM1. Further investigations with extended number of subjects are needed to confirm these results.

## ORAL CONTRACEPTION AND THROMBOPHILIA

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**Introduction:** In our work, we focused on the negative side effects of hormonal contraceptives, which is an increased risk of thromboembolic disease. We explored them especially in patients who had congenital hypercoagulable states such as mutation of coagulation factor V, factor II; deficiency of antithrombin III. We also focused on the function of protein C and protein S, and platelet hyperaggregability.

We searched for other acquired risk factors in patients with hypercoagulability. In addition to contraception, we focused on smoking, overweight, long travel, injury, family history.

**Material and methods:** Data were obtained from the information system Medea at Department of Haematology and Transfusiology, University Hospital in Martin. We used the method of retrograde analysis. All collected data were processed to tables and evaluated.

**Results:** There were 142 patients taking oral contraception at the time of thromboembolic events. 19% of them were smokers, 19% were overweight, 42% of them had thromboembolic disorders in family history. 7% of them had mutation of factor II, 23% mutation of factor V, 5% deficiency of antithrombin III, 16% deficiency of protein S and, 16% deficiency of protein C, respectively.

**Conclusion:** In our survey population, we found that hormonal contraception contributes to the increased risk of thromboembolic events. Moreover, in some patients we confirmed congenital hypercoagulable state and a set of other risk factors.



## INFLUENCE OF APPLICATION BOTULOTOXINUM TYPE A ON SECRETION OF TEARS IN NEUROOPHTHALMOLOGIC INDICATIONS

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**BACKGROUND:** Many patients with facial dystonia suffer from dry eye syndrome. Botulotoxinum type A is effective symptomatic therapy for reduction of spasms. We evaluated the influence of therapy of facial dystonia with botulotoxinum type A on lacrimal system.

**MATERIAL AND METHODS:** The injections of botulotoxinum were applied from neuroophthalmologic indications, subcutaneously to sixty patients. In group were enrolled sixty eyes. The influence of therapy on lacrimal secretion was rated by Schirmer test 2 times. Before therapy and 2 weeks after chemodenervative therapy.

**RESULTS:** In consideration of non gaussian distribution of values of Schirmer test (detected by Schapiro - Wilkov test), we evaluated difference between values before and after therapy with nonparametric Wilcoxon's test. In 55% there was significant decrease in secretion of tears, In 25 % increase of retention and there was no change in 20 %.

**DISCUSSION:** Our results are in accordance with results of Montoaya et al. 2002, which confirmed negative effect of botulinum toxin on lacrimal secretion.

**CONCLUSION:** In therapy of focal facial dystonia in neuroophthalmologic indications, after application injections botulinum toxin type A, secretion of tears has significantly decreased in 55% patients.

**KEY WORDS:** Botulinum toxin type A, Blepharospasm, Dry eye syndrome

## EXHALED NITRIC OXIDE AND COPD

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Exhaled nitric oxide (FeNO) is a noninvasive marker of airway inflammation however it's role in patients with chronic obstructive pulmonary disease (COPD) is still not fully understood. The aim of our study was to measure the levels of exhaled nitric oxide in patients with COPD and compare it with healthy individuals and assess the impact of smoking and treatment with inhaled corticosteroids on FeNO levels.

In our study we measured the levels of FeNO in 33 COPD patients and in 51 healthy individuals. FeNO levels were measured first using analyzer NIOX<sup>®</sup> in compliance with ATS/ERS guidelines. In every COPD patient we performed a functional examination of the lungs testing obstruction reversibility using 400ug of Salbutamol and a COPD assessment test score (CAT) of patient's subjective feeling of healthy status was recorded.

Patients with COPD had significantly higher levels of FeNO (19 ppb, IQR 8) than healthy controls (10,8 ppb, IQR 5,80) ( $p < 0,05$ ; Mann-Whitney-U test). There was no significant difference in FeNO levels between 22 COPD patients treated with inhaled corticosteroids (19 ppb, IQR 6,5) and 11 patients without anti-inflammatory therapy (17ppb, IQR 8,5). The sample of COPD patients was also divided according to smoking history and no significant difference in FeNO levels between 26 non-smokers (17ppb, IQR 9,75) and 7 smokers (19ppb, IQR 3) was found out. 28 patients had CAT score equal or higher to 10. We found out, that there is a strong negative correlation between the CAT score and FEV1 value.

## PARADOX OF OBESITY AND ISCHEMIC HEART DISEASE

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**Objective and an explanation of the study:** The obesity paradox shows that people with chronic obesity have a greater chance of survival than normal-weight people. The aim of the study is to determine whether paradox of obesity can be observed in patients with ischemic heart disease. Based ergometry, 2D echocardiography and coronary angiography will identify which of the monitored cohorts (subdivided by BMI) is in terms of coronary heart disease loaded at least in terms of subjective shortness (NYHA), both from the standpoint forecasts (Heart Score). We would like to show correlation relationship BMI and severity of coronary artery involvement.

**Material and methods:** One of the conditions is that the patient agreed to participate in the study. Let him therefore available: informed consent. Patients are selected throw the including and exclusion criteria from the hospital system Medea (we chosen the criteria) .Patients underwent ergometry, 2D echocardiography and coronary angiography. We determined BMI index in these patients, and they were selected to four groups. We also determined basic laboratory results (blood glucose, HDL, LDL cholesterol,whole cholesterol , ALT).

**Results:** In study we have 110 patients. We divided them to four groups, throw the BMI. First group contains 11 patients, second 26,third 17 and the forth 13 patients. Results of all tests (ergometry, 2D echocardiography, coronarography, laboratory test) shows, that negative results from the first group has 18 %, from the second group 26,9 %, from the third group 82%, and from the forth group 15,38 %.

