

**Abstracts**  
**of the**  
**Annual Meeting**  
**of the**  
**Iuliu Hațieganu University of Medicine and Pharmacy**  
**Cluj-Napoca**  
**23-27 November 2015**

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## Medical - Journal of Medicine and Pharmacy

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## OXYGEN THERAPY ADMINISTERED BY HOOD TO INFANTS WITH ACUTE RESPIRATORY FAILURE OF PNEUMOGENIC CAUSE

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**Introduction.** Acute respiratory failure of pneumogenic cause in infants raises sometimes therapeutic attitude problems. The objective was to evaluate the effectiveness of oxygen administration by hood on clinical and the value of laboratory parameters to assess blood oxygenation in acute respiratory failure in infants.

**Materials and methods.** The study included 30 infants with acute respiratory failure of pneumogenic cause, receiving oxygen through hood. The study was prospective cohort and was conducted during 2007 - 2012, the Pediatric Clinic III of Cluj-Napoca. The assessment of each case was done by determining a clinical score who appreciate score five parameters and hemoglobin oxygen saturation was monitored by pulse oximetry (SpO<sub>2</sub>), the same parameter was measured with the gas analyzer from arterialized capillary blood (SaO<sub>2</sub>) and the partial pressure of oxygen (PaO<sub>2</sub>). Measurements were performed before initiating oxygen therapy for establishing baseline and 30 minutes respectively and 60 minutes from the administration of oxygen by hood.

**Results.** Given oxygen therapy by hood statistical processing of clinical score showed significant differences after 30 minutes of initiating oxygen therapy compared to baseline, instead we recorded significant improvement after 60 minutes as compared to baseline and compared those recorded at 30 min. Statistical analysis revealed significant increases for the three parameters (SaO<sub>2</sub>, SpO<sub>2</sub> and PaO<sub>2</sub>) after 30 min and 60 min of initiating oxygen therapy. Also, significant increases were recorded for the three parameters compared to the values from 60 min to 30 min.

**Conclusion.** Oxygen therapy administered by hood to infants with acute respiratory failure of pneumogenic cause improves/corrects acute respiratory failure, assessed clinically and by blood gas measurement, over the course of 60 min and by elevated SaO<sub>2</sub>, SpO<sub>2</sub> and PaO<sub>2</sub> after 30 min and 60 min following the start of oxygen therapy.

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## ACID-BASE IMBALANCE IN CHILDREN – UNEXPECTED DIAGNOSIS

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**Introduction.** The most common pediatric acid-base imbalance is metabolic acidosis, which correlates with severe states of dehydration, shock, collapse and respiratory failure in advanced stages. Metabolic alkalosis is less common and most often correlates with an inadequate intake of bicarbonate ions and in respiratory alkalosis-hyperventilation. The objectives are to identify pathology revealed by acid-base disturbance in children.

**Material and methods.** The authors present two pathological situations in which diagnosis of severe condition was established starting from a disorder of homeostasis acid-base balance.

**Results.** D. A. 18 age old, with nonobstructive hypertrophic cardiomyopathy, severe pulmonary arterial hypertension, presented from the age of 4 years a accentuated progressive respiratory acidosis. After excluding common causes of respiratory acidosis, the authors found that this acid-base disturbance is secondary to brain tumor pathologies, namely bulbpontine glioma. T. A. 4 months old, shows at age of 2 month a decompensated metabolic alkalosis and hyponatremia, the latter resistance to electrolytic treatment. Clinical investigations and laboratory diagnosis allowed the diagnosis of cystic fibrosis.

**Conclusion.** In some cases acid-base homeostasis disorders of the body may reveal unexpected diagnoses.

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## PARTICULAR ASPECTS OF COMMUNICATION BETWEEN DOCTORS, PATIENTS AND FAMILY MEMBERS IN HEART FAILURE IN AN EASTERN EUROPEAN COUNTRY

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**Purpose.** Heart failure (HF) has multiple complications and high mortality. Patient-centered communication (PCC) and shared decision-making (SDM) provide a better control of HF and higher level of patient satisfaction about healthcare. Doctors often use a biomedical communication. Our study analyzes the doctor and patient ways with HF communicate in Romania.

