

ABSTRACTS

BOND DISORDERS OF PATIENT WITH ASTHMA: A CASE STUDY.

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The issue of treatment and providing preventive activities concerning bronchial asthma and related diseases is still not fully investigated. Besides the medical aspects, there are psychosomatic ones, such as sociopsychological, which can intensify asthma symptoms or arise as a consequence of the disease. Previous investigations in the sphere of psychosomatics show that a chronic illness makes the patients reveal an extended dependence on others. The patients also tend to perceive other people as being responsible for their health problems. This attitude is usually caused by the disease process and the feeling of a threat related to the symptoms. In the present article I presented a case study of a patient with diagnosed bronchial asthma who was subjected to psychodynamic therapy. The aim of the therapy is to help a patient in an interpersonal sphere and, when a therapeutic alliance is already established, to support the development of individualization and separation in his disordered process and help recover. The case presented in the article is one of many others, when patients with chronic diseases, including bronchial asthma, apply for therapy. Adult patients usually complain about discomfort in social situations, difficulties to keep job or, like in the presented case, about bond disorders which reveal themselves as intrusiveness and adhesiveness.

THE PREVALENCE OF TOBACCO SMOKING AMONG POLISH AND FOREIGN MEDICAL STUDENTS

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Smoking tobacco is one of the most important risk factors of many diseases (tumors of lung, larynx, circulation system diseases, obturation of the lung). According to WHO, about 1.1 billion of people in the world is addicted to tobacco products. Youth determines the individual life style in the adult age to a fundamental degree. The future of our society depends on young generations, but their health is not a key target. The aim of this study was an estimation of the tobacco abuse, gaining the knowledge about the risks connected with tobacco smoking and about health behaviors among medical students from the Polish and English curriculum of the Pomeranian Medical University in Szczecin, Poland. The survey was performed by means of a questionnaire which was composed of close-ended and multi-choice questions. Questionnaires studies were performed in Polish medical students (male-66, female-177) and foreign students (male-28, female-52). Numerous deficiencies in pro-health behavior were found. About 20% students smoked cigarettes. There was a significant difference between Polish and foreign students. A higher number of Polish women smoke cigarettes than the Norwegian women and a higher number of Norwegian men smoked than the Polish men. The first test of cigarette smoking among the youth took place at the age of 16. Students smoked about 10 cigarettes a day. Both groups smoked in similar situations. Parents of non-smoking students were significantly more often non-smokers. Also, partners of smoking students were substantially more common smokers.

THE IMPACT OF SLEEP APNEA SYNDROME ON OXIDATANT-ANTIOXIDANT BALANCE IN THE BLOOD OF OVERWEIGHT AND OBESE PERSONS

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Introduction: Oxygen free radicals are considered to be involved in pathobiochemistry of cardiovascular diseases. The discussion on cessation of breathing-related oxidative stress may explain coexistence of sleep apnea syndrome and a cardiovascular disease. **Aim:** This study was designed to assess chosen blood antioxidant markers in persons with excess body mass index (BMI) with and without obstructive sleep apnea (OSA) diagnosis. **Methods:** White overweight (BMI-1: 25.0-29.9 kg/m²) and obese (BMI-2: 30.0-34.9 kg/m²) non-smoking Caucasians with no acute or chronic disorder were qualified for the study. OSA suspected persons underwent overnight polysomnography with a Poly-Mesam device. The control (C) group was BMI and blood pressure-matched with no OSA. Newly diagnosed type-2 diabetics diagnosed with an oral glucose tolerance test were excluded. We formed two BMI-1 groups: BMI-1-C subjects (n=19, age 53±7; 9 females, 10 males), BMI-1-OSA subjects (n= 20, age 54±6; 8 females, 12 males) and two BMI-2 groups: BMI-2-C persons (n=20, age 49±7; 9 females, 11 males) and BMI-2-OSA patients (n=21, age 51±7; 7 females, 14 males). After an overnight fast, the following parameters were determined: concentrations of plasma lipids: T-C, TAG, HDL-C, LDL-C (bio Mérieux, UV-160A Shimadzu); concentration of plasma total antioxidant status, TAS (Randox, Statfax™ 1904 Plus); activity of erythrocyte Cu, Zn-superoxide dysmutase, SOD (Randox, Statfax™ 1904 Plus); plasma lipid peroxidation products measured as a concentration of thiobarbituric acid reacting substances, TBARS (Yagi method, Specord M40). A statistical analysis was performed using STATISTICA 5.0 for Windows. Data were shown as means ± SD. **Results:** I. BMI-1-OSA patients presented decreased SOD as compared with BMI-1-C persons (1022±476 vs. 1381±511 U/g ; p=0.006). BMI-2-OSA patients presented both SOD (1085±418 vs. 1601±535 U/g ; p=0.002) and TAS (1.14±0,27 vs. 1.35±0.21 mmol/l; p=0.047) decreased, and elevated TBARS (12.3±9,86 vs. 6.77±3.35 μmol/l; p=0.027) comparing with BMI-2-C subjects. II. Control subjects of BMI-1-C group had the calculated correlations TAS to SOD (r=0.49) and TAS to TBARS (r= -0.58). In BMI-2-Controls the correlation of TAS to TBARS (r= -0.72) was observed. **Conclusion:** Obstructive sleep apnea syndrome decreases the blood antioxidant status of persons with high BMI and may change the oxidative stress markers relationships.

TOBACCO SMOKING BEHAVIOR AMONG HIGH SCHOOL STUDENTS IN POLAND

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Background: The purpose of the study was to examine the Polish students' smoking behavior. **Methods:** A group of 671 students of the Poznań University of Medical Sciences (UM) and Adam Mickiewicz University (UAM) from Poznań, Poland aged 19-25 years participated in the study. They completed a questionnaire about tobacco smoking. **Results:** More than half (53.1%) of the participants tried smoking at least once. The most frequent age of the first attempt to smoke was 18 years. Males tried smoking earlier (between 10 and 14 years) than females (between 18 and 21 years); a significant difference. The most frequent reasons for starting to smoke were curiosity and influence by friends. The group of 155 students (23.1%) were active smokers during the study: 10.7% of them smoked regularly and 12.4% occasionally. The age of 18 was the most frequent time when the smoking became regular. Males smoked more frequently than females (insignificant difference). There were differences observed between the kind of school and the frequency of smoking. The UAM students smoked significantly more often than the UM students – 32.8% and 18.6%, respectively ($p < 0.05$). Students of smoking parents smoked more frequently than those of non-smoking parents. Most smokers (80%) were aware of detrimental effects of smoking and most of them declared a will to quit smoking. **Conclusions:** There is still a great percentage of smokers among high-school students in Poland. The age of 18 is the most dangerous time for the development of addiction. Anti-tobacco prevention must be started very early before the age of 18 and continued up to the age of 21.

THE INFLUENCE OF ANESTHESIA ON THE HYPOXIC VENTILATORY RESPONSE IN SENESCENT RATS

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There are reasons to believe that the hypoxic ventilatory response (HVR) declines with advancing age, as both neural respiratory pathways and the respiratory muscle pump weaken or deteriorate. The evidence is, however, controversial. Studies show a decline, no change, or even an increase in HVR in old age. In the present study we sought to determine to what extent the states of wakefulness and anesthesia, variably used in the experimental studies on the subject, could influence changes in the hypoxic reactivity with age. The study was performed in rats divided into two extreme age groups; young – 3 months old and senescent - >24 months old. Two different experimental paradigms, each using a separate set of rats, were employed: anesthetized (α -chloralose and urethane; 10 mg and 50 mg/100 g, i.p.) and awake. In the former, minute neural respiratory output, as an index of minute ventilation (V_E), was assessed from the product of amplitude and respiratory rate components of phrenic neurogram, and in the latter, V_E was assessed from tidal volume and respiratory rate recorded in a body plethysmograph. Both age-groups in both paradigms were subjected to two levels of acute hypoxia (14% and 11% O_2 in N_2). We found that in anesthesia the profile of minute neural respiratory output over the course of hypoxia was not appreciably suppressed in the old compared with young rats. However, the hypoxic respiratory gain, which is a magnitude of the increase in the peak responses with increasing level of hypoxia, was flattened out in the old rats. By contrast, in conscious rats, both the profile of V_E and the hypoxic gain were significantly increased in the old rats. Concerning the latter, the mean peak V_E increased to 1099.1 ± 105.6 and to 1463.3 ± 179.2 ml/min/kg at 14% and 11% hypoxia, respectively. For comparison, the respective increases in the young rats were to 833.2 ± 57.8 and to 980.1 ± 5.3 ml/min/kg, which was significantly lower than those in the old rats ($P < 0.05$). Thus, the study demonstrates that the ventilatory chemoreflex was well preserved in old rats, providing they had not been under anesthesia. Old rats may exhibit overcompensation of any age-related handicap in the respiratory system. We conclude that anesthesia heavily dampens hypoxic ventilatory reactivity in the aged, which may be one plausible explanation for divergent results reported in ventilatory studies.

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PREVALENCE AND CORRELATES OF ALLERGIC DISEASES AMONG CHILDREN

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The occurrence frequency of allergic diseases conspicuously grows, particularly in the countries with high average living standards. It is, to a large extent, attributed to the changes in lifestyle. The purpose of this paper was to determine the correlation between the factors that are indirectly (socio-economic status) and directly (passive smoking, nutrition habits) connected with the style and condition of living and the occurrence frequency of allergic diseases in children. It was also checked whether there were any differences in the biological development rate between the children with allergies and their peers. The material consisted of the data obtained during the cross-sectional surveys carried out in the towns and villages of Southern Poland in the years 2007-2008. 301 children aged from 4 to 9 year were examined. The height and body weight were measured in each of them, which served as a foundation for the evaluation of their development progress. The information on their social-economic status, the child's exposure to tobacco smoke and the way of feeding them during the first months of their lives was obtained by means of a questionnaire. The frequency of allergic diseases was determined from the responses given by the parents to the question of whether the doctor diagnosed the occurrence of such diseases in their children. The number of children with allergies found as declared by their parents was 51 (19,3%). The number of allergic children in the villages was by far lower than that in towns and cities (12.8% vs. 27.2%). The more frequent occurrence of allergies in children also depended on the level of their parents' education, good economic conditions, artificial feeding during the first months of their lives, and attending nurseries. The children with diagnosed allergies did not differ from their peers by height, but had a higher body weight, which can be a consequence of the medicines they took.

**AN ASSOCIACION BETWEEN CLINICAL SCORING OF DISEASE ACTIVITY
AND HIGH-RESOLUTION CT FINDINGS IN PULMONARY WEGENER'S
GRANULOMATOSIS**

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Wegener's granulomatosis (WG) is characterized histologically by necrotizing granulomatous angitis that most commonly involves the upper, lower respiratory tract and kidneys, but may affect any organ system. The aim of the study was to assess the usefulness of high-resolution computer tomography (HRCT) for evaluating pulmonary disease activity in WG patients. The pulmonary disease activity at the time of examination was scored in 66 patients with WG according to clinical, radiological, and bronchoscopic findings: activity group (n=43, group 1), past activity group (n=14, group 2). Of the 66 staging examinations, 57 (86%) revealed abnormal CT scans: masses or nodules (30 patients in group 1 and 6 patients in group 2), parenchymal opacifications (15 in group 1 and 1 in group 2), pleural irregularity (3 in group 1 and 10 in group 2). HRCT may be a useful adjunct to clinical scoring of disease activity in WG with pulmonary involvement.

CHRONIC CRUSTATION, NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS*, AND THE RELAPSE RATE IN PULMONARY WEGENER'S GRANULOMATOSIS

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Wegener's granulomatosis (WG) is a systemic disease characterized by necrotizing granulomatous inflammation of the upper and lower respiratory tract and necrotizing crescentic glomerulonephritis. Nasal carriage of *S. aureus* is considered a risk factor for *S. aureus* infections. The aim was to examine possible risk factors for relapse including refractory nasal carriage of *S. aureus* in patients with WG. Swab cultures of anterior nares for *S. aureus* were taken in consecutive patients (n=88) with limited form (n=45) and systemic one (n=33) and biopsy-proven WG. The occurrence of infection and relapses were identified according to the defined criteria. Of the 88 patients, 55 (59%: 95% CI, 41-76%) were found to be chronic nasal carriers of *S. aureus* ($\geq 82\%$ of nasal cultures positive for *S. aureus*). Hazard regression analysis identified chronic nasal carriage of *S. aureus* as independent risk factor for relapse (HR-9,26; CI 2.23-36,5) in patients with limited WG. Chronic nasal carriage of *S. aureus* characterized patients with WG who are more prone to relapses.

PROCALCITONIN AS AN INDICATOR OF A SYSTEMIC RESPONSE TO INFECTION OR REFRACTORY PULMONARY WEGENER'S GRANULOMATOSIS

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The differential diagnosis of infection from active immune disease, such as Wegener's Granulomatosis (WG), is one of major clinical problems. Laboratory measures, such as erythrocyte sedimentation rate or C-reactive protein, can be elevated in both active and nonactive disease. The aim of the study was to compare serum levels of procalcitonin (PCT) in patients with active and inactive disease. 22 sera were tested from 10 patients with active generalized biopsy-proven WG and 12 patients with a nonactive one. PCT serum levels were measured using an immunoluminometric assay. Procalcitonin levels were markedly elevated in 9 of the 10 sera taken during active WG, and 2 sera taken during nonactive WG (1.2-3.6ng/ml). PCT levels were in the normal range in 10 of the 12 sera in nonactive WG (0.28-0.56ng/ml). Procalcitonin serum levels may be a potentially useful marker in the diagnosis of refractory WG and bacterial infection or active immune disease with concomitant bacterial infection.

SEVERE RESPIRATORY DISTRESS CAUSED BY CENTRAL AIRWAY OBSTRUCTION TREATED WITH SELF-EXPANDABLE METALLIC STENTS

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We investigated retrospectively the efficacy of self-expandable metallic stents (SEMS) placement for treatment of severe respiratory distress in patients with central airway obstruction (AO). On admission, all thirty three patients presented with severe respiratory distress symptoms and hypoxia. In all cases central airway obstruction caused by neoplastic infiltration were treated with SEMS, using fiberoptic bronchoscopy method. We found an intraluminal obstruction present in 7 patients, extraluminal compression in 10 patients. Combined stenosis (intraluminal obstruction and extraluminal compression) was present in 16 patients. Tumor infiltration occupied more than 90% of the endoluminal diameter in 21 patients, 70% in 9, and 50% in 3 patients. Obstruction was caused by primary lung cancer in 23 patients, thyroid carcinoma in 5, and primary carcinoma of esophagus in 5 patients. In all cases 1 – 2 stents per patient were placed. Double stenting (esophagus and trachea) was required in six patients. All 33 patients exhibited symptomatic improvement after stent placement. Significant improvement was noticed in arterial blood gas analysis and O₂ saturation. The mean follow-up was 65 days, ranging from 5 to 752 days. We conclude that SEMS are useful for the treatment of severe respiratory distress caused by extraluminal and intraluminal stricture in the central airways and the overall effect of SEMS for stenosis is related to the degree of infiltration and of tumor progression itself.

INCREASED LEVELS OF INTERLEUKIN-12 AND INTERLEUKIN-18 IN BRONCHOALVEOLAR LAVAGE FLUID OF PATIENTS WITH PULMONARY SARCOIDOSIS

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Sarcoidosis is characterized by a diffuse alveolar inflammatory process, although bronchial airways are often involved. We studied prospectively 43 patients (23 women, 20 men) of the median age 39.7 years (range 29–64) with sarcoidosis (BBS) and 13 normal subjects (6 women, 7 men) of the median age 48 years (range 39–63). IL-12 and IL-18 levels were measured using ELISA kits. The Mann-Whitney U-test and the Spearman correlation test were used for statistical analysis (Statistica 5.0). Spirometry and body plethysmography were performed using Elite DL Medgraphics body box. BBS group was characterized by a significantly higher median range of: plasma ACE concentration (72 vs. 34 U /L, $p < 0.0001$), Lymphocyte percentage (34% vs. 14%, $p < 0.0001$), CD4+ cells (59% vs. 36%) and CD4/CD8 ratio (4.2 vs. 1.99, $p < 0.0001$). The IL-12 BALF levels were significantly higher in sarcoidosis patients than in healthy subjects (4.1 pg/mL vs. 3.2 pg/mL, $p < 0.001$). In addition, IL-18 levels were significantly increased in BALF samples (11.1 pg/mL vs. 6.15 pg/mL, $p < 0.0001$). Correlations within BBS group showed negative correlation between IL-12 BAL and ACE plasma levels ($r = -0.33$, $p < 0.05$) with no correlations between IL-12 BAL levels and other major disease activity markers. There was no such correlations in regards to IL-18 BAL levels. Our data suggest a potential role of IL-12 and IL-18 in the local immunologic response in pulmonary sarcoidosis. Further large-scale studies are needed to define the precise role of IL-12 and IL-18 in the immunopathogenesis of this disorder.

UPREGULATION OF TH1 CYTOKINE PROFILE (IL-12, IL-18) IN BRONCHOALVEOLAR LAVAGE FLUID OF PATIENTS WITH HYPERSENSITIVITY PNEUMONITIS

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Hypersensitivity pneumonitis (HP) is an immunologically mediated interstitial lung disease. The abnormalities in the bronchoalveolar lavage (BAL) fluid cell counts are almost always seen in patients with HP according to the stage of the disease. The aim of this prospective study was to find out how the BAL IL-12 and IL-18 levels correspond to the inflammatory activity of the disease. We studied 12 patients (3 women, 9 men) of the median age 41.7 years (range 28–52) with HP (Farmer's lung) and 13 normal subjects (6 women, 7 men) of the median age 48 years (range 39–63). IL-12 and IL-18 levels were measured using ELISA kits. The Mann-Whitney U-test and the Spearman correlation test were used for statistical analysis (Statistica 5.0). Spirometry and body plethysmography were performed using Elite DL Medgraphics body box. There were no significant lung function differences between both groups. HP group was characterized by significantly higher median range of: plasma ACE concentration (55 vs. 34 U/L, $p=0.0016$), lymphocyte percentage (57% vs. 14%, $p<0.001$), CD8+ cells (31.5% vs. 17%, $p<0.001$) and lower CD4/CD8 ratio (1.2 vs. 1.99, $p<0.0001$). The IL-12 BALF levels were significantly higher in HP patients than in healthy subjects (3.94 pg/mL vs. 3.2 pg/mL, $p = 0.003$). In addition, IL-18 levels were significantly increased in BALF samples (14.2 pg/mL vs. 6.15 pg/mL, $p < 0.0001$). Correlations within HP group showed strong correlation between IL-12 BAL and percentage of Lymphocytes ($R=0.678$, $p=0.015$), and negative correlation with percentage of Macrophages ($R=-0.6439$, $p=0.024$). There were no such correlations in regards to IL-18 BAL levels. We conclude that upregulation of Th1 cell cytokine profile may play a significant role in pathogenesis of HP. Studies based on large group of patients are needed to further establish the precise role of both cytokines in disease progression and its response to treatment.