**Conclusion:** In the study, within the number of our patients, we highlighted the fact that the best results, showing our test group three. Number of degrees in the BMI range of 26 to 27 subjects for these investigations, we actually managed to existing obesity paradox.

## MONITORING OF SELECTED CLINICAL AND LABORATORY PARAMETERS OF PATIENTS WITH MULTIPLE SCLEROSIS

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**Introduction:** Multiple sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative disease of the central nervous system.

Cigarette smoking and metabolism of vitamin D (25-OH-D) are important risk factors of MS susceptibility and progression of the disease.

The aim of my work was to study smoking behavior in 238 MS patients and in 232 healthy control subjects. We also investigated the plasma level of vitamin D.

**Material and methods:** Data were obtained by direct interview, through a structured questionnaire survey from patients and healthy control subjects, from medical records. Biochemical parameters (25-OH-D levels) were measured in the Institute of Biochemistry of University Hospital in Martin. All collected data were processed to table and statistically evaluated.

**Results:** In rapid progression group, there was a significant higher proportion of patients, which were exposed to cigarette smoke during their childhood and adolescence (OR=2,14, CI95%=1,11-4,13 p=0,031) and which were active smokers before the onset of the first symptoms of the disease (OR=2,17, CI95%=1,12-4,18, p=0,022) in comparison to subjects in the slow progression group.

There were a seasonal changes in the 25-OH-D levels in MS patients (lower levels in winter months, higher levels in summer months), which seems to be in negative correlation with incidence of MS relapses during the year.

**Conclusion:** The cigarette smoke exposition in childhood and adolescence and cigarette smoking are associated with rapid progression of the disease and worse prognosis of MS. The 25-OH-D lower levels in serum are risk factor for MS relapse.

## BONE MARROW INVOLVEMENT IN DIFFERENT HISTOMORPHOLOGICAL VARIANTS OF MANTLE CELL LYMPHOMA

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**Introduction:** Mantle cell lymphoma (MCL) is an uncommon B-cell non-Hodgkin lymphoma having poor prognosis. Different histomorphological variants of MCL are recognized and those showing blastic morphology (including blastoid and pleomorphic variant) are considered to be more aggressive.

**Material and methods:** We retrospectively reviewed biopsy reports of MCL diagnosed at our department in years 2004-2011 to identify cases with available staging BM biopsy. In these cases we analyzed presence and appearance of BM involvement in pretreatment and posttreatment trephine BM biopsies in relation to histocytology of MCL in primary nodal or extranodal tissue.

**Results:** From all 136 MCL, both histomorphological variant and representative staging BM biopsy were available in 78 cases, from which 55 cases (71%) infiltrated BM. From 44 cases of conventional small cell MCL, BM involvement was detected in 30, all represented by small cell MCL infiltrate. From 20 cases of blastoid MCL, the BM was infiltrated in 17 (85%), including 8 cases with small cell and 9 cases with blastic morphology of the infiltrate. From 14 cases of pleomorphic MCL, BM was involved in 8 (57%) of them, in 6 cases with small cell and in 2 cases with blastic infiltrate. Any significant difference in pattern or extent of BM infiltration was detected in analyzed variants of MCL. The extent of lymphoma infiltration ranged from 5% to 90% of BM cellularity. Posttherapeutic restaging BM biopsy was available in 42 cases, from which 21 (50%) showed persistence of MCL infiltration, including 13 conventional and 8 blastic MCL. These cases did not show any significant difference in course of BM involvement in posttherapeutic period.

**Conclusion:** MCL frequently involves BM. In contrast to different clinical behaviour, we did not identify any significant difference in BM disease course between small cell and blastic MCL. Supported by Grant VEGA Nr. 1/0378/12.

## BIOPTIC PARAMETERS ANALYSIS OF MALIGNANT CNS LYMPHOMAS REGISTERED IN THE LYMPHOMA DATABASE OF DEPARTMENT OF PATHOLOGY IN MARTIN

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**Introduction:** Malignant CNS lymphomas (ML CNS) may arise as primary or systemic. Primary ML CNS are malignant tumors constituting 3,1% of all primary brain tumors. They occur predominantly in diseased with immunodeficiency, particularly in patients with AIDS.

**Aim:** The aim of our study was analysis some morphological, phenotypic and genotypic characteristics of ML CNS followed by comparison of obtained results with the literature data.

**Methods:** Our database comprises altogether 78 cases which were being collected during the years 2004 -2013. Specimens were fixed in formalin and embedded in paraffin wax. Standard staining methods were applied on tissue cuts including HE, Gömöri, Giemsa, PAS and imunohistochemistry methods were applied as well.

**Results:** The whole set consisted of 34 (43,6%) women and 44 ( 56,4%) men, aged from 38 to 77 years with the median of age 63 years. The tumors were mostly localized supratentorially (36 cases, 75,6%) with the predominant localization in frontal and temporal lobe. In 66 cases (84,6%) there was a primary affection of CNS, systemic lymphomas occurred in 5 cases (6,4%), with the rest being undetermined (7 cases, 8,9%). The majority of ML CNS were classified as CD20+ diffuse large B-cell lymphoma (DLBCL). The most frequent histomorphological variant of DLBCL was centroblastic (40 cases, 54,7%) and non-germinal centre B-cell-like (non-GCB) phenotype showed to be dominant (39 cases, 53,4%). In our set none of the patients was HIV+.

**Conclusion:** Although we have managed to gather a lot of histopathological data about ML CNS, in the future we would like to carry on and enrich our study with the patient survival length.