**Methods.** 46 patients with HF aged over 18 were included in our study after signing an informed consent form. Patients and their families were asked to complete a semistructured questionnaire on doctor-patient communication and SDM.

**Results.** All patients wanted to know their diagnosis, regardless of gender, age, background, marital status and education ( $p > 0.05$ ) or age ( $p > 0.05$ ). They wanted to know all information on their HF, treatment and prognosis, which were provided by the doctors „with patience and empathy” for 91.3% of them. However, 44.4% of p. considered the information provided by the doctors allowed them to have a complete understanding of their condition. When asked to elaborate on their condition and severity, 44% failed to do so (mostly seniors, rural areas, less educated). Most patients believe family should be allowed to know their diagnosis only with their approval, unlike 66.7% of families. 80% of patients family was the most important provider of emotional and instrumental support. Families often ask doctors to “filter” information reaching the patient (because “patients becoming scared and discouraged”), which would break the law regarding patients rights in Romania. 30% of patients would want to have access to their medical records because they consider information is being hidden or withheld from them – this feature could be considered something specific to patients in our cultural area.

**Conclusions.** PCC and SDM needs to be adapted to patient’s specific features and preferences. Families can be a significant resource in SDM provided there is no breaking the law regarding patients rights.

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## THE FREQUENCY OF NEOPLASIA IN PATIENTS DIAGNOSED WITH MYOSITIS

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**Introduction.** There is a recognized link between dermatomyositis/polymyositis and various malignancies. In our country there are few data on this subject. The aim of this study was to identify the frequency and the types of cancers in patients with polymyositis (PM)/dermatomyositis (DM).

**Material and methods.** 134 observation sheets of patients with PM and DM were assessed. The patients were monitored in the Rheumatology Department, Cluj-Napoca, between 2002-2015. Myositis diagnosis was based on clinical and laboratory criteria Peter and Bohan.

**Results.** Most patients in the study were women (80%). The average age of diagnosis of myositis was 54 years. We identified nine cases of cancer (6.71%). The incidence of malignancies among the female population was 0.047 compared to the incidence in male population, 0.015. None of the patients presented symptoms suggestive of neoplasia. All subjects in the study had undergone extensive neoplastic screening. Most types of cancer diagnoses were lung cancer (n=3), gastric cancer (n=2), ovarian cancer (n=1), cervical cancer (n=2), brain cancer (n=1). Most malignancies were identified in the first year after the diagnosis of myositis. At the end of the study there were registered two deaths among the patients with neoplasia.

**Conclusions.** In our study we found a higher incidence of malignancy among middle-aged women diagnosed with DM. In our group were recorded two cases of death due to late diagnosis of neoplasia.

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## CHRONIC ARTHRITIS AND LEUKEMIA: A DIAGNOSTIC CHALLENGE

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**Introduction.** Osteonecrosis (ON) is a rare but serious complication of treatment for acute lymphoblastic leukemia (ALL). Multiple factors are implicated in ON pathogenesis, such as the malignancy itself, procoagulant states and treatment. However, in most of the cases the diagnosis is late, especially in patients with atypical clinical presentation.

**Material and methods.** We present the case of an 18-year-old male with ALL referred to Rheumatology Clinic for a second opinion regarding the etiology of a chronic arthritis.

**Results.** The patient was diagnosed with ALL when he was 17-years old and he was treated according to ALL-BFM 95 protocol, which included high doses of corticosteroids. In evolution he developed a deep vein thrombosis associated with homozygous carrier status for MTHFR gene mutation. One year after starting chemotherapy the patient developed pain and tumefaction in his left knee, left ankle and right elbow. He was diagnosed with “seronegative spondyloarthropathy” and treated with sulphasalazine and various NSAIDs, with no relief. At the time of presentation in our clinic he had chronic left knee, left ankle, right elbow arthritis with marked functional limitation. Knee ultrasound revealed large fluid collection and irregularities of the bone cortex. Synovial fluid analysis showed a non-inflammatory synovial fluid, with no crystals and negative cultures. Bone scan showed multiple areas of increased uptake, in the knees, ankles and elbows. Left knee MRI showed patchy areas of low signal intensity on T1 sequences and the double-line sign on T2 sequences. The patient was diagnosed with multiple osteonecrosis.