THE INFLUENCE OF N-OLEOYL-DOPAMINE ON THE RESPIRATORY RESPONSE TO HYPOXIA IN RATS

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N-oleoyl-dopamine (OLDA) is a fatty acid derivative of dopamine, a key neurotransmitter involved with the hypoxic respiratory response. The aim of our study was to investigate whether N-oleoyl-dopamine would exercise an influence on the respiratory hypoxic response. If that were the case, a second objective would be to investigate whether central or peripheral dopamine pathways were involved in this action. The study was performed on male Wistar rats. The animals were anesthetized, vagotomized, paralyzed, and ventilated. Changes in respiration were evaluated from the product of amplitude and frequency components of the phrenic neurogram. Minute respiratory output was expressed a percent change of the baseline level preceding each test. The experimental protocol consisted of acute responses to two levels of the hypoxic stimulus: 11% O₂ and 14% O₂ in N₂. After taking the control responses, either haloperidol (HAL), a general dopamine receptor antagonist, 0.5 mg/100 g, iv, or domperidone (DOM) – a peripheral dopamine receptor antagonist, 0.3 mg/100 g, ip, was administered, and the hypoxic tests were repeated. After a 20-min interval, OLDA (2 mg/100 g, ip,) was given. The last step was the repetition of the hypoxic responses after another 20-min interval from OLDA injection. In the control group, the administration of the antagonists was omitted. In this group OLDA alone decreased the maximum respiratory hypoxic responses from 147.8 ±10.2(SE)% to 124.1 ±6.8% and from 163.1 ±10.4% to 140.7 ±10.6% of the baseline levels for 14% and 11% hypoxia, respectively. The decrease might be related to the peripheral, carotid body-mediated dopamine-like action. In the antagonist groups, either HAL or DOM also decreased the maximum responses by about 13-17% and 22-30% of the baseline levels for 14% and 11% hypoxia, respectively. OLDA administered after HAL did not appreciably change the maximum responses from the HAL baseline. However, OLDA administered after DOM significantly increased the maximum response to 14% hypoxia, although it did not change that to 11% hypoxia. We conclude that OLDA alone decreases the hypoxic respiratory response but does not change the hypoxic sensitivity. Since the action of OLDA is blocked by HAL, this action may be mediated by dopamine pathways. The persisting increase in the respiratory response to the stronger hypoxia due to OLDA, given after DOM, may have to do with the central stimulatory effect of dopamine. OLDA would then interact with respiration at both central and peripheral levels.

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THE INFLUENCE OF MANGIFERIN, A POLYPHENOLIC ANTIOXIDANT, ON THE HYPOXIC VENTILATORY RESPONSE IN THE RAT

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Reactive oxygen species (ROS) have a role in the initiation of the hypoxic ventilatory response, which is generated by carotid body chemoreceptor cells. The exact nature of this role is unsettled. Nor is it full well clear whether ROS formation is decreased or increased by hypoxia in chemoreceptor cells, although the current prevailing opinion is that ROS increase in the mitochondrial electron transport chain during hypoxia. Furthermore, ROS interact with the very mechanisms of hypoxia-sensing, as they are required for hypoxia inducible factor-1 α stabilization by hypoxia; a step being essential for activation of the expression of hypoxia-responsive genes. If it is so, then antioxidant pretreatment could reasonably well be expected to downregulate the hypoxic ventilatory response. We investigated this hypothesis in the present study by comparing the hypoxic ventilatory responses before and after application of mangiferin (Sigma, St. Luis, USA), a polyphenolic antioxidant, belonging to a class of xanthenes that commonly occur in plants and having extensive free radical scavenging and iron chelating activities. The study consisted of biochemical and functional parts. In the former, we found that mangiferin did not cross the blood-brain barrier after intraperitoneal injection, as it could not be recovered from brain extracts, using thin-layered chromatography. That finding made us to reason that an interaction of mangiferin with the hypoxic ventilatory response would be centered at the peripheral, carotid body mechanisms. In the functional study, the acute ventilatory responses to two levels of the hypoxic stimulus, 12% O₂ and 8% O₂ in N₂, were carried out in conscious rats before and 40 min after intraperitoneal injection of mangiferin in a dose of 300 mg/kg. Minute ventilation and its constituent components, frequency of breathing and tidal volume, were studied in an unrestrained plethysmographic rodent chamber (Buxco, UK). We found that mangiferin significantly depressed the profile of the hypoxic ventilatory response over the 3-min test time. The peak hypoxic ventilation decreased from 1226.7 \pm 34.8SE to 924.0 \pm 75.7 ml/min/kg and from 2349.2 \pm 217.6 to 1616.4 \pm 91.8 ml/min/kg at 12% and 8% hypoxia, respectively (P<0.01). An enhanced decline in peak ventilation with increasing strength of the hypoxic stimulus shows that mangiferin depressed hypoxic sensitivity. Both components of respiratory pattern contributed to the ventilatory changes observed, but mangiferin exerted a greater influence on the respiratory rate. In conclusion, the study supports the notion that ROS are key modifiers of the hypoxic ventilatory response. ROS may be essential for a full development of the hypoxic hyperventilation, but do not seem the underlying determinants of the response, as antioxidant treatment does not abolish the ventilatory hypoxic reactivity.

SPIROMETRIC PARAMETERS IN MALNOURISHED GIRLS WITH ANOREXIA NERVOSA

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Repercussion of obesity for different organs and systems has been widely studied in the past. However, the effects of serious malnutrition on the respiratory system are less known. Anorexia nervosa is a frequent cause of malnutrition. The aim of the study was to determine spirometric parameters in a large cohort of 102 malnourished girls with anorexia nervosa admitted to the Department of Endocrinology between 2004 to 2008. Among patients, only 71(69,6%) of the girls aged 12-18 years (mean 15,6), mean BMI 16,1 kg/m², met the ATS/ERS forced expiratory maneuvers criteria for spirometry. Flow-volume loops obtained from these patients were taken into further statistical evaluation. The most frequently observed abnormalities were: decreased inspiratory capacity (IC) seen in 33 (46%) girls and decreased PEF observed in 45 (63%) of patients. Maximum voluntary ventilation was within the normal range in all but 2 (2,8%) subjects. Diminished values below a lower limit of FEV₁, FVC, FEV₁/FVC, MEF₅₀ were observed in 10 (14%), 13 (18%), 3 (4%), and 3 (4%) patients, respectively. We found relatively strong positive correlations between weight and the absolute values of examined parameters. We assume that spirometric abnormalities observed in anorexia nervosa are probably a result of respiratory muscle weakness and body mass loss.

INDUCED SPUTUM METALLOPROTEINASES AND THEIR INHIBITORS IN RELATION TO EXHALED NITROGEN OXIDE AND SPUTUM NITRIC OXIDES AND OTHER INFLAMMATORY CYTOKINES IN COPD PATIENTS

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An inappropriate MMP/TIMP balance in chronic inflammation may lead to disturbances in the turnover and remodeling of pulmonary extracellular matrix, as can occur in asthma and COPD. The aim of this study was to assess sputum levels of MMP-1, MMP-2, MMP-3, TIMP-1 and TIMP-2, and MMPs/TIMPs ratios in relation to exhaled NO (eNO) and sputum NOs (nitrates and nitrites) and IL8 obtained from COPD patients, healthy non-smokers, and healthy smokers. Higher levels of TIMP-1 (118.9 ng/ml) and TIMP-2 (3.75 ng/ml) were found in COPD patients than in healthy smokers (17.7 ng/ml, $p < 0.03$; 0.51 ng/ml, $p > 0.05$, respectively) and healthy non-smokers (84.6 ng/ml, $p > 0.05$; 1.61 ng/ml, $p > 0.05$, respectively). We observed significant positive correlations between concentrations of NOs and MMP-1, MMP-2, MMP-3 and TIMP-2 ($r = 0.37$, $p < 0.02$; $r = 0.60$, $p < 0.0001$; $r = 0.56$, $p < 0.0004$ and $r = 0.47$, $p < 0.004$, respectively) in COPD patients. IL8, MMP-2, MMP-3 and TIMP-2 levels in induced sputum were negatively correlated with airway obstruction, i.e., FEV1/FVC ($r = -0.61$, $p < 0.00009$; $r = -0.41$, $p < 0.01$; $r = -0.38$, $p < 0.02$; $r = -0.49$, $p < 0.002$). The correlation between NO and metalloproteases and their inhibitors and IL8 suggests an important role of the reactive form of nitrogen oxide in airway remodeling in COPD. Our study indicated the potential pathogenic role of stromelysin-1 (MMP-3) in COPD.

CBP AND PPAR γ mRNAs AFTER CORTICOSTEROID OR FORMOTEROL THERAPY OF COPD PATIENTS

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Inhaled corticosteroids (ICS), which effectively switch off pro-inflammatory genes in asthma are ineffective in chronic obstructive pulmonary disease (COPD). We have previously reported increased cAMP response element binding protein (CREB) and activated (CREB-P) protein levels in cells isolated from induced sputum, during COPD therapy with formoterol/inhaled corticosteroid (F/ICS). Thus, our data pointed to the possible role of increased CREB-associated signaling in poor response to the therapy. In this study we assessed how similar therapy affects expression of two CREB-related nuclear signaling molecules, particularly CREB binding protein (CBP) which interacts with integrated upstream nuclear signaling (at pre-chromatin level) and peroxisome proliferator-activated receptor gamma (PPAR γ) which negatively interfere with proinflammatory nuclear factor kappaB (NF- κ B) for CBP binding. Twenty five patients with stable disease were subjected to sputum induction before and after F or Budesonide (ICS) b.i.d. 4 weeks therapy. Sputum samples were solubilized, intact cells were extracted using millipore filtration and centrifugation and total cellular RNA was extracted from isolated cells using qPCR-grade RNA isolation kit (Superarray, USA). First strand cDNA samples were prepared and amplified by thermal cycling on ABI 7900 Cyclyer with SYBR-green detection and appropriate controls (Superarray, USA). In patients treated with F, CBP mRNA was not altered due to the drug therapy, but PPAR γ mRNA levels were increased by more than 2 fold ($P < 0.01$). ICS therapy resulted in significant decrease of CBP mRNA (almost 70% decrease; $P < 0.01$) and increased by more than 3 fold ($P < 0.01$) mRNA of PPAR γ . Since PPAR γ is negatively correlated with proinflammatory signaling and altered CBP expression may result in its reduced recruitment to the transcriptional initiation complex on the promoter region of various genes, elevated levels of PPAR γ mRNA and lowered CBP mRNA after therapy may reflect their involvement in nuclear anti-inflammatory signaling.

INDUCED SPUTUM IN PATIENTS WITH INTERSTITIAL LUNG DISEASES: A NON-INVASIVE SURROGATE FOR CERTAIN PARAMETERS IN BRONCHOALVEOLAR LAVAGE FLUID

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Background: Bronchoalveolar lavage is a standard tool for the assessment of patients with ILD. However, it is a relatively invasive procedure. Induced sputum (IS) has been proposed as a useful non-invasive method for the assessment of airway and parenchymal diseases. The aim of this study was to evaluate IS cellular composition and T-lymphocyte subpopulations and to compare them with those of BALF in patients with ILD. **Material and methods:** We studied prospectively newly diagnosed 59 patients with ILD: sarcoidosis (SA n=36), hypersensitivity pneumonitis (HP n=16) and idiopathic pulmonary fibrosis (IPF n=7). IS was performed at least 7 days after BAL by inhaling a 5% saline solution for 4 periods of 5 minutes by the selecting plugs method. 400 cells were differential counted in May-Grunewald-Giemsa stained cytopreps and T-lymphocyte subsets were analyzed by FACS. **Results:** 33 patients were able to produce an adequate sputum sample (SA-15, HP-11, IPF-7). The percentage of macrophages was significantly lower in IS than in BALF in SA group ($p=0.0045$), the percentage of neutrophils was significantly higher in IS than in BALF in SA and HP group (SA: $p=0.0007$, HP: $p=0.006$), and the percentage of lymphocytes was significantly lower in IS than in BALF in patients with HP ($p=0.004$). A significant correlation was found between BALF and IS CD4+, CD8+ T-lymphocyte subpopulations and CD4+/CD8+ ratio both in the whole group ($r=0.80$, $r=0.88$, $r=0.88$, $p<0.0001$, respectively) and in the 3 subgroups (SA, HP and IPF). Moreover, in HP patients there was a significant correlation between BALF and IS lymphocyte ($r=0.64$, $p=0.04$) and the percentage of eosinophils ($r=0.63$, $p=0.04$). **Conclusions:** A strong correlation of the T-lymphocyte subsets in IS and BALF in patients with different ILD may reflect inflammation in both the distal and proximal parts of the lung. IS may therefore be a non-invasive surrogate for certain parameters in BALF in these patients.

METABOLIC ABNORMALITIES AND INFLAMMATORY PARAMETERS IN OBSTRUCTIVE SLEEP APNEA PATIENTS

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OSAS is associated with an increased rate of metabolic syndrome consisting of central obesity, dyslipidemia and diabetes. These might be related to a proinflammatory state as inflammatory cytokines play a role in lipid's and carbohydrate's metabolism and energy expenditure. In this study, we have examined the presence of metabolic abnormalities in relation to IL-6, TNF and CRP serum level in 102 OSAS patients (71 men (M), 24 women (W) with OSAS (AHI \geq 5) and 77 nonapneic controls (39 M and 38 W) BMI matched. In both groups, obese and non-obese as well as diabetic and non-diabetic persons were included. Plasma total cholesterol and triglyceride were determined enzymatically on a Hitachi 912 analyzer (Roche Diagnostics,). HDL-cholesterol (HDL-C) was measured using a homogenous method with polyethylene glycol-modified enzymes and alpha-cyclodextrin. LDL-cholesterol (LDL-C) was calculated by the Friedewald equation. Cytokine level was determined by ELISA method. The mean IL-6 concentration in non-obese OSAS patients was lower than in obese OSAS group but significant difference was found only in the group of W ($p=0.001$). The IL-6 concentration was higher in OSAS patients with type 2 diabetes when compared to obese non-diabetic patients. The difference was significant in the group of W ($p=0.01$). The level of TNF in obese OSAS patients was highly significantly higher than in non-obese group ($p<0.001$). TNF level was significantly higher in obese patients with type 2 diabetes than in the non-obese group. In all subgroups results were statistically significant (whole cohort: $p<0.0001$; M: $p=0.0002$; W: $p<0.0001$). Obese OSAS patients with high IL-6 and high TNF level had higher total cholesterol level, higher LDL and lower HDL level then non-obese OSAS patients and then non-apneic control ($p<0.05$). The correlation between BMI, IL6 and C-reactive protein was positive ($p<0,01$). OSAS has a significant role in the occurrence of metabolic syndrome. This association may be related to inflammatory state.

CALCIUM SIGNALING IN NEUTROPHILS AND LYMPHOCYTES AND ITS MODIFICATION BY INSULIN

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The goal of the study was to evaluate the process of neutrophil and lymphocytes activation via Ca mediated transduction signal into those cells and its modification by insulin. The studies was performed with the use of isolated peripheral blood neutrophils and lymphocytes from 30 healthy volunteers. Neutrophils were activated by bacterial peptide fMLP. Lymphocytes T were activated by anti CD3 antibodies. Intracellular Ca²⁺ concentration kinetics was assessed by flow cytometry with the use of Fluo3 and Fura Red fluorescent dyes. Data were collected in histograms displaying Fluo3 fluorescence vs. time and Fura Red fluorescence vs. time and mean channels of fluorescence intensity were used for calculation. fMLP induced highly significant Ca²⁺ mobilization in granulocytes (p<0.0001). Insulin increased both free and bounded calcium level (p<0.05). fMLP induced stimulation of insulin pretreated granulocytes resulted in the increase of free calcium ions but not bounded calcium. Proportion of free to bounded calcium increased significantly (p<0.001). Anti CD3 antibodies induced significant increase of free calcium ions in T lymphocytes (p<0.0001). Bounded calcium level remained unchanged. Insulin increased both free and bounded calcium concentration in T lymphocytes (p<0.01). We conclude that insulin is a potent immunomodulator and its signaling pathways are mediated by calcium ions concentration changes. Derangements in the concentration of intracellular calcium may represent a link between the mechanisms of insulin resistance in diabetes.

ASTHMA, ALLERGY, AND RESPIRATORY SYMPTOMS IN CENTENARIANS LIVING IN POLAND

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According to National Census, there were 1541 people over the age of 100 years (centenarians) in Poland, in 2002 including 1215 females and 326 males. The aim of the present study was to assess the prevalence of asthma, allergy and respiratory symptoms in centenarians included in the Polish Centenarians Program, PolStu 2001, conducted between 2001 and 2004. The study group consisted of 301 subjects including 258 females and 43 males. Research data were gathered by means of a questionnaire designed exclusively for the study, filled in by an interviewer with the subjects or their families in case of subjects' cognitive impairment. According to medical history reported by the subjects, 10 persons (3.3%) suffered from asthma and 41 (13.6%) from allergy. There were none subjects with childhood-onset asthma. Allergy to food and medicinal products was the most prevalent. One in three centenarians reported dyspnea, and one in eight complained of cough. Correlation of reported symptoms with smoking status was analyzed. According to literature, various symptoms are underreported in elderly population. Moreover, in extremely old subjects, it is very difficult to perform a comprehensive medical assessment with additional tests to reveal the clinical importance of symptoms. Medical care of the very elderly should, therefore, be based on clinical assessment with support of a long-term medical history and reliable information on physician-diagnosed diseases.