## EXPRESSION AND SIGNIFICANCE OF ANTIAPOPTOTIC PROTEIN SURVIVIN IN PROSTATE LESIONS

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**Introduction:** The antiapoptotic protein survivin is rarely expressed in normal adult differentiated tissues, but it is often detected in their malignant counterparts. Owing to large quantitative differences in the degree of survivin expression in cancers on the one hand and in corresponding normal adult tissues or in benign lesions on the other hand, survivin appears to be a new promising prognostic biomarker.

**Material and methods:** Immunohistochemically, we evaluated survivin expression in 19 cases of benign prostatic hyperplasia (BPH) and 21 cases of prostatic adenocarcinoma. The intensity of staining, percentage of labeled cells and subcellular location of survivin were assessed. We analyzed the quantitative differences of survivin expression between BPH and adenocarcinomas.

**Result:** Survivin was detected in 4/19 cases of BPH (21,1%) and in 14/21 cases of adenocarcinomas (66,7%). The statistical analysis confirmed significant correlations between the assessed parameters in BPH and adenocarcinoma.

**Conclusion:** Our data point at usefulness of survivin staining, otherwise rarely performed in BPH. We confirm the importance of nuclear location of the survivin antigen which may be helpful for assessing the possible progression to adenocarcinoma. Our results suggest that survivin may be a valuable diagnostic marker, as well as a new independent prognostic parameter in prostatic adenocarcinoma.

## CLASSICAL HODGKIN LYMPHOMAS INVOLVING BONE MARROW

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**Introduction:** Classical Hodgkin lymphomas (CHL) are the most common malignant lymphomas. They rarely involve bone marrow (BM), but when it occurs, it is an unfavorable factor upgrading the clinical stage into IV. Only limited data are available about CHL infiltrating BM.

**Material and methods:** We retrospectively reviewed biopsy reports of CHL cases diagnosed at our department in years 2004-2013 to identify and to further analyze CHL cases showing BM involvement in pretreatment staging BM trephine biopsies.

**Results:** From all 704 reviewed CHL cases only 37 (5,3%) showed neoplastic BM involvement. In 31 cases (4,4%) it was represented by lymphohistiocytic proliferation suggesting BM involvement by primary CHL disease. The range of infiltration extent varied from 5% to 100% of BM cellularity. Remaining 6 (0,9%) cases showed BM infiltration with chronic lymphocytic leukaemia (CLL) alone, while they represented cases with colision or Richter's transformation of CLL to secondary CHL. 5 CHL cases infiltrating BM were exclusively diagnosed primarily in BM biopsy, what is considered to be an extremely rare finding. Majority (71%) of patients with CHL involving BM were older than 50 years, male to female ratio was 3:2. Surprisingly, 14 cases (45%) with true CHL infiltration of BM showed accented epitheloid-cell reaction in primary lymph node (LN) biopsy. 14 cases (45%) involving BM were classified as nodular sclerosis and 9 (29%) as mixed cellularity type in primary LN biopsy. 8 cases (26%) were unclassifiable or their diagnosis was problematic, sometimes with CD15 negativity or features of unclassifiable grey zone lymphoma. Any significant difference in primary site of CHL origine was identified in cases involving BM.

**Conclusion:** Bone marrow infiltration is a rare phenomenon in CHL and usually is a difficult differential diagnostic challenge. CHL involving BM may show some unique features requiring further study. Supported by Grant VEGA Nr. 1/0378/12.



## TUMOR ASSOCIATED MACROPHAGES TYPE 1 AND 2 IN CLASSICAL HODGKIN LYMPHOMA

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*Introduction:* In contrast to other malignant tumors, classical Hodgkin lymphomas (cHL) show unique histological patterns: tumor cells comprise a minority and admixed reactive cells a majority of the cell population. Among the reactive cells especially the macrophages – so called **tumor associated macrophages** (TAMs) are recently a subject of interest. They might be divided into suppressive TAM1 type (CD68+) and promotional TAM2 type (CD163+) cells.

*Material and methods:* From the archives of the Lymphoma Register we have selected a series of 71 consecutive cHL cases as a pilot retrospective study to prove a possibility of an evaluation of the TAM1 and TAM2 proliferation resp. in routine biopsy cases. The paraffin sections were stained immunohistochemically with antibodies against CD68 and CD163 antigens. In all the cases, the number of TAMs within the tumor tissue was evaluated semiquantitatively using cut-offs 0-10%, 10-50% and >50%.

*Results:* In 18 of the cases, the level of CD68+ and CD163+ cells was concordant: 0-10% 8 cases, 10-50% 13 cases and >50% 6 cases. In 32 cases the results were discordant and these cases may be divided into 3 groups: a) cases with 10-50% of CD68+ cells showing either 0-10% of CD163+ cells (n=16) or >50% CD163+ cells (n=5) and b) cases with >50% CD68+ cells showing either 0-10% of CD163+ (n=5) or 10-50% of CD163+ cells (n=4) c) cases with 0-10% of CD68+ cells showing either 10-50% of CD163+ (n=2).

*Conclusion:* We found that in routine cHL biopsies it is possible to evaluate the proliferation of TAM1/2 populations and to document its heterogeneity. As TAMs 2 have been reported to be associated with a worse prognosis for several tumors, the identification of TAM1+ versus TAM2 proliferation could be useful for prediction of cHL patients prognosis.

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## THE JAK2 V617F QUANTIFICATION IN Ph1 NEGATIVE MYELOPROLIFERATIVE DISORDERS

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The discovery of the JAK2 V617F mutation triggered an unexpected flowering of basic and clinical studies in the field of myeloproliferative disorders (MPD). This mutation is detected in 90% of patients with polycythemia vera and in approximately 50% of patients with essential thrombocythemia and primary myelofibrosis. The nucleic acid conversion of guanine to thymine results in a substitution of the highly conserved amino acid residue, valine to phenylalanine in codon 617 (V617F) of the autoregulatory pseudokinase domain JH2 of Janus Kinase 2. Expression of the mutant JAK2 V617F tyrosine kinase in cell lines induce autonomous growth factor independent proliferation.