**Conclusion.** We presented a case of multiple osteonecrosis, with atypical clinical presentation as chronic arthritis in a male adolescent treated for ALL. Special awareness of symptomatic ON is recommended in patients with ALL at high risk for ON. MRI is the imaging procedure of choice for early diagnosis of ON.

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## COGNITIVE PROFILES OF PATIENTS DIAGNOSED WITH UNIPOLAR (MAJOR DEPRESSIVE DISORDER) AND BIPOLAR DEPRESSION

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**Introduction.** Cognitive impairment is a common characteristic in many psychiatric illnesses, such as Bipolar Disorder and Major Depressive Disorder. Cognitive deficits were understood initially as secondary to typical mood symptoms. It was considered that they were present only in the acute episodes and improved as symptoms recovered. Reports have described persistent cognitive deficits even after significant improvement of symptoms and also during remission.

**Method.** We reviewed recent literature in order to explore the dimension of cognitive impairment in unipolar and bipolar depression and also to evaluate the differences between cognitive profiles of patients diagnosed with the above mentioned disorders.

**Results.** Both unipolar and bipolar depressed patients displayed cognitive impairment in several cognitive domains. Different subcomponents of attention were altered in both patients but impaired sustained attention appeared specific to bipolar disorder and may represent a marker of the latter. Two recent studies found that bipolar depressed patients performed significantly worse than unipolar depressed patients on tests assessing verbal memory, verbal fluency, attention and executive functions. The studies that compared cognitive performances of depressed patients diagnosed with different types of bipolar disorder and unipolar disorder indicated that bipolar I depressed patients performed worse than unipolar depressed patients in executive functions and verbal fluency, while bipolar II depressed patients performed similarly with unipolar depressed patients except visual memory which was worse in unipolar patients during acute depressive episodes.

**Conclusions.** Cognitive deficits are present during a depressive episode both in bipolar disorder and major depressive disorder. While those cognitive deficits are found in several domains, the two diagnosis categories display slightly different patterns of impairment.

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## EFFICACY OF A PLANT BASED EXTRACT IN MILD TO MODERATE FORMS OF PSORIASIS

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**Introduction.** Psoriasis is a chronic hyperproliferative dermatitis, with an important impact on the patients' quality of life. The conventional therapeutic options are not always satisfactory in terms of efficiency and safety, thus complementary and alternative medical approaches are frequently chosen by patients, mostly as self-medication. A number of plants containing key-phytochemical molecules belonging mainly to phenolics, triterpenoids, phytosterols and unsaturated fatty acids have been reported to act as antipsoriatic agents. Yet few of these have been actively tested in vivo. This study aims to assess the efficacy of an original plant-based extract in mild to moderate forms of psoriasis.

**Material and methods.** We developed an original plant-based extract, containing only highly safe ingredients, already approved for human use. We characterized it using high performance liquid chromatography-mass spectrometry. We included the extract in a standard base for topical use (ACT), and used the base alone as placebo (PLA).

We included 10 patients with mild to moderate forms of psoriasis, PASI score  $\leq 12$ , with no treatment for the disease. The patients applied the ACT twice a day on all lesions situated on the right side of the body, and the PLA also twice a day on those from the left side. We assessed the PASI and DLQI scores before treatment, and after one month of treatment with ACT vs PLA.

**Results.** We report in vivo antipsoriatic activity of our original plant-based extract. Its high concentration of anti-proliferative, anti-inflammatory and antioxidant molecules led to an improved PASI and DLQI scores. No side effects were noted.

**Conclusion.** These initial data, are promising and encourage us to extend the study. This would allow the development of a plant-based extract providing antipsoriatic activity and excellent safety to patients with mild to moderate forms of psoriasis.