FACILITATION OF THE COUGH RESPONSE DURING RHINITIS: THE ROLE OF THE FIRST AFFERENT NEURON

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Background: The idea of this study aroused from the paper published in *Respir Physiol Neurobiol* (2004), where we have reported augmentation of the cough response due to intranasal administration of capsaicin, a strong stimulant of the nasal mucosal afferent nerve endings. The aim of the present study was to assess the role of the first afferent nasal neuron in the process of facilitation of the cough response in the animal model. **Material and methods:** 26 male TRIK strain guinea pigs were used in this study. All animals were anesthetized with urethane (1.1mg/kg, ip). The animals were placed in the supine position on a heated operating pad and body temperature was maintained at 37-38°C. All animals were tracheotomized and the larynx was disconnected from the proximal part of the trachea, although the innervations of these structures were preserved. Cough induced by mechanical stimulation of the tracheobronchial mucosa was elicited three times: in the control condition, immediately after intranasal capsaicin challenge, and after intranasal capsaicin again prior local intranasal administration of Mesocain. During the intranasal Mesocain instillation, the animals were placed in the head down position and nasal cavity was filled with the anesthetic solution via a thin plastic catheter introduced into the nasopharynx through a small opening in the lateral laryngeal wall. This procedure was performed in 18 animals, in 8 animals the cough response was induced also three times, and as they were free of any intranasal manipulations, they were taken as controls for the stability and reproducibility of the tracheobronchial cough during urethane anesthesia. **Results:** We found that the intensity of tracheobronchial cough was significantly enhanced due to intranasal capsaicin challenge; the effect was abolished by a local intranasal pretreatment with Mesocain. The intensity of the cough attack was 21.95 ± 2.52 kPa vs. 37.65 ± 5.12 kPa vs. 22.89 ± 3.03 kPa, $p=0.007$ and the number of coughs in one attack was 2 (1-4) vs. 3.5 (2-7) vs. 2 (1-4), $p=0.001$. **Conclusion:** Facilitation of the cough response due to intranasal administration of capsaicin is of neuronal origin, however facilitation of the cough during rhinitis, that is complex pathological process could involve also other mechanisms.

CHANGES IN COUGH REFLEX SENSITIVITY IN A GUINEA PIG MODEL OF ALLERGIC RHINITIS

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Cough reflex sensitivity is increased in patients with allergic rhinitis. We reported that coughing is enhanced in the guinea pig model of allergic rhinitis. Here we address the hypothesis that the cough reflex sensitivity could be increased in this model. A retrospective analysis of the pooled data from our previous studies was carried out. In the ovalbumin-sensitized guinea pigs the allergic inflammation in the nose was induced by repeated intranasal instillations of ovalbumin (6-8 times in 7 days intervals). Control naïve animals received intranasal saline. Cough reflex sensitivity was determined by the inhalation of aerosols with doubling concentrations of citric acid (0.05-1.6M) 30 min-2h after intranasal instillation of ovalbumin. Cough threshold was expressed as the concentration of citric acid at which two cumulative coughs were recorded (C2cum, expressed as geometric mean [95% confidence interval]). We found that the cough threshold was reduced in the ovalbumin-treated animals. C2cum was 0.44M[0.33-0.58M] and 0.25M[0.18-0.35M] prior and after 6 repeated intranasal ovalbumin instillations, respectively ($p < 0.05$, $n = 36$). There was no difference in the cough threshold in the control animals (0.64M[0.5-0.82M] vs. 0.76M[0.51-1.14M], $p = 0.85$, $n = 29$). We reported that the leukotriene cys-LT1 receptor antagonist montelukast inhibited the enhanced coughing in this model. In this separate study, montelukast also partially reversed reduced cough threshold. C2cum was 0.33M[0.15-0.75M], 0.05M[0.02-0.11M] and 0.18M[0.08-0.4M] at the baseline, prior and after montelukast treatment, respectively ($p < 0.01$, $n = 8$). We conclude that the increase in the cough reflex sensitivity due to rhinitis can be modeled in the guinea pig. Our data indicate that the guinea pig model is useful for the evaluation of drugs aimed to treat cough associated with rhinitis.

RECURRENCE OF CEREBROVASCULAR EVENTS AND CASE FATALITY IN PATIENTS WITH SLEEP BREATHING DISORDERS AND ISCHEMIC STROKE OR TRANSIENT ISCHEMIC ATTACKS: A FOLLOW-UP STUDY

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Aim: The aim of the study was to assess the morbidity and case-fatality as well as functional outcome in patients with ischemic stroke or TIA and SBD as compared with patients with stroke or TIA without SBD. **Methods:** 89 patients (mean age 65.2 ± 10.3 ; 77 males 12 females, 69 patients with stroke and 20 patients with TIA) without previously diagnosed SBD, were screened for SBD in the first 7 days after transient ischemic attack or ischemic stroke. The clinical status was assessed with National Institute of Health Stroke Scale (NIHSS), the functional outcome was evaluated with Rankin and Barthel scales. The patients were stratified according to AHI into groups without SBD ($AHI \leq 5$) and with SBD ($AHI > 5$). We analyzed case-fatality rates and rates of TIA or stroke recurrence within 2 years of observation, as well as clinical (NIHSS) and functional outcome (Barthel and Rankin scales) in 3 and 24 months follow up. Clinical parameters: age, BMI, cardiovascular risk factors and fibrinogen plasma level were also analyzed. Statistical comparisons were performed with one-way non parametric ANOVA followed by Dunn's post hoc test and with chi-square tests. **Results:** SBD ($AHI > 5$) were present in 61 (68.5%) patients with stroke or TIA. The rate of recurrence of TIA or stroke in patients with SBD was significantly higher (12 patients-19.7%) as compared with patients without SBD (3 patients – 10.7%) within two years of observation, $p < 0.05$. Case fatality rates were not significantly different between these two groups (4 patients - 7% with SBD and 2 patients – 6.8% without SBD). There were no significant differences in neurological status (NIHSS) between patients with and without SBD (NIHSS 2.08 ± 1.6 and 1.96 ± 1.4 respectively; $p > 0.05$) during 3 months' follow-up. We did not find any differences either in the functional outcome (Barthel and Rankin scales) during 3 and 24 months' follow-up between patients with and without SBD. **Conclusions:** Our data show that SBD significantly increased the incidence and risk of recurrent TIA or ischemic stroke in patients with TIA or stroke during two years' follow-up. SBD in patient with stroke or TIA did not influence the clinical and functional outcome of stroke in the long term observation.

PHENOTYPIC ANALYSIS OF MACROPHAGES AND LYMPHOCYTES OF CIGARETTE SMOKERS

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The influence of tobacco smoke on human health is still an important problem worldwide. Complex inflammatory processes and changes in the immune system are crucial in the pathogenesis of smoking related disorders like chronic obstructive lung disease, lung cancer, asthma, interstitial lung diseases, atherosclerosis. Inflammatory cells are involved in these reactions and the cellular immune response seems to be most affected by cigarette smoke (CS). The objective of this study is to present the alterations in the immune cell populations in tobacco smokers with special account to pulmonary macrophages and lymphocytes. We compared healthy active smokers (n= 50) with healthy never-smoking volunteers (n= 48). Pulmonary macrophages and lymphocytes were harvested from induced sputum, bronchoalveolar lavage fluid (BALF) and peripheral blood (PB). BALF and induced sputum cell profile, morphology and phenotype of cells were assessed. For macrophage phenotyping the immunocytochemistry method with antibodies anti: CD11b, CD14, CD54 and CD71 was used. For lymphocyte subtypes measurement flow cytometry was used. Following subpopulations of lymphocytes were identified: T, B, natural killer (NK), T helper (Th), T suppressor/ cytotoxic (Ts/c), T activated (Ta, HLADR+, CD25+), regulatory (Treg) and cytotoxic (CD3+/CD16/56+). Expression of death receptor - Fas on immune cells was measured. We found a significantly increased macrophage (pigmented cells) count in smokers when compared with non smokers. This count significantly correlated with pack/years smoked. The induced sputum population of macrophages was characterized by an elevated proportion of cells with expression of CD11b, CD14, CD54 and CD71 surface markers. Fas positive macrophages were more numerous in the BALF of smokers when compared with non smokers. The analysis of BALF lymphocytes revealed significant differences between smokers and nonsmokers. There was a higher proportion of T, T c/s, Ta and cytotoxic T cells in smokers when compared with non smokers. The proportion of Th cells and the Th:Tc/s ratio in the BALF of smokers were lower than in non smokers (46.9% vs 62.7%, 0.51 vs 0.69). No significant differences in the proportion of PB cells between smokers and non smokers were found. A significant increase of Fas positive T cells of smokers was found. Proportion of Fas+ T lymphocytes was significantly higher in asymptomatic smokers when compared with non smokers (mean: 71.6% vs 61.0% for Fas+/ CD4+ and 73.8% vs 58.3% for Fas+/CD8+ lymphocytes). Significant correlations of Fas positive cells with pack years smoked were observed. Preliminary data showed a lower proportion of Treg in PB of smokers than in non smokers. The results of our study confirmed significant changes in the proportion of immune cell subtypes in the lung environment of smokers. These changes were dose-dependent. Changes in the proportion of Fas positive lymphocytes and T reg cells in PB indicated a possible influence of CS on the recruitment of lymphocytes.

INHIBITION OF SALIVARY AMYLASE ACTIVITY BY CIGARETTE SMOKE ALDEHYDES

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Cigarette smoke (CS) is involved in the pathogenesis of cardiovascular and respiratory diseases additionally to its local toxic effect on the oral cavity. The deleterious effects of cigarette smoke compounds rationalize the high incidence of periodontal diseases and neoplastic diseases of oral tissues in smokers. Some noxious compounds of cigarette smoke like unsaturated (acrolein, crotonaldehyde) and saturated aldehydes (acetaldehyde), could interrelate with thiol compounds of salivary proteins, leading to structural and functional alteration of these molecules. Prior studies have established an *in vitro* significant decrease of some salivary enzymatic activities, following exposure to cigarette smoke. Additionally, it was found that glutathione (GSH) has protective effect against the damaging role of CS to salivary enzymes, emphasizing the role of thiol groups in the mechanism of inactivation of some salivary enzymes. In this study salivary amylase activity showed a significant inhibition following an external addition of purified aldehydes known to be present in CS, probably due to the interaction between aldehydes and -SH groups of the enzyme. Our results indicate that although saturated aldehydes are the chief aldehydes present in CS, the significant decrease in amylase activity was due to unsaturated aldehydes, reacting, probably, through the double bond with the thiol group of proteins by the Michael addition reaction.

ALTERED EXPRESSION OF T LYMPHOCYTE SURFACE MARKERS IN CHILDREN WITH CHRONIC AUTOIMMUNE THYROIDITIS

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Introduction: CTLA-4 and CD28 surface molecules are among the main regulators of T cell activation. The CTLA-4 gene was found to be associated with a variety of autoimmune diseases, like type I diabetes mellitus, autoimmune thyroid diseases and with bronchial asthma. The aim of the study was to evaluate the changes in basic T cell subpopulations: CD4+ and CD8+, and the expression of CD152+ and CD28+ on T cells before and after in vitro stimulation of T cell from children with chronic autoimmune thyroiditis in comparison with healthy controls. **Material and methods.** The blood samples were obtained from 35 children with chronic autoimmune thyroiditis (AT) and from 25 healthy children age and sex matched, free from allergic, immune and hematological disorders and with normal thyroid gland function. The diagnosis of chronic autoimmune thyroiditis (Hashimoto's thyroiditis) was based on the presence of anti -thyroid antibodies and characteristic picture of thyroid gland in ultrasonographic examination. Commercially conjugated antibodies to fluorescein isothiocyanate (FITC) and phycoerythrin (PE) dyes (immunocytometry system, Coulter) were used, including isotype control. CD4, CD8, CD28, CD152 markers were evaluated at the baseline, after 48 hours culture with phytohaemagglutinine (PHA) or without stimulation. **Results:** At the baseline, expression of CD152 was significantly lower in AT patients than in the control group ($p < 0.0000002$). No difference in the expression of other examined markers was found. PHA stimulation significantly increased the number of CD152+ T cells ($p < 0.01$) and decreased CD28+ ($p < 0.001$) and also CD4+ T cells ($p < 0.01$) in healthy subjects. In AT children, after PHA stimulation, the number of CD152+, CD4+ and CD8+ T cells remained stable, CD28+ T cells decreased compared to non-stimulated culture. The number of CD4+CD152+ T cells correlated inversely with the antithyroglobuline antibodies level ($r = -0,34$, $p < 0.05$). **Conclusions:** The alterations in leukocyte surface markers are associated with AT. Lymphocyte activation in both examined populations leads to differing changes in the proportion of T-lymphocyte subsets. Defective CTLA-4 expression on activated T cells may promote the activation of co-stimulatory T cell signaling pathways leading to autoimmune diseases including AT. This effect may be primary or result from abnormal immune regulation.

COUGH REFLEX SENSITIVITY IN HEALTHY CHILDREN AND ADOLESCENTS: DEPENDENCE ON GENDER AND PUBERTAL STAGE

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Studies of healthy adult volunteers and patients with chronic cough have shown that women are more sensitive to inhaled tussigenic stimuli, including capsaicin. The explanation for this phenomenon remains unknown, although the influence of sex hormones may play the role.

AIM: Our purpose was to examine the effect of pubertal status on cough reflex sensitivity parameters in a population of male and female children and adolescents. **DESIGN:** The study comprised of cough reflex sensitivity (CRS) testing, spirometry, and a children-completed pubertal status questionnaire. The inclusion criteria to enter the study were no current symptoms of respiratory disease, no respiratory infection in last 2 weeks, no allergic disease, and no other diseases, which could modulate CRS. For assessing CRS, each subject inhaled up to 12 capsaicin aerosol concentrations (0.61-1250 $\mu\text{mol/l}$) during 400 ms at 1 min intervals. CRS was defined as the lowest capsaicin concentration that evoked minimum of 2 coughs. **RESULTS:** The results of 226 children (median age 12 yr, IQR 12-16 yr, 100 girls/126 boys) that completed pubertal status questionnaire were divided into 3 subgroups according to pubertal status (prepubertal, early pubertal, late pubertal). CRS [median(interquartile range)] was similar in prepubertal girls (n=20) and boys (n=14) [39.1 (4.9-117.2) $\mu\text{mol/l}$ vs. 29.3(9.8-78.1) $\mu\text{mol/l}$; p=0.74], group of early pubertal girls had lower CRS compared to boys, but the difference was not significant [156.3(68.4-312.5) $\mu\text{mol/l}$ vs. 39.1(19.5-156.3) $\mu\text{mol/l}$; p=0.09]. However, CRS in late pubertal girls (p=63) was significantly higher compared to boys (n=31) [39.1(19.5-156.3) $\mu\text{mol/l}$ vs. 156.3(39.1-312.5) $\mu\text{mol/l}$; p=0.05]. **CONCLUSIONS:** Our results point out that start of menarche in girls (late puberty stage) is associated with significantly higher CRS compared to boys. Female sex hormones, therefore, could play the role in increased cough reflex sensitivity among females.

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A WAVELET ANALYSIS OF VOLUNTARY COUGH SOUND IN PATIENTS WITH RESPIRATORY DISEASES

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Objective: Changes in the characteristics of the cough sound may refer to some specific pathological processes and their evolution. In this pilot study we analyzed voluntary cough sound properties in subjects with pathological conditions, such as asthma bronchiale (AB), chronic obstructive pulmonary disease (COPD) and discriminated them from the control cough sound in healthy subjects. **Methods:** The wavelet transform was used due to a non-stationarity present in the sound of cough. Duration of the sound, maximal power in the time and frequency domain, distribution of the power in different wavelet scale (frequency) bands and their ratio to the total spectral power were determined. Several discrete wavelet transform measures (up to 5th level of decomposition) were computed. Spectral characteristics were computed also for the sample (time instant) with maximum of wavelet coefficients. **Results:** The duration of the cough sound was longer during pathological conditions. The longest duration and the highest power of the cough sound were found in COPD. In AB patients, the higher frequencies were detected compared to chronic bronchitis and the power of cough sound was shifted to higher frequency range compared to control coughs. According to the selected parameters, cough sounds were classified using discriminant analysis with correct classification rate of about 85-90 %. **Conclusion:** Acoustic properties of a voluntary cough sound differ among all analyzed groups. Described method of analysis enables objective quantification of voluntary cough sound characteristics with potentially useful diagnostic and prognostic value.

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DISCRIMINATION BETWEEN VOLUNTARY COUGH SOUND AND SPEECH IN VOLUNTEERS BY SPECTRAL AND COMPLEXITY ANALYSIS

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Background: Cough is the most common and important symptom in many respiratory diseases. The objective monitoring of cough sound for extended period is an important step towards better understanding of this symptom. To-date, methods used to monitoring of cough sound were primarily subjective. Because ambulatory cough monitoring systems are not commercially available we have prepared own monitoring system, which is able to distinguish between voluntary cough sound and speech in healthy volunteers. **Methods:** The 18-min sound records (reading of the book with voluntary coughs marked at fixed places in the text) were obtained using portable digital voice recorder (Sony ICD-MX20, Sony Corporation, China) and stored to the digital recorder memory card. Sound records were transferred into PC, converted to 11 kHz 16-bit mono digital wave file (WAV format) and stored to PC memory. The non-sound (silent) segments of recording were excluded from further analysis. Parameters of sound events have been calculated using the time-domain, spectral and non-linear analysis (parameter sample entropy - SampEn). Based on these parameters, classification regression tree was constructed for classification of cough and non-cough sound events. We have validated the usefulness of our developed algorithm against manual counts of the cough sounds obtained by a trained observer in 18 sound recordings obtained from healthy volunteers (13 female - median age 24.7 yrs, range 18 – 56 yrs; 5 male - median age 47.2 yrs, range 26 - 66 yrs). **Results:** All assessed cough sound characteristics were different between cough and non-cough sound events. The median sensitivity of our classification tree value was 100% (the interquartile range is from 98 to 100) and the median specificity was 95% (the interquartile range is from 90 to 97). **Conclusion:** We have developed algorithm to distinguish between voluntary cough sound and speech with a very high degree of accuracy, which is comparable with recently published studies based only on the sound analysis.