Our study has been focused on the implementation and evaluation of the JAK2 V617F mutation analysis in a cohort of patients diagnosed with MPD.

Quantitative real-time Taqman based PCR assay was used, allowing an assessment of the percentage of JAK2 V617F mutant and JAK2 wild type alleles. DNA was isolated from peripheral blood cells and paraffin-embedded tissues from the patients with MPD.

The methodics design was developed and trial examinations confirmed it's validity, however, complete results were not available in time of abstract submission due to serious complications with probes delivery delay.

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## **ETHER PHOSPHOLIPIDS ARE ANTITUMOR AGENTS AGAINST COLORECTAL CANCER CELLS AND CAN ACT BY THE INDUCTION OF APOPTOSIS**

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The aim of this study was to assess a determination of influence of nature ether phospholipids (EP) to chemoresistance. Currently it is known a several mechanisms of action EP such as: induction of apoptosis, inhibition of protein kinase C or growth factors. Recently, we described a novel, nontoxic EP with selective antitumor activity, isolated from ischemic tissue of chick embryo. The mixtures of EP are produced by Areko<sup>®</sup> and designated as BAF<sup>®</sup>. BAF<sup>®</sup> exhibits a selective cytolytic effect on human colorectal adenocarcinoma cells (DLD-1 and HT-29). We tested cells to five different concentrations of active form of phospholipids (A- BAF<sup>®</sup>) but the lowest (0.025%) of them showed potent effect. We used also an inactive form of phospholipids (N- BAF<sup>®</sup>) which has not effects to both cell lines.

The progress of apoptosis we determined by histologic staining (May-Grunwald and Giemsa). Furthermore, the results on the HT-29 cells determinate a positive influence of active BAF<sup>®</sup> to daunorubicin, flououracil and cisplatin by MTT assay.

Although we found that A-BAF<sup>®</sup> behaves as a potent and highly selective antitumor agent able to increase sensitivity to cytostatics we don't know the exact molecular mechanism of action. Therefore we need further experiments such as quantitative RT-PCR, Western blotting or cells uptake which are capable to demonstrate a patomechanism of A-BAF<sup>®</sup>.

This work was supported by project "Center of Excellence for Research on Personalized Therapy (CEVYPET)", code 2622012053, co-financed from EU sources and European Regional Development Fund.

## **ABT-737 SYNERGIZE WITH THAPSIGARGIN IN INDUCTION OF DEATH OF LEUKAEMIC HL-60 CELLS**

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Intracellular  $\text{Ca}^{2+}$  plays important role in survival and death of mammalian cells including malignant cells. High cytosolic  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ) could initiate mitochondrial apoptosis that is controlled by proteins of Bcl-2 family. Sarco/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPases (SERCA) maintain intracellular  $\text{Ca}^{2+}$  homeostasis by active decreasing of ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ). Thus inhibition of SERCA causes increase of  $[\text{Ca}^{2+}]_{\text{cyt}}$  and depletion of endoplasmic reticulum (ER)  $\text{Ca}^{2+}$  stores that is associated with ER stress and might culminate in cell death.

The aim of this work was to study effect of thapsigargin, inhibitor of SERCA, in combination with ABT-737, inhibitor of anti-apoptotic proteins of Bcl-2 family, on survival of leukaemic cell line HL-60. Incubation of HL-60 cells with thapsigargin was associated with cell death in concentration dependent manner. Concentration of thapsigargin leading to the death of 50% cells ( $\text{LC}_{50}$ ) was estimated to be approximately 2 nmol/l. Incubation of HL-60 cells with ABT-737 was also associated with induction of death of HL-60 cells ( $\text{LC}_{50}$  was approx. 3  $\mu\text{mol/l}$ ). Combination of 1  $\mu\text{mol/l}$  of ABT-737 with thapsigargin led to increased sensitivity of HL-60 cells to thapsigargin that was manifested by decreased value of  $\text{LC}_{50}$  being 0,5 nmol/l. Thapsigargin in concentrations used in this study was able efficiently inhibit SERCA that was documented by spectrofluorometric measurements of  $[\text{Ca}^{2+}]_{\text{cyt}}$  increase, using Fluo-3 as calcium sensor. Since recent studies suggested role of Bcl-2 proteins in modulation of ER calcium handling, we have investigated effect of ABT-737 on ( $[\text{Ca}^{2+}]_{\text{cyt}}$ . ABT-737 alone was not able to elicit increase of  $[\text{Ca}^{2+}]_{\text{cyt}}$ . We have also documented that addition of ABT-737 in combination with thapsigargin did not elicit additional increase of  $[\text{Ca}^{2+}]_{\text{cyt}}$ . Thus ABT-737 potentiates thapsigargin induced cell death but the mechanism of this effect is not clear and remains to be further investigated.

This work was supported by project „Support of human resources development using the most modern methods and forms of education on JFM KU in Martin“ co-financed from European Union sources and European Social Fund.

## QUALITY OF LIFE OF PATIENTS WITH DIABETIC FOOT

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**Introduction:** Diabetic foot syndrome is one of the chronic complications of diabetes mellitus. The incidence is increasing. Diabetic foot syndrome affects type I and II of diabetics and it is the most common reason for hospitalization of diabetics. Even after 10 years of type I of diabetes mellitus is a high probability of diabetic foot. Quality of life of patients with diabetic foot is significantly adversely affected. In our work we investigated the quality of life of patients with diabetic foot and then we were looking for relationships between the identified outcomes.