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## IRRITABLE BOWEL SYNDROME AND FIBROMYALGIA, CENTRAL SENSITIVITY SYNDROMES

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**Introduction.** Irritable bowel syndrome (IBS) is one of the most frequent gastrointestinal disorders. Central sensitization to pain as a phenotype trait is present in patients with fibromyalgia (FM), and other conditions that associate generalized pain hypersensitivity, neuropathic pain or visceral pain hypersensitivity, such as IBS.

Our aim was to evaluate comorbidities in patients with IBS and also to evaluate the incidence of fibromyalgia, one of the most common comorbidities in IBS.

**Material and methods.** 58 consecutive patients diagnosed with irritable bowel syndrome according to Rome III criteria and 45 healthy controls were included. Fibromyalgia was diagnosed using the American College of Rheumatology (1990) classification criteria. Comorbidities were assessed also in relation to FM (present or absent) in patients with IBS.

**Results.** Fibromyalgia was the most frequent comorbidity in female patients. Psychiatric disorders have had also a high incidence, anxiety disorders being the most frequent in female patients while in male patients depressive disorders were the most frequent. In patients that associate IBS and fibromyalgia psychiatric disorders had a higher incidence in comparison with patients diagnosed only with IBS.

**Conclusion.** Comorbidities are frequent in patients with IBS, psychiatric disorders and fibromyalgia being the most frequent.

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## DEPRESSION AND SERUM LIPID PROFILE IN PATIENTS WITH CARDIOVASCULAR RISK FACTORS

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**Background.** Recent epidemiological studies show that psychosocial factors contribute significantly to the pathogenesis of cardiovascular diseases. Although they are treated as separate entities, the psychosocial factors tend to cluster in the same individuals.

**Objectives.** The aim of this study is to document the presence of subclinical depression (D) in a group of patients with cardiovascular risk factors and to examine possible associations between psychosocial factors and the serum lipids fractions in these patients.

**Material and method.** A total of 105 hypertensive subjects (64% female, 36% male) whose mean age was 57.23 years were subjected to a set of psychological assessments and laboratory tests. The diagnosis of hypertension was defined according to the ESH 2013 (European Society of Hypertension) criteria. The lipid profile was defined according to the NCEP/ATPIII (National Cholesterol Education Program, Adult Treatment Panel III) criteria. Depression was assessed using validated standard questionnaires (Beck Depression Inventory). Statistical analyses of the collected data were performed.

**Results.** Of the 105 hypertensive patients, 41 (59%) showed increased D scores. The mean values of the D score was significantly higher in female patients, compared with male patients. The mean serum concentration of total cholesterol (TC)  $204.59 \pm 51.19$  was significantly higher in the patients presenting higher D scores. There were no significant differences in the levels of and LDL-C, triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C).

**Conclusions.** High D score increases the levels of TC in hypertensive patients. Early diagnosis of subclinical D in hypertensive patients may lead to therapeutic interventions meant to reduce the risk of subsequent cardiac events in this population.

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## THE BENEFITS OF CALGARY SLEEP APNEA QUALITY OF LIFE INDEX IN SLEEP MEDICINE

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**Background.** In obstructive sleep apnea syndrome (OSA), the patient's quality of life (QOL) is affected due to impairment in physical and mental function and may improve under continuous airways positive pressure (CPAP) therapy. The aim of this study was to assess the power of Calgary Sleep Apnea Quality of Life Index (SAQLI) in the detection of QOL improvement in OSA patients after CPAP therapy.

**Material and method.** We conducted a cross-sectional study between January 2011 and December 2014 and we included 79 subjects diagnosed with OSA in Sleep Laboratory of Iuliu Hatieganu University of Medicine and Pharmacy with mean age  $54.13 \pm 10.87$  years.