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MEDULLARY RAPHE MIDLINE IS INVOLVED IN PRODUCTION OF EXPULSIVE EXPIRATIONS IN ANESTHETIZED RABBITS

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Effects of kainic acid lesion in the medullary raphe midline on reflex expirations induced mechanically from the trachea were examined. Spontaneously breathing rabbits were anesthetized by ketamine and xylazine, i.m., followed by pentobarbitone i.v. Excitatory neurotoxin kainic acid (2 mg/ml in artificial CSF, total volume of 55-100 nl.) was pressure microinjected into the medullary midline, rostral to the obex (2 microinjections at 2 different depths). The lesion (mostly affected the obscurus and magnus raphe nuclei) reduced the number of reflex expirations by 80% ($p < 0.05$) and expiratory amplitudes of esophageal pressure, abdominal EMG moving averages, and abdominal EMG powers by 71%, 62%, and 57%, respectively (in all cases $p < 0.05$). The duration of abdominal activity in post-lesion responses was not altered. Control microinjections of artificial CSF had no effect on the reflex responses. In rabbits, the medullary raphe nuclei participate in control of the number and the strength of expiratory expulsions originating from the trachea. Our results support the concept of separated neuronal circuits controlling the expression vs. motor pattern of the behavior.

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THE INFLUENCE OF MICROINJECTION OF D,L-HOMOCYSTEIC ACID INTO THE BÖTZINGER COMPLEX AREA ON THE COUGH REFLEX IN THE CAT

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Microinjection of D,L-homocysteic acid (DLH) was used to test the hypothesis that neuronal activation within the Bötzinger complex area (BOT) can modify the spatiotemporal characteristics of the cough reflex in 17 spontaneously breathing pentobarbitone anesthetized cats. DLH (50mM, 1.3-1.75nmol, 9 cats) reduced the number ($p<0.01$) and expiratory amplitudes of abdominal EMG ($p<0.01$) and esophageal pressure ($p<0.001$) during mechanically induced tracheobronchial coughs. The cough abdominal activity was shortened by 48% ($p<0.05$). DLH microinjections also temporarily reduced the respiratory rate ($p<0.01$) and increased the mean arterial blood pressure ($p<0.001$), baseline of esophageal pressure ($p<0.01$), and end tidal CO₂ concentrations ($p<0.01$). Lower DLH doses (0.27-0.35nmol, 7 cats) or the vehicle (25-35nl, 8 cats) induced few alterations in cardiorespiratory or coughs characteristics. Predominantly inhibitory effects of BOT neurons might account for observed depression of cough abdominal activity. The mechanism of cough number reduction due to DLH microinjections within the BOT is unclear.

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INFLUENCE OF SELECTIVE INHIBITORS OF PHOSPHODIESTERASE-3 AND PHOSPHODIESTERASE-4 ON COUGH AND AIRWAY REACTIVITY

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Introduction: Nowadays, there are several groups of drugs used in the therapy of cough. As their administration is often associated with occurrence of adverse effects, new alternatives and pharmacological targets are evaluated in experimental and clinical conditions. One of the ways is suppression of inflammation in patients with airway diseases associated with cough and airway hyperresponsiveness. In this, selective inhibition of special isoforms of phosphodiesterase (PDE) could be of benefit. The aim of this study was to assess the influence of selective inhibitors of PDE3 and PDE4 on cough and airway reactivity. **Methods:** Cough and airway reactivity were evaluated in non-anaesthetized guinea pigs in double-chamber whole body plethysmograph. The cough was evoked by inhalation of citric acid aerosol (AC; 0.6 mol/l) and trained observer visually counted and recorded number of coughs during AC nebulization (2 min) as well as after finishing the nebulization (2 min). As a marker of *in vivo* airway reactivity specific airway resistance was measured after 2 min lasting nebulization of AC and histamine aerosol (10^{-6} mol/l). *In vitro* airway reactivity was measured in organ chambers. Tissue strips from trachea and lungs were exposed to cumulative doses of histamine and acetylcholine (10^{-8} - 10^{-3} mol/l) and contractile responses were recorded. In blood, count and differential count of white blood cells were evaluated. All parameters were measured in healthy as well as in ovalbumin-sensitized guinea pigs before and after intra-peritoneal administration of cilostazol (selective PDE3 inhibitor) or citalopram (selective PDE4 inhibitor) at a dose of 1 mg/ kg. **Results:** Sensitization of guinea pigs with ovalbumin significantly increased number of cough efforts as well as specific airway resistance. *In vitro* studies confirmed significantly increased tracheal and lung tissue reactivity to histamine and acetylcholine. Pre-treatment with cilostazol decreased number of cough efforts only in healthy guinea pigs, whereas citalopram significantly suppressed cough in both healthy and ovalbumin-sensitized animals. Both selective PDE inhibitors decreased *in vivo* and *in vitro* airway reactivity, with more significant decrease observed after cilostazol in sensitized animals and after citalopram in healthy animals. Sensitization with ovalbumin led to significant increase of white blood cells count in blood, with predominant increase in percentual representation of neutrophils, monocytes and eosinophils. Both cilostazol and citalopram decreased the count of monocytes and neutrophils, confirming their anti-inflammatory potential. **Conclusions:** Administration of selective PDE inhibitors (PDE3 and PDE4) may participate in influencing the cough and airway reactivity in the model of ovalbumin-sensitized guinea pigs. However, their therapeutic potential as antitussive and anti-inflammatory drugs needs to be more corroborated. Nevertheless, advantageous could be simultaneous inhibition of PDE3 and PDE4, as both of them are involved in the pathomechanisms of inflammatory airway disease associated with airway hyperresponsiveness and cough.

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THE ORIGINS OF BIOETHICS: ADVANCES IN RESUSCITATION TECHNIQUES

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During the last years there has been observed an increasing interest in meta-bioethical issues. This turn in research focus is regarded as a sign of the maturation of bioethics as a distinct area of academic inquiry. The role of historical-philosophical reflection is often emphasized. It should be noted that there is rather a common agreement that the future of bioethics lies in critical reflection on its past, in particular, on the very origins of this discipline. Sharing A. Caplan's opinion, advance in medicine technologies, especially the introduction of respirators and artificial heart machines, is to be considered as one of the main issues that started bioethics. Using methods of historical as well as meta-ethical research this article aims at describing the role of advances in resuscitation techniques in the emergence of bioethics and exploring how bioethical reflection has been shaped by this technological development. The brief historical analysis permits to sustain that there is a close bond between the emergence of bioethics and the introduction of the sophisticated resuscitation technologies into medical practice. The meta-ethical reflection reveals that advances in resuscitation techniques not only caused the start of bioethics in the second half of 20th century but first and foremost has influenced the development of bioethics by: (i) posing the question of justice in health care, (ii) altering commonly accepted ontological notions of human corporeality, and (iii) reconsidering the very purpose of medicine.

THE INFLUENCE OF NEURAMINIDASE ON GROWTH AND METASTATIC ABILITY OF A NEOPLASTIC TUMOR

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Neuraminidase is an alpha-glycosidase enzyme, which cleaves terminal sialic acid residues from sialylated substrates, such as glycoproteins and glycolipids. By doing so, neuraminidase remodels cell surface charge and surface antigens, and regulates many biological processes. In the present study we set out to assess neuraminidase influence on the development of an experimentally transplantable tumor and its metastatic action in the lungs. The aim was to answer the question of whether neuraminidase, while administered both *in vivo* and *in vitro*, could influence the growth of Morris hepatoma and its metastatic ability in the rat. 124 female Buffalo breed rats, weighing 160 ± 10 g, 12 weeks old were used for the study. The experiment was continued for 21 days. The rats were administered *Clostridium perfringens* neuraminidase (Sigma) into the caudal vein or into tumor mass. Biometric measurements were taken of the tumor site in the left posterior extremity for the assessment of a tumor mass and volume. Internal organs were macroscopically inspected for the presence of metastases. A scrap of the left posterior extremity from the implanted site, lungs (in which Morris hepatoma metastases are usually found), and other organs were taken for histopathological examination. Some of the histological material was preserved for NM-23-H2 protein assay - a marker of Morris hepatoma growth and metastatic abilities. Blood was taken to determine Cathepsin B activity - a marker of hepatoma progression, and GOT, GPT, GGTP - as typical markers of liver damage. In all groups of neuraminidase-treated rats, which obtained different doses of it, only single metastases were found in the lungs, while in the control group 80% of the rats had metastases in this location. This trend was not observed in case of peritoneal metastases, which were similar in both control and experimental groups. GOT, GPT and GGTP levels were higher in the investigated groups with Morris hepatoma than those in the healthy rats. There was no correlation found between the levels of those enzymes and neuraminidase administration. The experiment also revealed an increased concentration of cathepsin B in all rat groups with Morris hepatoma implanted, which might indicate cathepsin B as a new auxiliary marker of Morris hepatoma growth in similar experimental models. Any influence of neuraminidase, administered *in vivo* or *in vitro*, on cathepsin B levels in this model was not detected. Thus, the results obtained in the experiment confirmed the GOT, GPT and GGTP abnormalities in a neoplastic disease. We conclude that neuraminidase influences hepatoma Morris growth and its metastatic abilities.

QUANTITATIVE REAL-TIME PCR EVALUATION OF FREE-CIRCULATING DNA IN PLASMA OF PATIENTS WITH RESECTABLE NON-SMALL CELL LUNG CANCER: PRELIMINARY RESEARCH

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Objective: Many reports of the last decade suggest that cell-free plasma DNA provides useful genetic biomarkers for early diagnostics and clinical outcome prediction in lung cancer patients. Thus, the choice of reliable and efficient method of plasma DNA quantification and its capacity to distinguish between health and cancer would be an essential step prior to any clinical evaluation of cell-free DNA measurement. Apart from the mean plasma DNA content determination, real-time qPCR enables rapid and convenient circulating DNA fragment-length assessment. Recent papers demonstrate the level of plasma DNA integrity may reflect the clinical state of cancer patient and possibly become a tool in prognosis and follow-up monitoring. The aim of present preliminary study was the quantitative analysis of DNA content in plasma samples from healthy volunteers and non-small cell lung cancer (NSCLC) patients before and after surgery. **Methods:** blood samples were collected from 16 healthy volunteers and 14 NSCLC patients (IA-IIIB) prior to and within one week after surgery. The extracted plasma DNA was measured quantitatively by real-time qPCR using β -actin gene as the amplifying target, likely present in all normal and neoplastic cells. The DNA integrity index, defined as the ratio in relative abundance of 400 versus 100 bp PCR products, was analyzed using one forward primer and two nested reverse primers for long and short amplicons. **Results:** the Kruskal-Wallis ANOVA analysis showed a significant differences in plasma DNA content between healthy volunteers (2.65 ng/ml) and NSCLC patients before (12.10 ng/ml) and after surgery (68.74 ng/ml; all $p \leq 0.02$). NSCLC patients presented higher content of 400 bp fragments after the surgery, than before treatment and healthy controls (all $p < 0.02$). The plasma DNA integrity index differed significantly between untreated cancer patients (0.15) and healthy control group (0.43; $p = 0.004$) and demonstrated a trend towards further decrease in patients after the surgery (0.07; NS). **Conclusions:** Physiological status significantly affects the cell-free DNA content in plasma. The mean plasma DNA content, determined by real-time qPCR, proved to be several-fold higher in cancer patients than healthy volunteers, indeed. Elevated levels of both 100 bp and 400 bp fragments of plasma DNA in cancer patients after surgery may indicate the intensified apoptotic and necrotic processes, mainly due to postoperative trauma. The highest value of DNA integrity index in healthy controls suggests the predominance of apoptotic origin of their plasma DNA. The preliminary results presented need further investigation in a study with larger cancer patient group.

COULD THE CONCENTRATION OF MATRIX METALLOPROTEINASE-9 IN THE SERUM OF COPD PATIENTS RELATE TO THE BASIC DISEASE PROGRESS AND A DEGREE OF AIRWAY OBSTRUCTION?

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Background: COPD is a chronic inflammatory process leading to irreversible airway obstruction. The previous studies showed that the increased level of matrix metalloproteinases (MMPs), especially MMP-9 in sputum and BAL (bronchoalveolar lavage) as a sign of local inflammation in COPD patients. To-date only some studies have estimated MMP-9 serum concentration in COPD patients which correlated with systemic inflammatory process. **Aims:** 1. The estimation and comparison of sera MMP-9 concentration in COPD patients and a healthy control group. 2. The evaluation of correlation between MMP-9 concentration and a degree of airway obstruction in COPD patients. **Materials and methods:** 23 smokers with COPD (17 male and 6 female), diagnosed on the basis of the GOLD 2007 criteria (mean age 59.6 ±9.3 yr) and 23 healthy controls (17 male and 6 female) (mean age 55.2 ±9.1 yr) were enrolled into the study. In both groups spirometry tests were performed using a Jaeger system. The MMP-9 concentration in the sera taken from both groups was studied using the enzyme-linked immunosorbent assay (ELISA) technique. **Results:** Patients with COPD had increased levels of serum MMP-9 compared with the control group (p=0.0005). In the COPD group, MMP-9 concentrations were negatively correlated with FEV1 (p=0.01) and with FEV1/FVC ratio (p=0.0002). **Conclusions:** The results suggest that MMP-9 plays an important role in the systemic inflammatory process in COPD. A higher serum concentration of MMP-9 is connected with the degree of airway obstruction and progression of the disease.

THE INFLUENCE OF OBESITY ON SLEEP QUALITY IN MALE SLEEP APNEA PATIENTS BEFORE AND DURING THERAPY

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Introduction: Evidence exists that obesity even in the absence of sleep related respiratory disorders affects negatively sleep. In this study we examined the influence of the obesity on sleep quality of male sleep apnea patients before and after breathing normalization with continuous positive airway pressure (CPAP). **Material and methods:** We compared the polysomnography from the diagnostic night, the second night with CPAP, and the control night (three months later) in 13 non-obese, 13 obese and 12 severely obese male OSAS patients. The groups were age-matched. **Results:** In the diagnostic polysomnography, the obese and severely obese showed increases in apnea hypopnea index (AHI) and NREM sleep and decreases in min SaO₂, REM sleep and partially slow wave sleep, compared with the nonobese group. In the second CPAP night, normalization of the AHI and a rebound of REM and SWS occurred, which was markedly more pronounced in the severely obese than in the nonobese and obese groups. The min SaO₂ was lower in the obese and severely obese groups. The polysomnography recorded three months thereafter revealed no differences in the AHI and in the sleep stages among the groups. The min SaO₂ in the obese was still lower than in the nonobese. **Conclusions:** After the long term CPAP therapy, no effect of obesity on the sleep quality was found. In the second night with CPAP, severe obesity was associated with greater rebound of SWS and REM sleep. The persistence of decreased min SaO₂ in the obese and severely obese groups under CPAP therapy suggests the concomitant obesity hypoventilation in these patients.

CARDIAC CHEMOREFLEX SENSITIVITY IN CRITICAL ILL PATIENTS

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Introduction: The autonomic nerve system enables the organism to adapt to stress and warrant organ perfusion and oxygenation. The sympathetic vagal balance is maintained beyond several peripheral and central mechanisms, e.g., baro- and chemoreflex sensitivity or heart rate variability (HRV). It has been shown in a previous study, that a reduced peripheral chemoreflex sensitivity is a predictor for sudden cardiac death in patients with congestive heart failure, but only few data exist to the meaning of autonomic dysfunction on the pathophysiology and outcome in critical ill patients. **Methods:** Routine clinical monitoring included the use of 3 french artery-catheter (a. radialis) for invasive blood pressure monitoring. Arterial and venous blood samples were analyzed by measuring the partial pressure of oxygen (PaO₂), partial pressure of carbon dioxide (partial CO₂) and PH using standard blood gas electrodes. For the determination of chemoreflex sensitivity, the ratio of the RR-interval shift in the surface ECG and the change of arterial and venous oxygen partial pressure during a 5 min inhalation of oxygen (6 l/min) were measured. **Results:** 19 critical ill patients aged from 29 to 71 years (median 52.6 yr) were referred to the intensive care unit because of sepsis (n=10, 52.6%), cardiogenic shock (n=6 31,6%) or other life threatening diseases (pneumonia, hemolytic uremia syndrome, postoperative). 31 measurements of chemoreflex sensitivity were done in 19 patients. There was a significant negative correlation ($r = -0.52$; $p = 0.003$) between chemoreflex sensitivity and severity of illness described by the SOFA-Score. In 21 of 31 measurements (67,7%) chemoreflex sensitivity was decreased. **Discussion:** Oxygen breathing causes a decrease in heart rate and a comparable rate dependant decrease in cardiac output in healthy volunteers. Further, during oxygen breathing systemic resistance and blood pressure increase. Our data suggests, that this physiological answer to changes in oxygenation is diminished in critical ill patients, who show reduced chemoreflex sensitivity in about 60% of all measurements. This loss of variability might be a consequence of the underlying disease process. On the other hand changes in coupling might be a cause of advancing disease. **Conclusion:** Reduced chemoreflex sensitivity has been suggested to indicate a poor prognosis. This is in line with the finding of our present study in which we found a significant correlation of the chemoreflex sensitivity and the severity of sepsis.

INTERFERON GAMMA PRODUCTION IN THE COURSE OF *MYCOBACTERIUM TUBERCULOSIS* INFECTION

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It is unclear why some individuals with unknown predisposition develop tuberculosis, while others remain healthy in spite of heavy exposure. Interferon gamma is considered to be a key cytokine responsible for resistance to *M. tuberculosis* infection, as confirmed by increased susceptibility to mycobacterial infections in rare inherited defects in IL-12-IFN gamma axis. The aim of this study was to assess the IFN gamma production by peripheral blood lymphocytes from immunocompetent TB (tuberculosis) patients. The study group included 51 TB patients. In all cases TB was confirmed by culture. Twenty healthy TB contacts were considered as control group. Commercially available ELISA - based assays were used to measure IFN gamma in the supernatant of whole blood cell cultures after stimulation with PWM (*Phytolacca Americana*), PHA (phytohemagglutinin) and PPD (Purified protein derivative). No difference in IFN gamma secretion between patients and control group was found when blood cells were stimulated by PWM or PHA. PPD induced IFN gamma production was higher in tuberculous patients than in controls. The secretion of IFN gamma after non-specific stimulation varied in different clinical and radiological presentation of tuberculosis and it was lower in most advanced and extensive forms of the disease. It is not clear whether the difference in production and release of IFN gamma is primary or secondary phenomenon in the course of the disease.