**Method:** The research was formed 50 patients with diabetic foot who were hospitalized in hospitals and social centers in Žilina, Čadca and Dolný Kubín. For measurement of quality of life these patients was used the questionnaire WHOQOL-BREF. The questionnaire contains from 2 items. The first is quality of life consisting of 4 domains - physical health, psychological area, social relations and environment. The second item is form about the course of diabetes mellitus and other associated diseases.

**Results:** The results were processed from 50 questionnaires. The return was 100%. In the sample were 15 women and 35 men and an average age was 62 ( $\pm$  9.6) years. In 12 respondents were incidenced diabetes mellitus type I and in 38 respondents type II. HbA1c was increased in 10 patients (higher than 8.00%). The average duration of diabetes in respondents is 14.7 ( $\pm$  9.3) years and BMI 29.91 ( $\pm$  4.7) which is defined as overweight in the WHO classification. Information about foot care from their diabetologist received 47 respondents but prescribed prosthetic shoes wear only 17 of them. The feet pain negated 6 respondents, 14 respondents rated the pain as mild (VAS 1-4), 23 respondents as moderate (VAS 5-7) and 7 as severe (VAS 8-10).

**Discussion and conclusion:** The results showed that diabetic foot significantly affects the quality of human life. Very important is prevention of diabetic foot. Several studies have confirmed that the most important aspect of prevention of diabetic foot is foot screening and effective education of diabetics. The necessity of prevention is important mainly because the number of diabetics is growing every year and therefore the probability of diabetic foot is bigger.

**Key words:** diabetic foot, quality of life, WHOQOL-BREF

## TITLE OF MEASUREMENT AND EVALUATION OF PAIN IN CLINICAL PRACTICE

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### **Introduction**

The objectivity of pain assessment is the basis of effective pain management. The planning and implementation of interventions related to elimination of pain depend on precise assessment and identification of patient's pain. The aim of our thesis was to identify what management of acute pain is carried out by nurses at the 1<sup>st</sup> Surgical Clinic UNM in Martin in the context of nursing process.

### **Material and methods**

To collect empirical data we have chosen quantitative-qualitative method – the retrospective analysis of nursing records. We have studied the categories such as assessment of pain, formulation of nursing diagnosis Acute Pain, assessment/evaluation scale and planning of interventions. The selection of nursing records was intentional. The sample has consisted of 50 patients hospitalized at 1<sup>st</sup> Surgical Clinic UNM in Martin. The inclusion criterion to select nursing record to the file was that the patient has undergone the surgery.

### **Results**

We have found out that nursing diagnosis Acute Pain was determined in each patient's record. In all the records there was a lack of assessment and lack of usage any assessment/evaluation scale. In all 50 cases interventions such as taking vital signs and administration of analgesics were used. In 10 cases there was a record about monitoring the effect of analgesics and monitoring the pain but with no further registration in documentation. Evaluation of the effectiveness of care provided was absent in all records.

### **Conclusion**

Nursing diagnosis Acute Pain belongs to diagnoses that are the most frequently determined. Rigorous assessment of pain together with the use of assessment/evaluation scale affect the selection of interventions and the whole course of nursing care.

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## EVALUATION OF SOCIAL SUPPORT IN ONCOLOGIC PATIENTS

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**Introduction:** Oncologic disease is accompanied with pain, decrease of life power, infirmity and fear of death. Social support is one of important protective factors in managing the stress from disease and treatment, mainly in the period, when insufficient protective sources are available for the patient. The aim of this paper is to analyse local and foreign studies dealing with evaluation of social support and to prepare a pilot study judging the range of social support in oncologic patients.

**Material and methods:** An analysis of systematic reviews and meta-analyses was done from data bases of Medline, Evidence based Nursing, ProQuest Nursing, focused on evaluation of social support in oncologic patients. The pilot study was realised by means of a free-available scale SELSA. It contains 15 items divided into three subscales: social relationships, family relationships, partnership relations, evaluated by a Likert scale from 1 to 5. A higher score indicates a lower social support. The achieved data were evaluated by means of descriptive statistics. The research group consisted of 50 patients, 30 women and 20 men. The mean age of the patients was 62, and the average disease length was 5 months.

**Results:** Totally 50 studies were analysed, confirming the fact that social support has positive influence on physical health of the patients, their comfort and effective management of the burdening situation (Steel et al, 2004, Rudge et al. 2005). Measure of social support in the whole set of our respondents was 30.16. As revealed in our study the partners' relationship domain seems to be a problem, where patients felt a lower level of social support. As for sex, men felt a higher level of social support. As for marital state, married patients experienced the highest level of social support, compared with the lowest level in single patients.

**Conclusion:** The results indicate a need to judge the range of social support in oncologic patients in clinical practice, which enables to set a relevant nursing diagnosis and to plan effective interference between the patient and his family.

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## NURSES' COMPETENCES

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**Introduction:** In the professional community the issue of competence is a frequently discussed topic. Competences turn out to be exceeded often by nurses in relation to other health professionals.

**The aim:** The aim of this bachelor thesis was to find out which competences are considered by nurses to be important for their profession and which competences are in fact carried out in practice.

**Population and methods:** To collect the empirical data, we used a questionnaire compiled by us. The questionnaire contained 64 items arranged in three subscales: scope of nursing practice provided by the nurse alone (30 items), the extent of nursing practice provided by the nurse in collaboration with the physician (18 items), and the nurse alone, based on the physician's orders (16 items) and items related to demographic characteristics. For the answer section of the questionnaire, we used a 7-point scale (1 – least important / 7 – most important). We included 80 respondents in the analysis (40 nurses from surgical departments and 40 nurses from internal medicine clinics).

**Results:** The most important difference between real practice competences and perceived importance for the profession was found in the last subscale – based on physician's orders. In first subscale, we got an average of 5.77 for application in practice and in importance for nursing we got average 6.45.