**Results.** Of all 79 subjects, 59 (74.7%) were men and 20 (26.3%) women; the mean apnea-hypopnea index (AHI) was  $52.46 \pm 20.83$  events/h. the mean abdominal circumference was  $123 \pm 14.90$  cm; the mean body mass index (BMI) was  $35.06 \pm 5.74$  kg/m<sup>2</sup>; the mean Epworth Sleepiness Scale (ESS) was  $11.33 \pm 4.59$ . In all 4 domains of SAQLI: A) daily functioning with pretreatment mean score  $4.13 \pm 0.58$  and post treatment mean score  $5.43 \pm 0.52$ ; B) social interactions with pretreatment mean score  $3.68 \pm 0.55$  and post treatment mean score  $5.36 \pm 0.57$ ; C) emotional functioning with pretreatment mean score  $3.83 \pm 0.53$  and post treatment mean score  $5.38 \pm 0.56$ ; D) symptoms with pretreatment mean score  $0.81 \pm 0.12$  and post treatment mean score  $1.15 \pm 0.14$  was seen an improvement in scores of SAQLI after treatment, with significantly statistical correlation ( $p < 0.001$ ).

**Conclusions.** The change in the total SAQLI score from pre to post intervention was 1.13 which is well above the minimal important difference and according to domains A,B,C and D the mean score difference was  $1.29 \pm 0.69$ ,  $1.67 \pm 0.83$ ,  $1.54 \pm 1.54$  and  $0.34 \pm 0.19$ . SAQLI is a sensitive tool in the detection of QOL improvements in OSA patients after CPAP therapy.

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## CONTINUOUS AIRWAYS POSITIVE PRESSURE VENTILATION: POSSIBLE NEW HEALTH RISK FACTORS?

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**Background.** Nowadays, obstructive sleep apnea syndrome (OSAS) has become a key pawn in health care services. Continuous positive airway pressure ventilation (CPAP) machines for the treatment of OSAS require particular attention not only in terms of technical effectiveness, but also due to the accessories attached to the device such as masks and tubing. The aim of this study was to analyze by complementary methods the composition, morphology and real size of inorganic elements, as well as microbiological morphology on the inner surface of CPAP masks and tubing.

**Material and method.** The samples from the internal surface of 35 masks and the tubing used plus 1 mask with sealed tubes were harvested with chopsticks infiltrated with water peptin for the microbiological examination of the surface. Also, other investigation methods used were scanning electron microscopy, energy dispersive X-ray spectroscopy microanalysis, and X-ray photoelectron spectroscopy.

**Results.** We used a modern and innovative surface sampling method, i.e. the double-sided adhesive carbon tape technique, which allowed high performance studies for the characterization of inorganic elements as well as microbial structures that have not been studied so far in this respect. The presence of microbiological (main *Staphylococcus epidermidis* 5000 CFU/plate, *Staph. hominis* 6000 CFU/plate and *Staph. haemolyticus* 6500 CFU/plate, and *Candida*) versus inorganic structures (Ca, Si, S, Al, Mg) on the surface of CPAP masks and tubing was demonstrated, which raises a flag on the risk of their inhalation into airways.

**Conclusions.** There is statistical significance between the mask and the tubing regarding the duration of the use, methods of hygiene, local infections and the obtain concentration of the bacterial species ( $p < 0.0001$ ,  $p < 0.00001$ ,  $p < 0.001$ ). Safety measures need to be implemented in order to reduce this risk, including the possibilities to incorporate currently known antimicrobial nanoparticles in the structure of masks and tubing.

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## TREATMENT PATTERNS OF TNF- $\alpha$ INHIBITORS IN ANKYLOSING SPONDYLITIS IN EVERY DAY PRACTICE

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**Background.** The efficacy of TNF- $\alpha$  inhibitors in ankylosing spondylitis (AS) was demonstrated in randomized trials. There is little research about how they are used in every day practice.

**Objective:** Evaluation of treatment patterns of TNF  $\alpha$  inhibitors in ankylosing spondylitis in a specialty center.

**Methods.** A monocentric retrospective analysis was set out of patients with AS treated with one of the TNF  $\alpha$  inhibitors – infliximab (IFX), adalimumab (ADA), etanercept (ETN). We included patients that were followed-up for at least 12 months in our department between 2009-2013. Our database included: dosages, administration period, time of treatment with the first, second or third TNF  $\alpha$  inhibitor, reasons of discontinuation or switching (inefficacy or adverse events), clinical and biological parameters.