DAILY PATTERN OF BREATHING IN HEALTHY YOUNG MEN

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Daily changes in pulmonary indices of pulmonary function were examined in 30 healthy young men subjects. The breathing pattern technique (Lungtest system, MES, Kraków) was used to measure daily changes of ventilatory parameters. The measurements of VT, BF, MV, TI, TE, TTOT, VT/TI, TI/TTOT, P0.1/VT/TI, MV/P0.1 were performed every 3 hours during a 24-hour period. Each repetitive cycle test was carried out in the sitting position, subjects with a nose clip were breathing 6 min through mouthpiece, shutter and measurement element. All participants were isolated from sunlight and external time cues. Results of statistical analysis (Fridman test and Student-Newman –Keuls test) revealed significant daily variables in VT, MV, MV/P0.1, VT/TI, P0.1/VT/TI and lack of significant daily variations in other parameters. $P < 0.05$ was accepted as the level of statistical significance. The daily minima of statistical significant variables occurred within the usual sleep period 2:00, 5:00, and also at 8:00 a.m. (although subjects remained awake). In conclusion, the results of this study demonstrated that the respiratory breathing pattern remains relatively stable during 24 hours. Healthy adults have a small but significant daily variation in some breathing parameters.

COMORBIDITIES AS AN ELEMENT OF MULTIDIMENSIONAL PROGNOSTIC ASSESSMENT OF PATIENTS WITH COPD

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Introduction: Chronic obstructive pulmonary disease (COPD) is characterized by chronic airflow limitation and a range of pathological changes in the lung and some significant extra-pulmonary effects, and important comorbidities which may contribute to the severity of the disease. BODE index, a 10-point scale which integrates body mass index, degree of airflow obstruction and dyspnea, and exercise capacity, has been proposed to better identify severity of the disease and to predict subsequent survival of the individuals affected by COPD. The higher scores in BODE index indicate a higher risk of death. Up to date there is limited information concerning comorbidities as prognostic factor as well as estimating correlation between comorbidities and BODE index. **AIM :** The goal of the study was to evaluate prevalence of concomitant diseases in patients with COPD as well as to assess correlation between comorbidities and BODE index, to determine conclusively which coexisting diseases have the highest negative influence on COPD course and subjects general condition. **Material and methods:** In 2007, we enrolled into the cross-sectional study 80 patients with COPD. They were at least 40 years old and had to have more than 10 years history of smoking. 50% of subjects smoked between 20-60 pack-years. 15 patients (18,8%) did not quit smoking (13 male and 2 female). To determine the presence of comorbid diseases, all participants were asked questions according to prepared questionnaire. Every subject underwent lung function test (spirometry), 6- minutes walking test and ECG at rest. The study group consisted of 55 male (69%) and 25 female (31%). The mean age was 63 ± 8.3 years. The study is still ongoing. **Results:** The most frequent comorbidities in patients with COPD were: systemic hypertension (52.5%), oedema of lower limbs (43.8%), movement disorders (38.8%), varices of lower limbs (38.8%), sleeping disorders (32.5%), angina pectoris (26.2%), vertigo (26.2%). Subjects with higher scores in BODE index had significantly greater prevalence of arrhythmias ($p < 0.05$), episodes of pneumonia, mainly male and group of patients of 60-65 years old regardless of gender ($p < 0.05$). Evident correlation was also observed between low FEV1 and number of episodes of pneumonia ($p < 0.05$). Additionally, there was found that patients with higher BODE scores had greater risk of COPD exacerbation and they had increased probability of hospitalizations because of pulmonary problems. **Conclusions:** Results of the study indicate close connection between BODE scores and some comorbidities (especially arrhythmias) and other pulmonary disorders what can suggest that these conditions may aggravate COPD course as well as increase risk of mortality. Therefore, there is a great need to take into special account these conditions in the course of assessing and treating COPD patients. Moreover, number of hospitalizations and COPD exacerbations can be well predicted based on BODE index what can help in choosing better therapy regimen.

EFFECTS OF LOW-DOSE AND HIGH-DOSE AMINOPHYLLINE ON LUNG FUNCTION IN A MODEL OF MECONIUM ASPIRATION SYNDROME

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Introduction: Methylxanthine derivatives may be beneficial in the treatment of meconium aspiration syndrome. Since there is no information on appropriate dosing, this study compared effects of high-dose and low-dose aminophylline on lung function of meconium-instilled rabbits. **Methods:** Artificially ventilated rabbits (2.6±0.3 kg) received intratracheally a suspension of human meconium (4 ml/kg, 25 mg/ml). When respiratory failure developed, animals intravenously received aminophylline at high-dose (HD, 2.0 mg/kg, n=8) or at low-dose (LD, 1.0 mg/kg, n=7) at 0.5 and 2.5 h after meconium instillation, or were left without treatment (n=7). All animals were further oxygen-ventilated for additional 5 h after the first dose of treatment and respiratory parameters, blood gases, and white blood cell count (WBC) were evaluated. At the end of experiments, animals were killed by an overdose of anesthetics and trachea and lungs were excised. Left lungs were saline-lavaged and differential WBC in the sediment was estimated. Right lungs were used to determine lung edema by wet/dry weight ratio and oxidative damage by estimation of thiobarbituric acid-reactive substances, dityrosine and lysine-lipid peroxidation products. In addition, strips from trachea and right lungs were used for measurement of *in vitro* airway reactivity to histamine. **Results:** HD-aminophylline significantly improved gas exchange, reduced ventilatory pressures and right-to-left pulmonary shunts and decreased edema formation, and number of lung neutrophils compared to non-treated group. LD-aminophylline enhanced oxygenation, diminished ventilatory pressures, and pulmonary shunts, but to lower extent than HD-aminophylline, and failed to reduce significantly the lung edema and number of lung neutrophils. Both HD- and LD-aminophylline decreased oxidative damage to lung proteins and lipids, with more pronounced effect of HD-aminophylline on TBARS and of LD-aminophylline on dityrosine levels. Tracheal reactivity to histamine significantly decreased after HD-aminophylline, while lung tissue reactivity was more reduced after LD-aminophylline. **Conclusions:** HD-aminophylline enhanced pulmonary function, diminished lung edema and several inflammation-related parameters in meconium-instilled rabbits more effectively than LD-aminophylline. However, stronger response of LD-aminophylline on lung tissue reactivity and dityrosine suggests participation of other anti-inflammatory mechanisms on the level of lung parenchyma.

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COMBINATION OF BUDESONIDE AND AMINOPHYLLINE IN EXPERIMENTAL MECONIUM ASPIRATION SYNDROME

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Introduction: High-dose glucocorticoids and methylxanthines have improved lung function in meconium-instilled animals, but influenced several cardiovascular parameters. Considering their additive effects we have supposed that combination of low-dose budesonide and aminophylline may keep favorable effects on lung function in reduced adverse effects. **Methods:** Adult rabbits were intratracheally instilled 4 ml/kg of human meconium (25 mg/ml) or saline (n=7). When respiratory failure developed, meconium-instilled animals were treated at 0.5 and 2.5 h after meconium instillation by intravenous aminophylline (1.0 mg/kg, n=7), or by budesonide (0.125 mg/kg) administered intratracheally using inpulsion regime of high-frequency jet ventilation 5 min later followed by intravenous aminophylline (1.0 mg/kg, n=8), or animals were left without treatment (n=7). All animals were further oxygen-ventilated for additional 5 hours after the first dose of treatment. Blood gases, respiratory and cardiovascular parameters and white blood cell count (WBC) were regularly evaluated. At the end of experiments, animals were killed by an overdose of anesthetics and trachea and lungs were excised. Left lungs were saline-lavaged and differential WBC in the sediment was estimated. Right lungs were used to determine lung edema by wet/dry weight ratio and oxidative damage by estimation of thiobarbituric acid-reactive substances, dityrosine and lysine-lipid peroxidation products. In addition, strips from trachea and right lungs were used for measurement of *in vitro* airway reactivity to histamine. **Results:** Although aminophylline improved some respiratory parameters, budesonide combined with aminophylline more effectively reduced right-to-left pulmonary shunts and improved gas exchange, with no significant cardiovascular effects. Combined treatment reduced lung edema and number of lung WBC (particularly neutrophils) to higher extent than aminophylline. Both treatments reduced peroxidation and *in vitro* airway reactivity to histamine, but these effects were slightly more pronounced after single aminophylline. **Conclusions:** Budesonide combined with aminophylline improved respiratory and several inflammatory parameters in meconium-instilled rabbits more effectively with less cardiovascular side effects than single aminophylline. However, no additive effect of budesonide to aminophylline was observed in lipid and protein peroxidation and *in vitro* airway reactivity compared with aminophylline.

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OXIDATIVE STATUS AND LUNG FUNCTIONS DURING THE LONG-TERM INHALATION OF MEDICINAL OXYGEN (O₂) IN COMPARISON WITH PARTIALLY IONIZED OXYGEN (O₂⁻ AND O₂⁺) IN GUINEA PIGS

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Aim: This study was performed to prove the hypothesis that the long-term application of partially ionized oxygen is a safe method with less serious impairment of lung functions than classical oxygen therapy. **Methods:** Experiments were carried out on 40 male guinea pigs (220±35 g). Animals were placed in metabolic cage and inhaled 100% medicinal oxygen (O₂), or partially negatively (O₂⁻) or positively (O₂⁺) ionized oxygen during 17 and 60 hr. Control animals inhaled atmospheric air. Wet/dry weight (W/D) ratio was determined to evaluate the degree of lung edema. Accumulation of dityrosine and lysine-LPO (lipid peroxidation) products demonstrating oxidative modification of proteins were determined in lung homogenate by fluorescence method. Relative numbers of cells were evaluated in bronchoalveolar lavage (BAL) fluid and in peripheral blood. **Results:** After 17 hr the concentration of dityrosines (in arbitrary units; AU) increased in group with O₂⁻ and decreased in group with O₂⁺ as compared with controls (both P<0.01). In group inhaling non-ionized medicinal oxygen the raise was not statistically significant. After 60 hr, the fluorescence of dityrosines significantly rose after inhalation of O₂ and after O₂⁻ as compared with controls (both P<0.01), while there was no increase after inhalation of O₂⁺. After 17 hr values of lysine conjugates with LPO products significantly increased in comparison with controls after inhalation of O₂ (P<0.05) and O₂⁻ (P<0.001). In the group with O₂⁺, the fluorescence of lysine conjugates with LPO products did not rise significantly as compared with controls. After 60 hr the changes were comparable to those after 17 hr. After inhalation of O₂⁻ and O₂⁺ W/D ratio did not change significantly, while after inhalation of O₂ it was reduced. Relative number of neutrophils in BAL fluid was elevated in all groups with oxygen therapy. However, in the O₂⁺ group this value was reduced when compared with O₂ and O₂⁻ groups. **Conclusions:** The results indicate that the long-term inhalation of positively ionized oxygen is associated with less adverse effects on lung functions than non-ionized or negatively ionized oxygen.

THE INFLUENCE OF PLEURAL MACROPHAGES ON PROLIFERATIVE ACTIVITY AND APOPTOSIS REGULATING PROTEINS OF MALIGNANT CELLS

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Malignant tumors contain numerous macrophages as a major component of the leukocytic infiltrate. Our objective was to study soluble factors produced by pleural macrophages. We sampled pleural effusions from patients with cancer, and used human tumor cells lines as targets. Pleural macrophages were cultured, and supernatants were used as a conditioned medium (CM) for cultures of human cell lines A549, MCF7, MDA-MB231, HT29, HCT116, SW620, Jurkat and HL-60. We investigated proliferative activity and expression of apoptosis regulating proteins Fas, Bcl-2, caspase 3, and survivin of malignant cells cultured in the CM. The influence of CM on proliferative activity of malignant cells was assessed by flow cytometric analysis of S phase fraction of the cell cycle. CM had appreciable effect on proliferative activity of malignant cells. An increase of proliferative activity was observed in all assessed cell lines after co-culture with the CM *vs.* control medium. As measured by the percent of positive cells, level of Fas receptor on malignant cells membranes was lower in treated cells than in untreated control cells in all cell lines. Intracellular expression of Bcl-2 protein was significantly increased in A549, HCT116 and MDA-MB 231. Elevated level of intracellular expression of survivin was observed in A549, HT29 and HCT116. Decrease of expression of caspase 3 protein was observed in HT29, SW620 and MCF7. Our findings raise the possibility that macrophages from malignant pleural effusions can act as a stimulator of growth of malignant cells.

THE EVALUATION OF RESPIRATORY-CARDIOVASCULAR INTERACTIONS IN THE DIAGNOSIS OF AUTONOMIC DYSFUNCTION IN OBESITY

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Introduction: The breathing is a human rhythm that exerts profound influences on the cardiovascular system – the heart rate and blood pressure. The aim was to test whether the evaluation of the cardio-respiratory interaction using the heart rate and blood pressure variability analysis and respiratory maneuvers can reveal early subclinical autonomic dysfunction in obese adolescents. **Methods:** Obese patients (20; 12 girls, 8 boys) aged 12-18 years were investigated. The control cohort consisted of 20 healthy probands matched for sex and age. The continuous ECG signal was obtained using ECG device Chirastar 60 (CHIRANA, Slovakia) and continuous finger blood pressure was monitored by Finapres 2300 (Ohmeda, USA). Spectral power in high frequency band (0.15-0.5 Hz) of the heart rate variability (HF-HRV) reflecting respiratory sinus arrhythmia was taken as an index of the cardiac vagal control and spectral power in high frequency band of the blood pressure variability (HF-BPV) as a reflection of mechanical effects of the respiration. Respiratory maneuvers - deep breathing test and Valsalva manoeuvre - were examined. **Results:** The obese group had a significantly reduced spectral power in the HF-HRV and marginally significant lower coefficient of variation (CV_{R-R}) in the deep breathing test. No significant differences were found in either parameters and in the Valsalva maneuver. **Conclusions:** Our study has revealed reduced respiratory sinus arrhythmia indicating cardio-vagal dysfunction in obese adolescents. No differences were detected in the HF-BPV spectral analysis. The evaluation of respiratory sinus arrhythmia using various methods (deep breathing test, spectral analysis of the HF-HRV) can provide important diagnostic information concerning early subclinical autonomic dysfunction in the obesity.

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THE ROLE OF SHORT CHAIN FATTY ACIDS IN THE PATHOGENESIS OF COPD

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Introduction: Significant amounts of SCFA are synthesized in the colon by microbacterial flora from non-absorbed carbohydrates and proteins. Due to colon absorption, the end products of bacterial metabolism are diffused into general circulation and get rid off with by expiration. Quantitative and qualitative changes of SCFA can impact the pathogenic mechanisms of COPD. **Results:** Total levels of SCFA in patients with COPD were decreased 1.6-fold compared with those in the normal in sputum (0.15 ± 0.01 mg/g), 4.3-fold compared with those in the normal serum (0.05 ± 0.01 mg/g), and 4.5-fold compared with those in the normal stool (2.34 ± 1.12 mg/g). **Conclusions:** A decreasing of SCFA could be linked with the quantitative and qualitative changes in the colon microbacterial flora in COPD patients due to hypoxia in the colon mucosa, a change of pH and gas metabolism. Besides, an increase in respiratory pathogens due to COPD exacerbation can additionally change a local SCFA production in the lungs, which was confirmed by changing profiles of SCFA. An increased level of C2 could be linked with the activation of aerobic microflora. Increasing butyrate concentration can stimulate differentiation of airway epithelium, hyperplasia, and hypersecretion of goblet cells and mucus production. Absorbed C3 can directly activate bronchial smooth muscle contractile activity that aggravates bronchial obstruction and induces exacerbation of COPD.

EOSINOPHILIC AIRWAY INFLAMMATION IN COPD AND ASTHMA

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Eosinophilic airway inflammation is regarded as a typical feature of asthma, while in COPD neutrophils seem to be predominant inflammatory airway cells. **Objectives:** To compare the cellular components of airway inflammation in patients with newly diagnosed COPD and asthma. **Materials:** 17 patients with mild and moderate COPD (M/F 10/7, age 57±11 yr) and 22 patients with mild to moderate asthma (M/F 12/10, age 36±14 yr). None of the patients has been treated with steroids for at least 3 months. **Methods:** All patients underwent clinical examination, lab studies, skin-prick tests, pre- and post-bronchodilator spirometry, methacholine challenge test. Sputum induction was performed according to the ERS protocol with total and differential cell count assessment. **Results:** Eleven (69%) COPD patients and all asthma patients ($p<0.05$) showed airway hyperresponsiveness. An increased number of eosinophils was found in both study groups. However, there were no significant differences in the cellular composition of induced sputum between asthma and COPD patients. The total number and percentage of eosinophils in COPD patients ($2.1\pm 3.1 \times 10^6$ cells/ml and $30\pm 17\%$, respectively) was not different from that in asthma patients ($1.0\pm 1.4 \times 10^6$ cells/ml and $22\pm 16\%$, respectively). In COPD group positive correlation between the total number and the percentage of sputum neutrophils and the number of pack-years of cigarettes smoked ($r=0.7$ and 0.6 , respectively, $p<0.05$) was noted. Surprisingly, in COPD pts the number of eosinophils in sputum corresponded with methacholine PC_{20} ($r=0.6$, $p<0.05$). **Conclusions:** Eosinophils seem to be important inflammatory cells not only in asthma but also in COPD.