On the contrary, the lowest importance in practice was reported by nurses in the third subscale. In this subscale, we got an average of 4.11 for application in real practice and average 4.74 in importance for nursing.

**Discussion and Conclusion:** Based on the survey results, we can conclude that nurses are trying not to exceed their competences defined by the regulation of the Ministry of Health 364/2005, unless it is required directly by the patient's condition (intubation, extubation...)

Keywords: competences, nurses, nursing practice



## COMMUNICATION SKILLS OF NURSING STUDENTS

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Effective communication skills in professional practice of a nurse are considered to be the main precondition of nurse's therapeutic approach to the patients. Their crucial role is to contribute to establishment and development of the nurse – patient relationship and creation of stable base of the relationships among healthcare professionals. Thus it is essential to shape nursing students' attitudes towards communication and learning communication skills as early as during their undergraduate studies.

The aim of study was to determine nursing students' attitudes to education concerning communication skills and their consequent utilization in professional practice. The scientific data were collected by 26-items questionnaire CSAS (*Communication Skills Attitude Scale*) searching positive (PAS) and negative (NAS) attitudes of nursing students towards the utilization of communication skills in their future profession and the way in which they are learned during the studies. The research sample consisted of 271 full-time students of bachelors' degree study programme in nursing.

Based on data analysed we have found out that nursing students have middle intensity of positive attitudes towards communication and learning communication skills (CSAS<sup>+</sup> 95,36 ± 10,02; PAS 48,20 ± 6,50; NAS 30,90 ± 5,26). Statistical analysis of data from the perspective of demographic categories selected (year of study, previous education, passing the subject Communication in Nursing, the methods by which this subject is taught, evaluation of the level of communication skills achieved, perception of the need to improve communication skills) proved statistically significant differences in attitudes in our research sample.

Systematic education of nursing students in the field of communication and communication skills can contribute to establishment and development of positive attitudes to communication skills that are crucial to perform nursing profession efficiently. The study has proved the significance of including the subject concerning Communication in Nursing in the curricula of undergraduate nursing study programme and identified positive attitude of nursing students towards learning communication skills and their utilization in nursing profession.

## **AWARENESS OF GRAVIDITY, PARTURITION AND CONTRACEPTION AMONG GIRLS AT THE AGE OF 16 – 18**

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**Introduction:** Awareness of girls of contraception, gravidity and parturition is considered important from the point of view of a correct decision in the field of sexual behaviour, forestalling of unwanted gravidities and the awareness of possible dangers.

**Material and methods:** Objective of this work has been to identify sources of information of the 16 – 18-year-old girls about gravidity, parturition and contraception. To survey the awareness of girls, a questionnaire method was used. The questionnaire of an own construction implied 3 areas – gravidity, parturition and contraception. The questionnaire contained 26 questions, it was distributed to secondary students aged 16 - 18. Altogether 100 questionnaires were distributed, out of which 98 questionnaires were returned as evaluable. 98-per cent responsibility was reached. The average age of the respondents was  $16,95 \pm 0,76$ .

**Results:** We have found out that the most frequent source of information about *gravidity* among girls in the given category was the Internet (53%), parents (52%) and books and magazines (44%); about *parturition* it was parents (53%), the Internet (49%), books, magazines (27%) and a friend (27%) and about *contraception* it was the Internet in 53%, a friend in 53%, gynaecologist and parents in 35%. The respondents evaluated their level of knowledge of the given problems as average in 58%, as sufficient in 28%, as above-average in 6% and as insufficient in 8%. On average, 73% of the respondents would welcome more information on gravidity, parturition and contraception.

**Conclusion:** The results point to the fact that the most frequent sources of information in the given problems are the Internet and parents. However, these sources do not provide adequately objective information. Medical workers erudite in this field are more appropriate providers of information. Since as many as 73% of the respondents are interested in further information, it is important to strengthen educational activity and to increase the awareness of the given age group of these problems. Similar survey in the Czech Republic shows that adolescent girls are interested in information about these problems (Chaloupková, 2007, pp. 74-76). Their source of information was television and magazines which are also not considered to be a reliable source.

## NATURAL PARENTHOOD PLANNING

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**Introduction:** Natural parenthood planning (NPP) is often neglected even though it fully respects physical health, unlike other ways of parenthood planning. The notion of fertility based upon fertility factors such as mucus, basal temperature, and cervix can lead to better female reproductive health. The goal of the study was to gain an insight into NPP public awareness and public attitudes on NPP use and personal experience.

**Methods:** The survey consisted of two groups of respondents. 136 respondents (aged  $m=30.5$ ,  $SD=11.50$ ) represent public and 430 respondents (aged  $m=31$ ,  $SD=11.75$ ) are people who practise natural parenthood planning. An anonymous self-made questionnaire was used as a research method in both groups of respondents.

**Results:** Results show that the public has a poor notion about NPP (58.48% of respondents have never heard of NPP) and has wrong perception on NPP. Couples who practise methods of NPP are mostly satisfied (66.05% of satisfied) with using these methods.

**Conclusion:** NPP does not suppress sexuality but leads to its better knowledge and understanding. Furthermore, it does not represent a health risk, is rather reliable, inexpensive, morally acceptable, and brings a husband and wife closer together. Midwives should not regard NPP counselling only as providing information and leaflets about contraception but should focus on female physical, mental, and reproductive health.

**Key words:** natural parenthood planning, natural methods, health, awareness

## INFORMING WOMEN ABOUT THE NEGATIVE SIDE EFFECTS OF CONTRACEPTION

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**Aim:** Hormonal contraception is offered today as something obvious, harmless or even beneficial for the health of a modern woman or a teenage girl. Recent experience as well as the latest scientific research show the presence of severe health problems in patients taking this sort of contraception.