**Results.** Seventy five AS patients (72% males) received TNF  $\alpha$  inhibitors therapy (50% IFX, 21% ADA and 29% ETN). The median duration of follow-up was 27 months. Six out of 75 (8%) discontinued biological therapy with TNF  $\alpha$  inhibitors. A second TNF  $\alpha$  inhibitor was given for 12 out of all patients, and 2 of them needed a third TNF  $\alpha$  inhibitor. Reasons for switching TNF inhibitors were inefficacy (3/75) and adverse events (10/75). Patients who failed the first TNF  $\alpha$  inhibitor (3/75) had a good clinical response after switching the TNF  $\alpha$  inhibitor. Adverse events causing discontinuation or switching were: tuberculosis (3/75), high transaminases (2/75), pustular psoriasis (2/75), recurrent uveitis (1/75), severe allergy (1/75), neuropathy (1/75) and spinocellular carcinoma (1/75).

**Conclusions.** The majority of AS patients have good response and go on with the first TNF  $\alpha$  inhibitor. The most frequent cause of switching or discontinuation of the TNF inhibitors was adverse events. Switching the first TNF inhibitor with another one has good outcome, self-reliant the reason of treatment changes.

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# IMPROVEMENT OF THE QUALITY OF GAIT AND BALANCE BY TREATMENT WITH NATURAL THERAPEUTIC FACTORS IN BAILE TUSNAD IN POST-STROKE PATIENTS

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**Introduction.** Stroke is one of the main causes of morbidity and mortality worldwide. Carbonated mineral waters in Baile Tusnad are used for their peripheral and cerebral vasodilator effects in the rehabilitation of cardiovascular patients. This clinical study was aimed at monitoring the efficiency of natural therapeutic factors with the purpose of continuing rehabilitation treatment in a balneoclimatic resort.

**Material and methods.** The study included 30 patients with a history of stroke, treated in the Baile Tusnad treatment facility. The patients underwent rehabilitation treatment consisting of kinesitherapy, carbonated mineral baths for 15 minutes, aerotherapy for 30 minutes, massage therapy, all performed daily for 16 days. Each patient was clinically evaluated before and after treatment based on the Tinetti Balance Assessment Tool, the 10-m walking test, the Motor Assessment Scale, the Barthel Index, the Quality of Life Scale, and adverse reactions.

**Results.** At the end of treatment, a statistically significant improvement in the walking speed, in the quality of gait was found,  $p < 0.05$ . Statistically significant results,  $p < 0.05$ , were also obtained when evaluating balance. A statistically significant value was obtained for the Motor Assessment Scale. The evaluation of the patients' performance in ten activities of daily living depending on the required external help, using the Barthel Index, evidenced a statistically significant  $p$  value  $< 0.05$ . For the Quality of Life Scale, a  $p$  value  $< 0.05$  was obtained. There were no side reactions to the treatment.

**Conclusions.** Natural therapeutic factors influenced the clinical and functional picture, determining a significant improvement of the quality of gait and balance, an increase of independence and an improvement of the quality of life in these patients.

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## INCREASING THE QUALITY OF LIFE OF PATIENTS WITH PARKINSON'S DISEASE BY TREATMENT WITH NATURAL THERAPEUTIC FACTORS IN BAILE TUSNAD

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**Introduction.** Primary Parkinson's disease is a progressive disorder of the central nervous system. Baile Tusnad resort is recognized for its role in the rehabilitation treatment of cardiovascular, neurological, renal, digestive, rheumatic diseases through the presence of natural therapeutic factors: carbonated mineral waters due to their peripheral and central vasodilator effects, mofettes, and a stimulating bioclimate. The aim of this study was to evaluate the clinical efficiency of natural therapeutic factors in Baile Tusnad in order to continue the rehabilitation treatment of patients with Parkinson's disease in a balneoclimatic resort.

**Material and methods.** The study included 17 patients (5 women and 12 men) with Parkinson's disease, Hoehn-Yahr stages I-III, treated in the Baile Tusnad treatment facility. The patients underwent rehabilitation treatment consisting of carbonated mineral baths for 15 minutes, aerotherapy for 30 minutes, massage therapy and kinesitherapy, performed daily for 16 days. All patients were clinically evaluated before and at the end of treatment using the Tinetti Balance Assessment Tool, the 10-m walking test, the Webster Scale, the Quality of Life Scale, and adverse reactions.