A COMPARISON OF CELLULAR AND BIOCHEMICAL MARKERS OF AIRWAY INFLAMMATION IN PATIENTS WITH MILD TO MODERATE ASTHMA AND COPD: AN INDUCED SPUTUM AND BRONCHOALVEOLAR LAVAGE FLUID STUDY

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Although the clinical pictures of asthma and chronic obstructive pulmonary disease (COPD) may be similar, the pathogenesis and the course of these diseases differ in many aspects. **Aim:** The aim of the study was to compare the cellular and biochemical features of airways inflammation in patients with asthma and COPD, based on the induced sputum (IS) and bronchoalveolar lavage fluid (BALF) evaluation. **Material and methods:** The study was conducted in 22 patients with asthma (M/F 12/10, mean age 36 ± 14 yr) and 17 patients with COPD (M/F 10/7, mean age 57 ± 11 yr). The further inclusion criteria were as follow: (1) stable disease, (2) mild to moderate severity of the disease, (3) no anti-inflammatory treatment within at least 3 months prior to inclusion. Each patient underwent sputum induction followed by flexible bronchoscopy and bronchoalveolar lavage (BAL). Total and differential cell counts, as well as the concentration of interleukin-8 (IL-8) (ELISA QuantiGlo, R&D Systems) and myeloperoxidase (MPO) (MPO-EIA kit, OXISResearch, USA) were measured in IS and BALF. **Results:** No significant differences in the total and differential cell counts in IS were found in patients with asthma and COPD. However, COPD patients showed an increased total macrophage count in BALF as compared to asthma patients (14.0×10^6 cells/ml vs. 7.1×10^6 cells/ml; $p < 0.05$). The relative eosinophil count in BALF was significantly higher in patients with asthma versus patients with COPD (5% vs. 1%, $p < 0.05$). The concentration of IL-8 in IS and BALF was significantly higher in patients with COPD vs. patients with asthma (1015.6 vs. 123.6 pg/ml in IS and 15.2 vs. 3.9 pg/ml in BALF, $p < 0.05$). BALF concentration of MPO (but not IS MPO concentration) was significantly higher in patients with COPD as compared to patients with asthma (7 ± 3 pg/ml and 4 ± 3 pg/ml; $p < 0.05$, respectively). **Conclusions:** The analysis of the cellular composition of IS from patients with mild to moderate asthma and COPD does not warrant differentiation of these two conditions. BALF study reveals more differences in the cellular and biochemical features of airways inflammation in patients with asthma and COPD than IS evaluation.

AN ANALYSIS OF AIRWAY OBSTRUCTION PARAMETERS IN HEALTHY CHILDREN DEPENDING ON MOTHER-SMOKING DURING PREGNANCY

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Background: Smoking seriously damages human health. In danger is not only the active smoking person, but also people in his environment. It is well known fact that children of mothers smoking during pregnancy have predispositions to higher incidence of pulmonary diseases. Vasoactive substances as nicotine and NO in mother's body are inductive or "triggering" factors of pulmonary diseases. Challenge for pediatricians is to diagnose these disorders as soon as possible. Examination of respiratory functions in uncooperative pediatric patient could be still problematic. A relatively new method for examination of respiratory functions in those patients is a non-calibrated respiratory inductive plethysmography (RESPITRACE). **Method:** The aim of our study was to analyze the effect/impact of smoking of mother during pregnancy on changes of values of the phase angle (φ) and T_{me}/T_E index in group of healthy children (with negative personal, family and allergic histories). A group of 127 healthy children (average age 11.3 ± 0.6 month, 81 boys and 46 girls) was divided into two groups according to age: up to 6 month-old healthy children and children older than 6 months. In the first group were 7 healthy children those mothers smoked during gravidity a 34 children those mothers did not smoked. In group of children older than 6 months were 10 children which mothers smoked during pregnancy and 76 children of non-smoking mothers. Each of them was investigated by non-calibrated respiratory inductive plethysmography (RESPITRACE) in the supine position. We followed the changes of φ and T_{me}/T_E . Sedative medication was not applied to eliminate possible negative influence on results of the study. **Results:** We have found statistically significant decrease of phase angle j ($p < 0.05$) and significantly higher values of T_{me}/T_E index in healthy children of non-smoking mothers against value of phase angle j and T_{me}/T_E index of smoking mothers children in group of 0-6 months old children. These differences were not confirmed in group of children older than 6 month of age. Moreover, we found statistically significant decrease ($p < 0.05$) of phase angle φ in group of smoking mother's children up to 6 month of age in comparison with children older than 6 month without any influence of smoking. Similarly, the values of T_{me}/T_E were found significantly higher ($p < 0.05$) in older smoking as well as non-smoking mother's children in comparison with the younger, smoking mother's children (0-6 month). **Conclusion:** The results of presented study revealed negative impact of mother's smoking during pregnancy represented by changes of airway obstruction parameters. This difference appeared especially in group of youngest children declines with age.

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THERAPEUTIC POSSIBILITIES IN CHILDREN'S ARDS: A REPORT OF TWO CASES

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Background: Disorders of respiratory system are frequent in childhood, followed by progressive or acute respiratory failure in many cases. Furthermore, many severe non-respiratory diseases with acute course could seriously influence the respiratory functions of children resulting in acute lung injury (ALI) and moreover to the acute respiratory distress syndrome (ARDS). Changes in the surfactant system, e.g., inhibition of formation or structural damages, were followed and described during progression of ALI/ARDS, which requires a prompt therapy. Despite the recent knowledge about the pathophysiology and new therapeutic approaches, mortality of ARDS remains still high (30-50%). The latest studies report on positive effects of exogenous surfactant therapy, represented by a decline of mortality, improvement of oxygenation, decreased requirement of aggressive mechanical ventilation and a shortened time of artificial lung ventilation. Therefore, application of exogenous surfactant is frequently recommended for ARDS patients. **Methods and results:** The authors present their own experience with administration of exogenous porcine surfactant (Curosurf) in therapy of children with ARDS in two cases. Diluted surfactant was administered through endotracheal canula with a flexible bronchoscope directly to the both main bronchi. The first patient was 14 months old boy with retropharyngeal abscess and progression of septic shock, ARDS, and multi organs dysfunction syndrome (MODS). His clinical course did not show signs of improvement, despite a complex therapy. Therefore, we decided to apply the exogenous surfactant. We noticed a very prompt regression of ARDS symptoms and stabilization of patient's clinical course after administration of two single doses. A second patient was 17 years old boy with ARDS due to a near-drowning in a lake. Similarly, two single doses of exogenous surfactant were applied to him, which resulted in a reduction in signs of ARDS in a relatively short time. **Conclusion:** Exogenous surfactant seems to be of benefit in children with ARDS. Since the uniform pediatric guidelines regarding the application and dosing of surfactant have not yet been published, indications, dosing regimen, and route of administration remain to be explored.

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CORRELATION BETWEEN THE STAGE OF A DISEASE AND THE INTENSITY OF MORPHOLOGICAL CHANGES IN ADULT CYSTIC FIBROSIS PATIENTS

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Objectives: The aim of this study was to evaluate the correlation between the stage of a disease and the intensity of morphological changes –including transmission electron microscopy – within bronchial mucosa. **Subjects:** We studied 14 (8 female, and 6 male) adult patients with cystic fibrosis, aged 18-38 years, mean 23.6 years. **Methods:** Stages of the disease was assessed on the base of clinical status, radiological, and endoscopic examination. We focused on morphological changes within epithelial cells, the presence of metaplasia and/or dysplasia, the type of inflammatory infiltrate, the presence of epithelial ulcerations, thickening of epithelial basement membrane and collagenization of lamina propria. We used an analysis of similarities according to the selected clinical and morphological data. Ward's agglomeration method was used. As the discriminating factor we used morphological changes in the bronchial mucosa and submucosa. All statistical analyses were done using Statistica 6.0. **Results:** In present study we found two clinically different patients groups. The first one (8 patients) with poor clinical condition, advanced inflammatory bronchiofiberscopic and radiological changes and multiple exacerbations. In bronchial mucosa biopsies, evidence of chronic inflammation and only focal appearance of ciliated epithelium was found. The basement membrane was markedly thickened and the lamina propria was collagenized. In 2 patients, the lack of inner dynein arms was found. Moreover, squamous cell metaplasia and dysplasia was diagnosed and 3 and 4 cases respectively. In the other group (6 patients) with good clinical condition, normal BMI and small changes on chest x-ray, the cystic fibrosis diagnosis was made at older age. In such patients, we predominantly found neutrophils in BAL. In bronchial biopsies, ciliated epithelium covered the whole specimen. Histologically, there were features of chronic inflammation and in 2 cases with acute phase. **Conclusions:** In cystic fibrosis patients there is a correlation between the clinical course and morphological changes in bronchial mucosa. Bronchial ulcerations, squamous cell metaplasia and dysplasia were found in the group with more severe clinical course.

ELECTROCARDIOGRAPHIC CHANGES IN PATIENTS WITH SPONTANEOUS PNEUMOTHORAX

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Pneumothorax associated ECG abnormalities have been widely described. However, the mechanisms and clinical significance of ECG changes are still not fully understood. **Aim:** The aim of our study was to evaluate the prevalence and a pattern of the ECG alternations in patients with SP. **Material and methods:** Prospective study of 40 consecutive patients with SP admitted to our Department was undertaken. In all cases the diagnosis of SP was confirmed by chest X-ray. The relative volume of pneumothorax was calculated with the two different methods (Light index and Rhea nomogram). ECG tracing was recorded before and after full lung's re-expansion. Arterial blood gases were measured to assess the potential influence of pneumothorax-associated hypoxemia on ECG tracing. **Results:** The mean age of patients was 43.7 ± 19.1 lat (range 18-86). There were 22 cases (55%) of left-sided pneumothorax (LSP) and 18 cases (45%) of right-sided pneumothorax (RSP). Mean relative volume of pneumothorax was $51.4 \pm 24.7\%$ (measured according to Light index) and $53.5 \pm 22.9\%$ (according to Rhea nomogram). Heart rate (HR) was significantly higher in patients with pneumothorax as compared to that noted after lung re-expansion (91 ± 20 /min and 72 ± 16 /min; $p < 0.001$, respectively). However, there were no correlations either between HR and pneumothorax size, or HR and PaO₂. Abnormal left axis deviation was found in 3 patients with LSP and one with RSP, while abnormal right axis deviation was found only in two patients with LSP. Relevant QRS abnormalities (incomplete RBBB) or T-wave abnormalities (inversion) were found in 4 patients (10% of all). All of these abnormalities resolved following lung's re-expansion. QRS voltage in precordial leads V2-V6 was significantly decreased in patients with LSP, while RSP was associated with significant increase in the voltage of QRS in V5 and V6. **Conclusions:** The ECG tracing in patients with pneumothorax often reveals relevant changes. The most relevant abnormalities were observed in patients with large right-sided pneumothorax.

PLEURAL FLUID ADENOSINE DEAMINASE AND INTERFERON-GAMMA AS DIAGNOSTIC TOOLS IN TUBERCULOUS PLEURISY

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Several biological markers have been proposed to improve the efficacy of diagnosing tuberculous pleurisy. The study was undertaken to evaluate the accuracy of pleural fluid ADA activity and IFN- γ concentration in differentiation between tuberculous pleural effusion (TPE) and non-tuberculous pleural effusion (non-TPE). **Material and methods:** 94 patients (50 M and 44 F, mean age 59 \pm 18, range 18-95 years) with pleural effusion (PE) were studied. TPE was diagnosed in patients with: 1) positive pleural fluid or pleural biopsy culture, 2) granulomas in the pleural biopsy specimen, after exclusion of other granulomatous diseases. Pleural fluid ADA activity was measured with colorimetric method by Giusti, while IFN- γ concentration was measured with ELISA (R&D Systems, USA). **Results:** TPE was recognized in 28 pts. The non-TPE group consisted of 35 pts with malignant PE, 20 pts with parapneumonic effusion/pleural empyema, 5 with pleural transudate, and 6 with miscellaneous PE. The mean ADA activity and concentration of IFN- γ were significantly higher in TPE than in non-TPE (75.1 \pm 39.1 vs. 11.0 \pm 16.6 U/l, $p < 0.0001$ and 614.1 \pm 324.5 vs. 15.1 \pm 36.0 pg/ml, $p < 0.0001$, respectively). The diagnostic sensitivity and specificity of ADA were 100% and 92.2%, respectively (cut-off value 40 U/l) and were similar to those of IFN- γ (100% and 98.5%, respectively; cut-off value 100 pg/ml). **Conclusions:** The pleural fluid ADA activity and IFN- γ concentration are highly sensitive and specific markers of tuberculous pleurisy.

RELATIONSHIP BETWEEN THE NUTRITIONAL STATUS AND LUNG FUNCTION IN ADULT CYSTIC FIBROSIS PATIENTS

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Background: Cystic fibrosis (CF) is a world-wide disease occurring in virtually all ethnic groups. In Caucasians it is the most common lethal hereditary disorder with autosomal recessive inheritance. The steadily more effective treatment of the respiratory infection and more intensive nutritional support over the past 15-20 years have resulted in an impressive and continuing improvement in both the physical condition and survival of many individuals with cystic fibrosis. Along with lung function, nutritional status appears to be one of the most important prognostic indicators in cystic fibrosis patients. Different studies use different indices of nutrition, but, overall, a poor nutritional status appears to be independently associated with poor prognosis. **Aim:** To analyze the nutritional status and the stage of lung disease in cystic fibrosis (CF) adults. **Material and Methods:** The research was conducted in Department of Respiratory Diseases, Poznan University of Medical Sciences. A group of 39 CF patients (21 females and 18 males) was studied. The mean age was 23.9 ± 3.7 years (range 18-33 years). Patients were grouped according to the presence or absence of malnutrition. Body mass index (BMI) was used to single out the groups: normal weight ($n_1=28$) with $BMI \geq 18.5 \text{ kg/m}^2$; malnourished patients ($n_2=11$) with $BMI < 18.5 \text{ kg/m}^2$. The severity of lung disease was determined by spirometry (FEV₁% and FVC) and microbiological review. **Results:** The mean value of body mass index was $19.5 \pm 2.9 \text{ kg/m}^2$ (range 12.8 – 24.9 kg/m^2). Malnutrition was established in 11 patients (28.2%), 5 patients suffered from severe malnutrition. 28 patients (71.8%) have a normal nutritional status. In this group (according to ESPEN guidelines) 9 patients was at risk of malnutrition (18.5 - 19.9 kg/m^2). A statistical analysis revealed significant differences between malnourished and non-malnourished patients concerning the FEV₁% ($p = 0.009$) and FVC% ($p = 0.002$). Patients with malnutrition were more frequent colonized by *P. aeruginosa* and fungi ($p=0.0001$), but seldom by MSSA ($p<0.0001$). **Conclusions:** These data emphasize a close relationship between nutrition, lung function, and clinical course in CF. Normal body weight and the absence of *P. aeruginosa* infection was associated with better preservation of lung function.

RESPIRATORY SYMPTOMS AND FOOD ALLERGENS

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Food allergens can provoke many symptoms in various systems and organs of human body. Food allergy may present with a variety of respiratory tract symptoms, which can be provoked either by IgE-mediated reactions or cellular (non-IgE) mediated reactions. Among the diagnostic procedures in management of food allergy, the skin tests have very important position, especially a skin prick test for diagnosis of IgE-mediated, acute reactions) and newly introduced atopy patch test (APT) for diagnosis of cellular, delayed immune reactions. We studied the prevalence of positive skin prick and atopy patch tests with food (cow's milk, hen's egg, wheat flour, tomato) and inhalant (*Dermatophagoides pteronyssinus* and mixed grasses) allergens in an unselected children population of Italian schoolchildren (n = 532, 50.6% boys, age 10.232.27 years). On the population level, we investigated the correlation between the positivity of skin tests and questionnaire derived atopic and non-atopic respiratory symptoms and diseases (nocturnal cough, cough after physical effort, nasal obturation, bronchitis, pneumonia, otitis media, allergic rhinoconjunctivitis, laryngitis, bronchial asthma). The children with positive APT to wheat flour had more cough after physical effort in the past (p = 0.033) or in the last year (p = 0.019). Children with positivity to wheat flour more frequently suffered from allergic rhinoconjunctivitis (p = 0.031) in the last year. They also had frequently bronchitis recidivans in the past (p = 0.019). The subjects with positive APT reactions to hen's egg suffered from allergic rhinoconjunctivitis in the past (p = 0.020) or in the last year (p = 0.050) compared to those with negative results of the APT with hen's egg. This children also had bronchial asthma in the past (p = 0.028). In children with positive APT with mixed grasses we observed higher prevalence of bronchial asthma in the past (p = 0.011) in comparison with children with negative APT results. In children with a history of the other respiratory symptoms or diseases mentioned, we were unable to detect the association with positive APT results either with food allergens or aeroallergens. Food and inhalant allergens play important role in the induction and exacerbation of some respiratory allergic diseases. The positive correlation of positive results of skin tests with these allergens, also on the level of an unselected population, confirm the importance of these tests in the diagnostic algorithms of allergic diseases involving respiratory system.

DOES THE CAUSE OF CHRONIC COUGH DEPEND ON THE DIAGNOSTIC APPROACH?

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Chronic cough is a common complaint. Although the main reasons of chronic cough are well known, it remains a diagnostic challenge. Further studies are still needed to improve the efficacy of chronic cough diagnosis and treatment. **Objectives:** We aimed to: 1) assess the prevalence of the different conditions listed as the potential causes of chronic cough in patients who were unsuccessfully treated by general practitioners, and 2) to compare the efficacy of two different diagnostic approaches:

- based on clinical data, basic diagnostic tests and empiric therapy versus
- based on clinical data and results of detailed examinations of cough etiology.

Material and methods: Eighty patients with chronic cough (>8 weeks) referred to a respiratory specialist after unsuccessful ambulatory treatment. The etiology of cough was determined either on an ambulatory basis or in the hospital setting. In all patients the obtained data included cough characteristics, history of smoking and concomitant treatment (e.g. ACE-inhibitors), chest X-ray and spirometry were performed. In the ambulatory group empiric treatment (H-1 antagonists, inhaled glucocorticosteroids or proton pump inhibitors) was provided, based on the most probable cough reason. Subsequent drugs were added or additional examinations performed, if the first therapy was ineffective. In the second group (hospitalized patients) additional tests included: metacholine challenge test, skin prick tests with common aeroallergens, induced sputum for eosinophil count, ENT examination, sinus CT scans, videolaryngoscopy and 24 hour-esophageal pH monitoring. Specific treatment was initiated on a basis of the results of these studies. **Results:** Both groups consisted of 40 patients, mean age 52 years, F/M 67/13 (34 vs. 33 women in ambulatory and hospitalized group, respectively). Mean cough duration exceeded 4 years. The most common reasons of cough were gastroesophageal reflux disease (GERD) in 27 vs. 32 pts in the ambulatory and hospitalized group respectively, upper airway cough syndrome in 19 vs. 23 pts, asthma in 12 vs. 11 pts. Nonasthmatic eosinophilic bronchitis (NAEB) was markedly more frequent in the hospitalized group (18 vs. 2 pts in "out-patient"). In few cases cough of other origin was diagnosed. More than one reason of chronic cough was recognized more often in hospitalized than ambulatory group (34 v 22 pts, respectively). **Conclusions:** The main causes of chronic cough were gastroesophageal reflux disease and upper airway cough syndrome. The prevalence of cough due to GERD was higher than observed by other authors. An extensive diagnostic approach allows to recognize NEAB more frequently and reveals the complex nature of chronic cough in the individual patient.