**The method:** 100 respondents using hormonal contraception took part in our survey (average age 30 ). We made up and used our own survey sheet to measure the level of women's knowledge about the negative side-effects of hormonal contraception.

**Results:** Based on the questionnaire we found out the insufficient knowledge of women about the contraception they are using, about its side effects (46% had no information, 40% had insufficient information), about the risk factors, which can increase its side-effects and about the drug action and its mechanism.

**Conclusion:** Using hormonal contraception belongs to the most common ways of preventing pregnancy, therefore, it would be appropriate to improve the education of women about hormonal contraception by midwives, its negative side-effects as well as to educate secondary schools and university students. It would be appropriate to give them information about natural methods of birth control, which are comparably effective, but causing no side effects to the woman.

**Key words:** contraception, side effects, informing, women

## IMPLEMENTATION OF TOBACCO CONTROL LEGISLATION IN CENTRAL EUROPE

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**Introduction:** Analysis of selected indicators of effects of tobacco control legislation in Czech Republic, Slovakia, Poland and Hungary in 2002 and 2012 period. Global Tobacco Survey (GYTS) data in 13-15 years old adolescents were analysed.

**Material and methods:** The GYTS is a school-based survey of schoolchildren aged 13–15 years developed by the World Health Organization and the Centers for Disease Control and Prevention. Series of the questionnaire surveys were conducted in Czech Republic (2002, 2008 and 2011), Slovakia (2002, 2007 and 2011), Poland (2003 and 2009) and Hungary (2003, 2007 and 2012) on representative samples.

**Results:** Majority of respondents reported passive smoking outside homes within 7 days prior survey, the most of them in Czech Republic (75.6%). Within 2008 and 2012 passive smoking occurrence decreased significantly only in Hungary from 73.0% to 65.7%. Percentage of current smokers being not refused because of their age while buying cigarettes in a store significantly declined in Hungary from 76.2% in 2003 to 35.2% in 2012. This indicator in other countries did not change significantly and in Slovakia reached 78.6% in 2011. About three quarters of respondents in Czech Republic, Poland and Hungary reported to see some of a cigarette brand logo on TV. In Slovakia, occurrence of this indicator declined gradually from 78.0% in 2002 to 59.8% in 2011.

**Conclusions:** High occurrence of passive smoking in public places remains a common problem of all studied countries, indicating a need for a comprehensive smoke-free legislation. Results indicate low compliance with a legislation banning sale of tobacco products to minors. Indirect tobacco advertisement in media represents another significant problem. Possible factors leading to favourable trends found in Hungary (access of tobacco to minors) and Slovakia (indirect advertisement) should be further analysed to provide implications for tobacco control activities in other Central European countries.

## TRENDS OF TOBACCO USE AND ITS FORMS IN CENTRAL EUROPE

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**Introduction:** Analysis of selected indicators of tobacco use in 13-15 years old adolescents in Czech Republic, Slovakia, Poland and Hungary in 2002 and 2012 period. Global Tobacco Survey (GYTS) data were analysed.

**Material and methods:** The GYTS is a school-based survey of schoolchildren aged 13–15 years developed by the World Health Organization and the Centers for Disease Control and Prevention. Series of the questionnaire surveys were conducted in Czech Republic (2002, 2008 and 2011), Slovakia (2002, 2007 and 2011), Poland (2003 and 2009) and Hungary (2003, 2008 and 2012) on representative samples.

**Results:** Current cigarette smoking (one or more days within last 30 days) prevalence ranged from 18.5% in Hungary to 30.6% in Czech Republic, holding no significant gender differences. Within studied period current cigarette smoking declined in Hungary (from 27.2% in 2003 to 18.5% in 2012) and current smoking of other tobacco products increased in all countries, particularly in Czech Republic (from 8.5% in 2002 to 26.8% in 2011). Water-pipe use increase in Slovakia from 4.8% in 2007 to 12.7% in 2011, holding similar extend as in other countries.

**Conclusions:** Tobacco use is widespread in Central European countries. Increase of use of other tobacco products is most probably attributable to exotic forms of tobacco, namely water-pipes. Possible determinants of decline in cigarette smoking in Hungary should be further analysed to provide implications for tobacco control activities in other Central European countries.

## **CHANGES IN SUBCELLULAR DISTRIBUTION OF PROTEINS AND THEIR EFFECT ON MALIGNANT TRANSFORMATION**

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Introduction: it is well known that changes in intercellular adhesions are associated with functional and structural aberrations of tumour cells and thus can significantly contribute to the tumour progression. The aim of our work was to assess the importance of changes in expression of Snail, Claudin-1 and E-cadherin. These proteins are considered to be involved in changes of intercellular adhesions and interactions. E-cadherin is considered to be a key transmembrane molecule; its reduced expression leads to loss of intercellular communication and increased metastatic potential. Snail is one of the most important regulators of E-cadherin. It also regulates the expression of Claudin-1, a tight junction protein which affects cell differentiation. Effector functions of the proteins are known to be affected not only by their quantity, but also by their subcellular distribution. And this is what we focused on in our work.

Material and methods: for protein detection, an indirect immunohistochemistry method on formalin-fixed, paraffin embedded tissue sections was used. Biopsic samples including 120 cases of colonic carcinoma and 42 adenomas were examined. The protein expression was compared to non-tumorous colonic tissue. The results were statistically analysed using Fisher exact and chi-square tests.

Results: E-cadherin: there were neither significant differences in the intensity of expression, nor in the distribution of the protein. Claudin-1: the expression was significantly higher in adenomas and in adenocarcinomas compared to normal epithelium. In tumour lesions, there also was translocation from membrane to cytoplasm. Snail: there were no significant differences in expression between non-tumorous and tumorous tissue. On the other hand, redistribution from nucleus to cytoplasm in carcinomas was shown.