DIVERSE EFFECTS OF MUTATED PAI-1 ON THE CANCER CELLS BIOLOGICAL ACTIVITY

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Plasminogen activator inhibitor type 1 (PAI-1) *via* complexing with urokinase and its receptor inhibits plasmin driven proteolysis and therefore regulates tumor-related angiogenesis, invasion and metastasis formation. It was however suggested that PAIs biological effect depends on its active concentration in the cellular milieu, with lower concentrations inducing cancer cells bioactivity and supraphysiological exerting opposite effect. In order to assess the impact of PAI-1 anti-proteinase activity on the process of lung cancer cells proliferation we analyzed the effect of PAI-1 cysteine mutant (VLHL PAI-1) characterized by the prolonged half-life time ($T_{1/2}= 6931.47$ h) and therefore enzymatically much more stable than wild type PAI-1 ($T_{1/2}=1.57$ h). Two functionally different lung cancer cell systems A 549 and H1299 characterized respectively by normal and high urokinase production were evaluated. In H1299 cultures significant inhibitory effect was exerted by high (100 mcg/ml) and extremely high (200.30 mcg/ml) PAI-1 concentrations ($p<0.001$). Its time-dependence was also observed as the inhibition was considerably more pronounced after 72 hr of culture than 48 or 24 hr (respectively ($p<0.001$, $p<0.05$)). In A 549 proliferation was suppressed only by the high PAI-1 concentration following 72 hr of culture ($p<0.01$). Accordingly, the dosage- and time-dependent down-regulation of VEGF production was demonstrated, though inhibition rate was significantly higher for the H1299 cells. No changes in the bFGF, MMP-9, nor TIMP-1 by cultured lung cancer cells were seen. PAI-1 is a negative regulator of lung cancer cells due to its anti-proteinase activity. Its biological effect is time and dose-dependent.

DRIVING SIMULATOR INVESTIGATIONS IN PATIENTS WITH COPD

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Introduction: Patients with chronic obstructive pulmonary disease (COPD) have cognitive deficits especially in terms of attention performance. The present study investigated whether simulated driving performance is also impaired in these patients. **Patients and methods:** Driving simulator investigations (C.A.R.[®]) were done in 17 patients with COPD (age: 55.2 ± 9.3 years, Tiffeneau-Index < 70% pred.) and 10 normal controls (age 55.1 ± 7.8 years). The simulation lasted 60 minutes under monotonous driving conditions thus pronouncing the attention aspect of vigilance which is long-term attention under monotonous conditions. **Results:** In the simulated driving situation patients with COPD caused significantly more accidents than normal controls (COPD: 3.9 ± 2.7 , controls 1.3 ± 1.5 , $p < 0.01$). The number of concentration faults was comparable in both groups (COPD: 8.5 ± 4.7 , controls: 7.1 ± 3.2 , n.s.). **Conclusions:** Compared to healthy controls patients with COPD cause significantly more accidents in the simulated driving situation. Thus the present study proves cognitive deficits in COPD patients with regard to the attention aspect of vigilance. These deficits may have negative impact on driving performance in real traffic in COPD patients.

OXIDATIVE AND NITROSATIVE STRESS AND BRONCHIAL ASTHMA IN CHILDREN

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Bronchial asthma (AB) is a common disease that affects children and adults of all ages. Although the pathogenesis of AB remains incompletely understood, it is associated with chronic airway inflammation and increased oxidant stress. Although oxygen is a prerequisite to life, at concentration beyond the physiological limits it may be hazardous to the cells. Since the lung are directly exposed to very high amounts of oxygen, it is imperative for the organ to possess defenses against possible oxidative challenge. Many studies conducted on the human asthmatic subjects or laboratory animal models of asthma revealed that numerous biologically active pro-inflammatory mediators lead to increased and uncontrolled production of reactive oxygen species (ROS) and the gaseous molecule nitric oxide (NO). Oxidant-antioxidant imbalance may play an important role in the pathogenesis of AB, especially during acute exacerbations. The ROS likely play a vital role because these have been shown to be associated with many pathophysiological changes that are relevant in asthma, such as increased lipid peroxidation, increased airway reactivity and secretion, increased production of chemoattractants and increased vascular permeability. Persistently increased ROS and NO in asthma lead to reactive nitrogen species (RNS) formation and subsequent oxidation and nitration of proteins, which may cause alterations in protein function that are biologically relevant to airway injury and inflammation. Another important source of NO-derived oxidants is eosinophil peroxidase and myeloperoxidase and leucocyte-derived enzymes. Concomitant with increased oxidative stress in asthmatics, loss of protective antioxidant defense, especially superoxide dismutase, contributes to the overall toxic environment of the asthmatic airway. Oxidative metabolites may play a direct or indirect role in the modulation of airway inflammation. It was shown also that decreased intake of antioxidant is connected with increased risk of exacerbation of wheezing symptoms. Conversely, e.g. the consumption of fruit rich in vitamin C (important dietary antioxidant), even at a low level of intake may reduce wheezing symptoms already susceptible individuals. There are alterations in a wide array of oxidants and antioxidants, with imbalance shifting toward increased oxidative stress in AB. Increased oxidative stress is likely to contribute to perpetuation and amplification of the inflammatory response in asthmatics. Therapeutic augmentation of the antioxidant defenses might be beneficial. Further studies are necessary to clarify the exact position of oxidative stress in the pathogenesis of asthma: it plays important role in the initial phase of clinical expression of asthma, co-works in asthma exacerbation and contributes to the maintenance of chronic inflammation in asthmatics.

HOSPITAL STAFF AND SMOKING HABITS: DO WE NEED A MODIFICATION OF SMOKING BEHAVIOR IN POLISH HOSPITALS?

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The purpose of the study was to analyze smoking behavior among medical staff in hospitals. The typical group of employees in one of the biggest hospitals in Poland was examined thoroughly. The medical examination was carried out by standardized investigative instrument, a questionnaires processed by European Network for Smoke-free Hospital. In researched group, 26% admitted to smoke, while addiction confirmed near 12%. Statistically it is confirmed, that addiction to smoking is conditioned by practice but the percentage of smoking people is lowest in the professional group of physicians. However, the physicians admitted to smoke in hospitals most often. It is crucial factor of psychological preventive treatment as well as changes of health behavior among patients. Also, there were identified some factors which determined smoking as, e.g., a large number of duty hours. It was proposed the estimation of medical employees maintenance, whose knowledge and medical case study should have impacted on avoiding smoking. On the basis of health behavior analysis, the authors evaluate the possible solutions which can lead to a reduction of the percentage of smoking individuals in public health institutions.

RECURRENCE OF ARTERIO-VEINUS MALFORMATIONS WITH LIFE-THREATENING COMPLICATIONS IN A PREGNANT WOMAN WITH HEREDITARY HEMORRHAGIC TELEANGIECTASIA

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Hereditary hemorrhagic teleangiectasia is an autosomal dominant vascular disorder with incidence of 1 to 2,300 and high penetration, characterized by teleangiectasia, arterio-venous malformations and aneurysms. Arterio-venous malformations may occur in any organ but with the lungs, the brain and the liver as the most common ones. It has been noted that there is a significant morbidity and mortality risk associated with pregnancy in women in this otherwise relatively benign condition. To support this suggestion we present the case of 43-year-old woman, diagnosed with hereditary hemorrhagic teleangiectasia and treated with lobectomy at the age of 5 and with transcatheter coil closure of pulmonary feeding artery at the age of 30, who developed recurrence of arterio-venous fistulas during pregnancy. The woman was admitted to hospital with fatigue, dyspnea and severe right-sided headache in 15 HBD. 3-dimensional chest computed tomography revealed multiple arterio-venous fistulas. A head computed tomography scan revealed enhancing right parietal mass with surrounding edema and she underwent craniotomy. The diagnosis was brain abscess, suggesting an odontogenic etiology after dental treatment procedure. On the second day after craniotomy she developed right-sided ischemic stroke with left-sided hemiparesis and ischemia induced epilepsy. She went into premature labor at 35 weeks and the child was delivered by elective cesarean section. Seven days after delivery she developed severe pulmonary insufficiency, was diagnosed with hemothorax and treated with transcatheter embolotherapy. During the last 7 years of surveillance she developed progressing intrapulmonary shunt deterioration and hypertrophic pulmonary osteoarthropathy. The presented patient developed the whole variety of pregnancy induced complications. The possible reasons for deterioration during pregnancy are: increased plasma volume, progesterone induced venous distensibility and a decrease in smooth muscle contractility. It is a matter of future research to establish genotype-phenotype correlations and assess disease severity using genetic tests. This would influence early diagnosis, prevention and treatment. For the time being regardless of the treatment modality, long term follow up with spiral CT should be mandatory to monitor the development of new lesions and recurrence of those previously treated.

SUCCESSIVE TREATMENT OF CHURG-STRAUSS SYNDROME WITH CYCLESONIDE AFTER ORAL STEROIDS DISCONTINUATION DUE TO SIDE EFFECTS

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Churg-Strauss syndrome, ANCA-associated is characterized by history of asthma, peripheral blood and tissue eosinophilia with particular predilection to lungs in context of multisystem disorder manifested by skin involvement, mesenteric ischemia, peripheral neuropathy, and myocarditis. We would like to present a case of 35 year-old patient with severe asthma, referred to our hospital with symptoms of the status asthmaticus. On admission, the patient was in severe state, physical examination suggested bilateral pneumonia, confirmed by chest X-ray, blood gas analyze showed features of a respiratory failure. Patient was successfully treated with epidephrine, predonisone, three antibiotics, and oxygen. On subsequent hospitalization one month later, also due to asthma exacerbation he presented with fever, cough, dyspnea, mialgia, and symptoms of sinusitis. Chest X-ray revealed profuse pulmonary infiltrates, peripheral blood smear showed eosinophilia 50%. The history of severe asthma and peripheral blood eosinophilia made us suspect Churg-Strauss syndrome. Further investigations revealed negative test for both antineutrophil cytoplasmatic anybodies in a perinuclear distribution and antibodies against myeoperoxidase, granulomas in the bronchi, which could correspond to Wegener's granulomatosis. The therapy with oral steroids was started with resolution of symptoms. After 10 months of oral steroids use, the patient again developed dyspnea, fever, marked obturation, hypoxemia, disseminated patchy infiltrates seen on chest X-ray and sinusitis with nasal polyps. Ultimately the diagnosis of Churg-Strauss syndrome was established on clinical basis and cyclophosphamide was added to the corticosteroids. Clinical improvement in the patient's general health and normalization of laboratory tests were achieved again. The patient was under surveillance for 2 years and over this time symptoms and lesions to the lungs did not recur. Cyclophosphamide was discontinued after 12 months of use. The patients decided to withdraw oral steroids because of whole range of adverse symptoms: significant weight gain, striae of the skin on abdomen, and aseptic necrosis of both femoral heads and diabetes mellitus. To prevent disease recurrence the new regimen with ciclesonide 2 x 320 µg was introduced. Today the patient is in good health with no symptoms, the laboratory findings show eosinophilia of 6%, which is the lowest in his disease course. The usage of cyclesonide is not recommended for Churg-Strauss syndrome, but in the case of the patient with mainly pulmonary manifestations who discontinued oral steroids due to side effects proved successful. Further investigations are needed to find out whether cyclesonide my replace oral steroids in long term control of Churg- Strauss syndrome with mainly pulmonary manifestations.

SPIROMETRY, SYMPTOMS, AND TREATMENT OF 3305 PATIENTS WITH ASTHMA DIAGNOSED BY FAMILY PHYSICIANS

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Objectives: The study aimed at evaluating of symptoms, spirometry, and treatment of 3305 patients with diagnosed asthma. **Patients and methods:** 3305 patients were examined. All patients answered a questionnaire. Physicians provided information about the diagnosis and treatment. Spirometric tests were performed in all examined patients. **Results:** Everyday, 18.4% of patients complained of daily cough, 11.0% night cough; 12.0% of the patients complained of dyspnea during the day, and 8% had dyspnea at night; 11% percent awoke at night, 11.0% did not experience cough during the day, and 23.0% at night; 22.5% did not experience dyspnea at all during the day, and 34.0% at night. 31.5 % of the patients did not awake in the night. Conversation with a doctor (79.0%) remains a main source of information about the disease. 818 patients (24.7%) actually smoke cigarettes, and 941 (28.5%) are passive smokers. Long-acting beta₂-mimetics regularly use 67% of the patients, inhaled glucocorticosteroids 83.0%, short-acting beta₂mimetics 74.0% , antileucotriens 25.0%, methylxanthines 21.0%, and oral corticosteroids uses regularly - 13.0%. Diagnosis according to the attending physicians: intermittent asthma 11.6%, mild persistent 39.0%, moderate persistent 38.0%, severe persistent 11.0% of the examined patients. The mean spirometric values were in the normal limits. **Conclusions:** About 20.0% of patients complain of everyday symptoms. Over 50% of patients are exposed to tobacco smoke. The main source of information about asthma is the doctor. 83% of patients take inhaled glucocorticosteroids regularly.

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MASK LEAKAGE IN CPAP AND C-FLEX

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Introduction: Many patients with obstructive sleep apnea syndrome (OSAS) receiving continuous positive airway pressure (CPAP) complain of leaky masks or too high pressure during expiration. C-Flex is a breathing mode with a constant CPAP pressure during inspiration and a reduced pressure during expiration. We compared the leakage data between CPAP and C-Flex and their influence on patients' compliance. **Methods:** 30 patients (22 men, 8 women, age 55.4 ± 11.7 yrs, BMI 32.0 ± 7.4 kg/m²) with polysomnographically diagnosed OSAS got a CPAP or C-Flex therapy in a randomized double-blind trial. After 6 weeks an adjustment to the other mode followed. Leakage data were sampled during all polysomnographic examinations. **Results:** 12 patients stopped the study (7 after C-Flex, 5 after CPAP), 4 of them gave up CPAP therapy completely (2 after CPAP, 2 after C-Flex). The leakage in CPAP mode was 27.5 ± 11.5 l/min and in C-Flex mode 28.0 ± 10 l/min (n.s.). The average nightly use in CPAP mode was 350.0 ± 70.2 min and in C-Flex mode 347.0 ± 70.8 min (n.s.). In the final decision of therapy 9 patients chose C-Flex and 4 patients CPAP ($p=0.001$). 5 patients had no preference regarding the therapy mode. **Conclusion:** There is no difference in leakage and compliance between CPAP and C-Flex. But significantly more patients decided for a therapy with the C-Flex mode. There must be other actually unknown factors that influence the decision for the mode of therapy.

MODULATORY EFFECTS OF SERA FROM PATIENTS WITH VARIOUS TYPES OF PULMONARY FIBROSIS ON MONONUCLEAR CELL-INDUCED ANGIOGENESIS IN RELATION TO RADIOLOGICAL AND FUNCTIONAL PULMONARY CHANGES

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Pulmonary fibrosis occurs in many lung diseases. Angiogenesis plays an important role in the pathogenesis of idiopathic pulmonary fibrosis, but whether that is the case in other fibrotic pulmonary disorders is unclear. The aim of the study was to examine the effect of sera from patients with different type of pulmonary fibrosis on angiogenesis induced by human mononuclear cells (MNC) in relation to radiological, clinical and functional status. The study population consisted of 32 patients with idiopathic pulmonary fibrosis (IPF), 11 patients with drug-induced pulmonary fibrosis (DIPF), 6 with cryptogenic organizing pneumonia (COP), 12 patients with silicosis, 13 with systemic sclerosis (SSc) and 20 healthy volunteers. As an angiogenic test we used animal model of leukocyte induced angiogenesis assay. Spirometry, whole-body plethysmography, static lung compliance (Cst), and diffusing capacity of the lungs for CO (DLco) were performed in all patients. Sera from IPF, SIL, and COP patients significantly stimulated angiogenic activity of MNC compared to sera from healthy donors and from DIPF patients ($p < 0.001$). However, sera from healthy donors and DIPF significantly stimulated angiogenic activity of MNC compared with the control with PBS and SSc ($p < 0.001$). Angiogenic activity of sera did not correlate with pulmonary function of patients with pulmonary fibrosis. However, proangiogenic effect of sera from systemic diseases patients depended on the radiological changes. Sera obtained from patients with pulmonary fibrosis constitute the source of mediators modulating angiogenesis, but the pattern of reaction is different in various diseases. Sera from IPF, SIL, COP patients stimulate neovascularisation, but sera from SCL patients exert an inhibitory effect on angiogenesis.

INHALED INSULIN – DOES IT BECOME REALITY?

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After more than 80 years of history the American and European Drug Agencies (FDA and the EMEA) approved the first inhalable version of insulin (Exubera[®]) from Pfizer/Nektar early 2006. Marketing activities started in September 2006. In October 2007, Pfizer announced it would be dropping Exubera[®], citing that the drug had failed to gain market acceptance. Since 1924 various attempts have been made to get away from injected insulin. Three alternative delivery methods were always discussed: Delivery to the upper nasal airways or the deep lungs and through the stomach. The delivery of an insulin pill through the stomach has many hurdles to overcome and will not be discussed here. Delivery of insulin to the small area of the upper nasal airways suffers from poor transport across the nasal membranes and dosing issues. And even a mild cold could easily change the intended insulin dose. Furthermore, over 100 I.U. of insulin must be deposited into the nose to deliver 10 I.U. into the blood. The delivery of insulin through the deep lungs has access to a large surface area and the absorption into the blood happens through the extremely thin alveolar membrane. This approach seems to be the most promising. However, there is concern about the long-term effects of inhaling a growth protein into the lungs. It was assumed that the large surface area over which the insulin is spread out would minimize negative effects. But recent news indicates that, at least in smokers, the bronchial tumor rate under inhaled insulin is increased.