Conclusion: We have proved that transformation of colonic epithelium is caused not only by quantitative changes, but also – and more importantly – by changes in subcellular localisation.

## BLOOD PRESSURE IN RELATIONSHIP TO VITAMIN D LEVELS

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**Introduction:** Vitamin D plays a crucial role in calcium and bone metabolism. Recently, the growing evidence suggests the link between vitamin D deficiency and cardiovascular diseases including hypertension. The purpose of our study was to investigate the relationship between plasma vitamin D levels and blood pressure in selected population of healthy volunteers from Bratislava and neighborhood.

**Materials and methods:** Data from 671 apparently healthy subjects (506F/165M) aged 15-81 were included into the retrospective study. We analyzed basic anthropometric and lifestyle data (age, weight, height, BMI, smoking), systolic and diastolic blood pressure (SBP, DBP) and vitamin D plasma levels (25(OH)D). Exclusion criteria were: vitamin D supplementation at least 2 months before the study and engagement in the study during the period from May to October. Study group was divided into 2 subgroups according to the gender.

**Results:** Both subgroups did not differ in age and smoking. Men had significantly higher BMI, SBP, DBP and 25(OH)D levels than women. High prevalence of vitamin D deficiency and insufficiency (<30 ng/ml) was found predominantly in women (68% in women vs. 47% in men). 25(OH)D significantly inversely correlated with SBP, age and BMI, but not with DBP. General linear model showed significant influence of 25(OH)D on SBD independently on age, BMI, smoking and male gender.

**Conclusion:** The prevalence of vitamin D deficiency in Slovak population is alarming. The significant relationship between 25(OH)D levels and SBP independent on known risk factors of hypertension was found. It is very important to study the relationship of vitamin D and blood pressure because of the potential clinical impact. It is relatively affordable to determine plasma concentrations of 25(OH)D and the supplementation with vitamin D, needed mostly in winter season, is not expensive. Maintenance of the optimal plasma concentration of 25(OH)D could possibly help in prevention and therapy of hypertension.



## **THERAPY OF MULTIPLE SCLEROSIS COULD AFFECT LIPOPROTEIN PROFILE-PILOT STUDY**

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Multiple Sclerosis (MS) is an autoimmune disease in which was described increased risk of cardiovascular disease (CVD) after diagnosis. That risk retreats with the progression of therapy in which is mostly used interferon  $\beta$  (IFN) that lowers LDL and raises HDL and so could prevent CVD. The aim of our study was to detect lipoprotein profile and lipoperoxidation in multiple sclerosis patients with focusing on the incidence of atherogenic lipoproteins as possible risk factors of CVD.

The tested group consisted of 10 patients with relapsing-remitting MS, 8 females and 2 males who were cured by interferon  $\beta$ . The control group consisted of 10 healthy subjects, 8 females and 2 males, too. Weight, height and body mass index (BMI) were recorded in each proband. Blood sample was collected for biochemical and serological tests. Estimated were triglycerides, total cholesterol and lipid hydroperoxides. Lipoprotein fractions and subfraction of VLDL, IDL 1-3, LDL 1, LDL 2, LDL 3 - 7 and HDL were detected by polyacrylamide gel electrophoresis.

Results of our measurements showed reduction in VLDL, IDL-3 and elevation of LDL-1 lipoprotein subfractions in tested group compared to control group. We suggest that interferon may enhance conversion proatherogenic VLDL via IDL to LDL. It could explain the decreased levels of the first two mentioned lipoproteins and high level of the third one, the less atherogenic LDL subfraction. Increased production of free radicals and oxidative stress in these patients has been also detected. Oxidative damage of lipoproteins may enhance their atherogenicity, but lipoperoxidation damages membrane phospholipids or free plasma lipids, too.

In conclusion, interferon  $\beta$  and the disease itself may affect the lipoprotein profile and lipoperoxidation in multiple sclerosis so negatively as well as positively, but further detailed investigation on the larger group is necessary.

## CLINICAL APPLICATION NT-proBNP AS AN AUXILIARY MARKER IN THE SCREENING OF PRIMARY ALDOSTERONISM

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**Introduction:** Primary aldosteronism is one of most common causes of secondary arterial hypertension. It often leads to resistant hypertension and appropriate diagnosis of this disease is of growing importance. Screening by determining the aldosterone-renin ratio (ARR) - ratio of plasma aldosterone concentration to plasma renin activity is often encumbered with false positive results and therefore confirmatory tests must be done. Our aim was to find out whether the examination of NT-proBNP could be helpful in diagnosis of primary aldosteronism.

**Methods:** We used results of plasma aldosterone concentration (PAC) and plasma renin activity (PRA) which were generated from the hospital database of 692 patients with resistant hypertension between 2.1.2008 and 31.10.2011. In these patients, levels of NT-proBNP were available in the hospital database in 221 patients. Data from 131 patients in whom the PAC, PRA and NT-proBNP were analysed within 2 months apart were used for statistical analysis.

**Results:** In our group of 131 patients there were 83 men and 47 women. Average age was  $54.2 \pm 14.8$  years. Positive results of aldosterone/renin ratio (ARR) over 30 were in 33 patients. We found large dispersion of levels NT-proBNP in both patient groups with ARR > 30 and ARR < 30. After regression analysis we did not find any significant relation between NT-proBNP and PRA ( $r = 0.0539$ ), PAC ( $r = 0.1170$ ) or ARR ( $r = 0.136382$ ).

**Conclusions:** We did not find any correlation between NT-proBNP and PAC, PRA or ARR. Determination of NT-proBNP does not appear to be helpful in the screening of primary aldosteronism.

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