Several companies worked on providing inhalable insulin. The most advanced technology was Exubera[®] consisting of an insulin powder aerosol and a special inhalation device. Treatment has only been approved for adults aged over 18 years. It was a short-acting powder form of insulin that was inhaled before each meal. A long-acting insulin was still needed to be given each day by injection for type 1 and some type 2 diabetics. The Exubera[®] inhaler was about the size of a 200 ml water glass when closed. It opened to about twice the size for delivery. The lack of discreet delivery was another issue repeatedly brought up by detractors of the device.

Pharmacokinetics and pharmacodynamics of Exubera[®] are similar to those found with short-acting subcutaneous human insulin or insulin analogues. The duration of action is about the same as that of short-acting human insulin. The time to onset of action is about the same as with short-acting insulin analogues. It is thus possible to use Exubera[®] as a substitute for short-acting human insulin or insulin analogues.

Similar to other inhaled insulins, a number of side effects (coughing, shortness of breath, sore throat and dry mouth) were reported. Exubera[®] was not approved for smokers or anyone who has smoked in the last six months because almost twice as much of the inhaled insulin can enter the bloodstream and increase the possibility of an overdose. It was also not approved for anyone with a lung disorder (e. g. asthma, emphysema, COPD). Exercise also increases transport and likelihood of hypoglykemia.

Another major problem with Exubera[®] was the inability to deliver precise insulin doses. The smallest blister pack available contains the equivalent of 3 I.U. of regular insulin. This dose would make it difficult for many people using insulin to achieve accurate control which is the real goal of any insulin therapy. Someone on 60 I.U. of insulin per day would lower the blood glucose about 90 mg/dl (5 mmol) per 3 I.U. pack, while someone on 30 I.U. a day would drop 180 mg/dl (10 mmol) per pack. Precise control was not possible, especially compared to an insulin pump that can deliver one twentieth of a unit with precision.

Because of the low acceptance Exubera[®] was dropped from the market. To our information also the other companies (Eli Lilly in cooperation with ALKERMES, Mannkind (Technosphere, Powder), Novo Nordisk (AERx, Liquid), Andaris (Powder)) stopped further development and it is unclear whether an inhaled form of insulin will ever be marketed.

SYSTEMIC TREATMENT BY INHALATION OF MACROMOLECULES – PRINCIPLES, PROBLEMS, AND EXAMPLES

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Aerosol inhalation is an established tool for the treatment of pulmonary diseases since many years. In contrast, aerosol inhalation for treatment of systemic diseases is a novel therapeutic approach. Clinical use of the latter therapy for many years has been limited by the lack of accuracy and reproducibility of the administered doses and a small proportion of inhaled drug related to the dose remaining in the aerosol delivery system. Further problems were the risk of potential allergic reactions in the respiratory tract and the strong variability of drug absorption from the alveoli into the circulation. However, these problems have been solved in the last years by modern aerosol delivery systems allowing the production of an aerosol with a defined and optimized aerosol particle size combined with an optimized breathing manoeuvre and optimization of the efficacy of the nebulizers revealing a high proportion of aerosolized drug near the total amount.

Beside physical and physicochemical factors (e.g., solubility, stability within the nebulization process and electrical charge) a number of physiological parameters affect the uptake of inhaled biomolecules (e.g., peptides and proteins) after pulmonary deposition. In detail, these are inactivation (e.g., by proteolysis, oxidative inactivation by reactive oxygen species (ROS), and phagocytosis) and absorption inhibition by different absorption barriers (e.g., bronchial epithelium and alveolocapillary membrane). Pulmonary absorption can be increased by very different approaches like addition of protease inhibitors (e.g., aprotinin) or absorption enhancers (e.g., bile acids, cyclodextrin and detergents), packing of the biomolecules into particles (e.g., liquid or solid liposomes and microspheres) or synthesis of Fc-fusion proteins. These approaches enhance the bioavailability of the inhaled substances by inhibition of proteolytic degradation and oxidative inactivation or phagocytosis (in case of antiproteases, liposomes and microspheres), solubilization of absorption inhibiting membranes (in case of detergents, bile acids, and liposomes) or increased uptake by specific cellular receptors (in case of Fc-fusion proteins). A number of these approaches are experimental and used only in animal experiments whereas others are investigated in clinical studies. However, it should be considered that absorption enhancers like the administered pharmaceuticals themselves can cause biological reactions (e.g., membrane damage by cyclodextrins and inflammatory response by hydroxymethylaminopropionic acid (HMAP)) which depend on the administered doses and the duration of the treatment and are reversible after the end of therapy.

An increasing number of studies investigated the systemic effect of inhaled high molecular weight substances (e.g., insulin, heparin, interleukin-2) and demonstrated that controlled aerosol therapy may serve as a non-invasive alternative for drug application by means of a syringe. Results of clinical studies demonstrated that the inhalation of a number of systemically active drugs is well tolerated. There are only few factors (e.g., exogen allergic alveolitis, active sarcoidosis, and active smoking) influencing alveolar drug deposition and bioavailability of the inhaled substances. In consequence, the inhalant administration of drugs for systemic treatment will be an interesting field in future research.

Our review summarizes the principles and underlying mechanisms for pulmonary absorption of macromolecules including the effects of absorption enhancers and gives an overview on prior research in the large field of inhalant treatment of systemic diseases.

SAFETY OF REAGENTS FOR INFECTION TESTING: RESULTS OF THE MARKET SURVEILLANCE BY THE BFARM UNTIL THE END 2006

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The European Directive 98/79/EC on in-vitro diagnostic medical devices (IVD) stipulates the marketing and post market surveillance of IVD in the European Economic Area. In cases of issues and field corrective actions the manufacturers have to inform the responsible Competent Authorities (CA). In Germany, the Federal Institute for Drugs and Medical Devices (BfArM) is the responsible CA for most IVD. Only a small subset of IVD for immune hematological and infectiological testing as well as tissue typing specified in Annex II (Parts A and B) of the Directive is in the responsibility of the Paul-Ehrlich-Institute (PEI).

In this study all notifications regarding reagents for infection testing (e.g., culture media, susceptibility testing, serological testing, and molecular analysis), but not analyzers, received by the BfArM between the beginning of 1999 and the end of 2006 were analyzed with respect to the sources of notification, the underlying product defects, and the corrective actions performed by the manufacturers.

In the observation, a total number of 888 notifications were received from the BfArM. These regarded to professional use products (n=642) and lay use products for self-testing (n=246; e.g., blood glucose and pregnancy). From the professional use products (n=90) reports related to the included IVD for infection testing. Reports were predominantly received from manufacturers (n=55) and CAs (n=29), whereas only few reports came from other sources (e.g., users). The affected products were most frequently those for serological analysis (n=42) and culturing techniques (n=36, including culture media) whereas tests based on molecular means played only a minor role (n=12). The products most frequently served for detection and susceptibility testing of bacteria (n=54) and diagnostics of viruses (n=18). Products for diagnostics of fungi and parasites as well as common culture media were less often affected (n=3, n=2 and n=13, respectively).

Manufacturers were able to identify the underlying root causes of product failures in 68 cases (75.6%). In 16 cases (13 of those were immunological tests) the root cause remained unclear or was not reported to the BfArM (especially at the beginning of the observation period because of other reporting criteria). In the remaining cases, a product failure was excluded by the investigations of the manufacturers (n=4) or a user error was the underlying cause of product failure (n=2). The proven product failures were most frequently caused by material defects (n=25), production errors (n=11) and microbiological contaminations (n=6). Other causes were e.g., labelling error (n=5), miss of specification (n=4), software error (n=4), and incorrect instructions for use (n=3). Based on the underlying causes manufacturers settled corrective actions in 73 cases (81.1%). Corrective actions were also performed for prevention in some cases without product failure. Most frequent corrective measures were (multiple entries) customer information (n=69), recall (n=56; in case of a recall a customer information is mandatory), modifications in production or quality management (n=39), change of the used raw materials (n=17), and modification of the instructions for use (n=11).

The obtained results and experience since 1999 suggest that the regulatory system for post marketing surveillance of IVD is an established tool to ensure product safety. However, the experience shows that the current system should be further optimized e. g. by improving the number of user reports and the availability of the information regarding field corrective actions performed by the manufacturers (e.g., *via* publication on the homepage of the CA as it is performed by the BfArM).

EXHALED CARBON MONOXIDE AS A NEW POTENTIAL MARKER OF RESPIRATORY DISEASES IN CHILDREN

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Among modern methods included in the diagnostic algorithms for various diseases, analyses of expired breath and its condensate acquire increasing importance. Various markers can be determined in the exhaled air, especially volatile gaseous compounds: nitrogen oxide (NO), carbon monoxide (CO), hydrocarbons, and 8-isoprostanes. Most of CO in the body originates from the enzyme degradation of heme. The level of exhaled CO reflects the extent of activation of heme oxygenase, induced by many factors (e.g., cytokines, infections, reactive oxygen and nitrogen species, bacterial toxins). In contrast to NO, CO can serve as a marker of inflammation and oxidative stress. The representation of CO in the exhaled breath (eCO) changes in various diseases of the respiratory and other systems. Among the respiratory diseases in which the use of the eCO measurement seems to be of benefit are bronchial asthma, airways infections, cystic fibrosis, primary ciliary dyskinesia, etc. The observation of eCO concentrations represents a modern, simple, available and a well reproducible method for the diagnosis of respiratory system disorders and for the following their progression and response to therapy.

RIGHT VENTRICULAR FUNCTION IN PATIENTS WITH SEVERE INTERSTITIAL LUNG DISEASES: A TISSUE DOPPLER IMAGING STUDY

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Introduction: Pulmonary diseases cause right ventricular (RV) dysfunction. Tissue Doppler Imaging (TDI) can be useful in the evaluation of RV deformation. The aim of the study was to assess RV dysfunction in patients with interstitial severe lung diseases (ILD) using TDI echocardiography. **Methods:** The study group consisted of 40 patients with end-stage ILD referred for lung transplantation (LT). Eighteen of them fulfilled the ATS/ERS criteria for LT (Group I: 11 M and 7 F, mean age 46.6±12 years, mean forced vital capacity (FVC) 1.37±0.76 l, mean diffusing capacity for carbon dioxide (DLCO) <50% of predicted) and 22 patients who did not fulfill the criteria for LT and were placed on a waiting list (Group II: 14 M and 8 F, mean age 48.5±12 years, mean FVC 2.76±0.96 l, mean DLCO >50% predicted). The transthoracic echocardiography examination (TTE) was performed in all patients and it included RV dimension in the long parasternal axis (RVD1) and in the apical axis (RVD2), RV diastolic area (RVAd) and systolic area (RVAs), fraction area change of RV (FAC), tricuspid annulus plane systolic excursion (TAPSE), pulmonary artery acceleration time (AT), RV systolic pressure (RVSP). Longitudinal TDI parameters included maximal velocity of myocardium (VEL), time to maximal velocity (TV), maximal strain (S) and strain rate (SR) and they were evaluated for the inflow and outflow RV tract and for the medial and apical segments of interventricular septum (IVS) during the ejection period. **Results:** Among the TDI parameters, SR of the RV outflow tract and TV in the medial segment of IVS were significantly different between both groups. The maximum SR in Group I was significantly lower compared with Group II (-1.1±0.3 vs. -3.2±1.2 s⁻¹, p=0.03) and TV in the medial segment of IVS was significantly longer in Group I in comparison with Group II (159.2±38.1 vs. 129.9±47.9 ms, p=0.01). The patients in Group I had significantly lower values of TAPSE (14.3±3.3 vs. 20.6±6.2 mm, p=0.01) and AT (70.3±23.3 vs. 96.9±12.4 ms, p=0.01). No differences were found for other parameters of RV function such as RV area, FAC or RVSP between the examined groups. **Conclusions:** The severity of the disease in patients with ILD correlates with the RV systolic dysfunction, which was reflected by the lower values of SR in RV outflow tract and by a delay of IVS motion in the medial segment. Among conventional echocardiographic parameters only the TAPSE and AT related with the severity of the lung disease.

OBSTRUCTIVE SLEEP APNEA IN HEART FAILURE PATIENTS: EVIDENCE FOR PERSISTENT CONDUCTION DISTURBANCES OR SINUS NODE DYSFUNCTION?

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Introduction: Bradycardia is a common finding in patients with obstructive sleep apnea and might be pronounced in heart failure patients. The aim of this study was to determine the relationship between nocturnal hypoxemia, apnea-hypopnea index and electrophysiological parameters of sinus node and atrioventricular conduction properties. **Methods:** Electrophysiological studies were performed in 12 patients with heart failure. Polygraphic studies were done in all of the patients irrespective of sleep apnea symptoms. Patients with an AHI >10/h were classified as sleep apnea patients. Exclusion criteria were existing CPAP therapy or atrial fibrillation. **Results:** Mild Sleep apnea was diagnosed in 50% of the patients (AHI $17.8 \pm 4.4/h$ vs. $5.1 \pm 3.6/h$). There was no difference with respect to resting heart rate, PQ-interval, or QRS-duration between the two groups. Sinus node recovery time was normal in all of the patients (993 ± 291 vs. 1099 ± 62 ms, $p = 0.45$). There was no abnormal atrioventricular conduction. Nevertheless, sleep apnea patients showed decreased AH-intervals ($134 \pm 42ms$ vs. $102 \pm 25ms$, $p = 0.1$) and HV- intervals ($59 \pm 9ms$ vs. $43 \pm 7ms$, $p = 0.01$).

EFFECTS OF CIGARETTE SMOKE ON LUNG AND SYSTEMIC IMMUNITY

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The influence of cigarette smoke (CS) on human health is still an important problem worldwide. Complex inflammatory processes and changes in the immune system are crucial in the pathogenesis of smoking related disorders like chronic obstructive lung disease (COPD), lung cancer, asthma, interstitial lung diseases (ILD), atherosclerosis as well as infectious lung diseases. The objective of this review is to present the alterations in the immune system of smokers. CS consists of about 4000 substances known to be: antigenic, cytotoxic, mutagenic, carcinogenic which are mainly dispersed in the gas phase. The particle phase contains important constituents of CS, such as: tar, nicotine, aromatic hydrocarbons, phenol. CS contains a high concentration of reactive organic radicals (ROR) and substances producing ROR. Discrete changes in peripheral blood of smokers may be found: leukocytosis, elevated proportion of T cells with slightly elevated proportion of cytotoxic/suppressor cells (CD8+). The respiratory tract is the main affected system in tobacco smokers. In the bronchial epithelium, metaplastic and dysplastic changes are accompanied by an elevated expression of adhesion molecules and secretion of many proinflammatory cytokines. CS causes activation of signal transcription pathways in bronchial epithelial cells. In the population of pulmonary macrophages an elevated proportion of cells, changes in the morphology of cells, elevated expression of surface markers of cell activation with impaired phagocytotic and antigen presenting functions have been observed. Chronic exposure to cigarette smoke causes increased production of metalloproteinases (MMP) by macrophages. The influence of tobacco smoke on the population of granulocytes results in an elevated proportion of neutrophils and the concentration of the products of neutrophils: NE, IL-8, TNF α in the airways and lung parenchyma. CS seems to alter the way of death of neutrophils from apoptosis to necrosis. On the other hand, an elevated expression of the receptors of apoptosis on lymphocytes is found. CS enhances the recirculation of lymphocytes, which results in an augmentation in activated and cytotoxic/suppressor cells in the bronchial lumen. In the recruitment of cytotoxic cells, the following cytokines are involved: IL-1b, IL-6, IL-8, TNF α . Bronchoalveolar lavage fluid and bronchial biopsies taken from smokers are characterized by an increased proportion of CD8+ suppressor/cytotoxic T cells and a significant decrease of CD4/CD8 ratio. Nonspecific feature is influx of eosinophils into the airways of smokers. The most important way in prophylaxis and treatment of smoking related diseases is smoking cessation. Changes in the immune system, however, are not entirely reversible.

TRACHEOBRONCHIAL EXPIRATION REFLEX: A NEW INSIGHT INTO AIRWAY DEFENSIVE REFLEXES

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The expiration reflex (ER) was defined as the strong expiratory effort not preceded by an inspiration, which can be only elicited by mechanical stimulation of the larynx or vocal folds (1). Cough reflex (CR) starts with an inspiration which is followed by strong expiration. It is clear that the ER differ from CR in many physiologic and pharmacologic ways. However, in some experimental and clinical studies was questioned the exclusive place for ER induction in the larynx. The aim of our study was to find out whether laryngeal mucosa is really exclusive place for induction of ER, and whether stimulation of tracheobronchial (TB) mucosa does not lead to ER, too. To find the answers to the question mentioned we reanalyzed our previous records with mechanical and chemical stimulation of mucosa in the tracheobronchial tree (TB) and in the larynx (LX) in cats and rabbits to see if an ER can consistently be elicited from both these places or from the larynx alone. The reanalyzed records came from experiments done on anesthetized and non-anesthetized cats, and on anesthetized rabbits of either sex. Respiratory reflexes were provoked by mechanical and chemical stimulation both laryngeal and tracheobronchial mucosa. In one group of anesthetized animals we inflated lungs by pressure 1.5 kPa to find out the effect of slowly adapting stretch receptor stimulation on the intensity of CR and ER. Details on the experimental methods used have been fully described elsewhere (2, 3). Our results showed that mechanical stimulation of TB mucosa frequently leads to ER or ER plus CR (in 11 to 39% stimulations), but ER was more frequent from LX than from TB. The occurrence of ER or ER plus CR was higher in the anesthetized than in non-anesthetized cats. Lung inflation increases the occurrence and strength of ER or ER plus CR in cats and rabbits compared with animals without lung inflation. The results obtained in our study support the view that ER can be elicited from TB tree, but not so frequently as it is with the stimulation of LX mucosa. This conclusion, if accepted, is important, because TB expiration reflex must be taken into account during laboratory and clinical research on defensive reflexes from the lower airways.

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