**Results.** At the end of treatment, a statistically significant improvement of the walking distance and speed, of the quality of gait was found,  $p < 0.05$ . Statistically significant results ( $p < 0.05$ ) were also obtained for balance. The Webster Scale, which assesses the limits of movement and autonomy, evidenced a  $p$  value  $< 0.05$ . For the Quality of Life Scale, a statistically significant  $p$  value  $< 0.05$  was obtained. There were no side reactions to the treatment.

**Conclusion.** Natural therapeutic factors influenced the clinical and functional picture, determining a significant improvement of the quality of gait, balance, bradykinesia and the quality of life.

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## CARDIAC MRI HIGHLIGHTS THE TARGET ZONE FOR VENTRICULAR TACHYCARDIA ABLATION

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**Introduction.** A 65 years-old man was referred from Alba Iulia Hospital for Ventricular Tachycardia ablation. He had a history of persistent atrial fibrillation, and COPD grade II. In September 2015 he presented a presyncope, in the condition of a ventricular tachycardia that lasted 20 hours and he was hospitalized at Cugir Hospital with monomorphic VT. He was transferred to our cardiology department for coronary angiography and electrophysiological study.

**Material and methods.** EKG during tachycardia showed a 380 msec cycle length (heart rate of 160/min), with a morphology that fulfills the electrocardiographic criteria of TV (Wellens, Brugada, Griffith algorithm). EKG during rest without VT showed atrial fibrillation without further changes. Echocardiography: normal size of the left ventricle with EF=45% likely in the context of sustained ventricular tachycardia. Coronarography: epicardial coronary arteries showed no significant lesion. Cardiac MRI highlighted a zone of fibrosis in the anterior-lateral apex.

**Results.** Given these preliminary exams, electrophysiological study was performed using three-dimensional mapping system NavX. It showed: Not inducible VT by programmed ventricular stimulation before and after Adrenaline infusion. When we touched with the catheter the apical zone of fibrosis sustained repetitive VT was induced. The ablation performed at this level stopped the VT tachycardia during radiofrequency application. He was discharged with amiodarone and beta-blocker treatment and will be implanted with an internal defibrillator.

**Conclusion.** Cardiac MRI can highlight the zone of origin of VT by showing zones of fibrosis. Catheter ablation targeting these zones can stop the arrhythmia by acting on the substrate of VT.

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## A RARE CASE OF CHILDREN WEIL'S DISEASE (SEVERE LEPTOSPIROSIS WITH CHOLESTATIC HEPATITIS AND RENAL FAILURE)

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**Introduction.** Leptospirosis is considered the most common zoonosis with worldwide distribution. It is caused by a pathogenic spirochete of the genus *Leptospira interrogans*. The most important reservoirs are rodents, predominantly rats. Clinical spectrum can range from an asymptomatic, subclinical infection to a fatal hepatorenal syndrome (Weil's disease) or severe pulmonary form (pulmonary hemorrhage, acute respiratory distress syndrome). In presence of kidney and liver failure it is known as Weil's disease, which counted for 10% of all infections and has 10% mortality.

**Case report.** We report the case of a 16-year old boy with severe leptospirosis (Weil's syndrome). He presented with fever, severe myalgia, jaundice, and oliguria. When he was asked about any epidemiologic factors that placed him at risk for leptospirosis, the patient admitted that in the last two weeks he was hand fishing. Laboratory results revealed thrombocytopenia (80,000 cells/mm<sup>3</sup>), hemoglobin of 14.5 g/dL, leukocytosis (11.5 × 10<sup>3</sup> cells/mm<sup>3</sup> with lymphopenia 2.9%), cytolysis (AST 510 U/L, ALT 78 U/L, and creatine phosphokinase 3071 U/L), hyperbilirubinemia (total bilirubin 2.55 mg/dl, conjugate bilirubin 1.79 mg/dl with increasing of total bilirubin level in two days up to 6.86 mg/dl), increased serum creatinine (2.3 mg/dL). The urine analysis showed moderate hematuria but no proteinuria. The diagnosis was confirmed serologically: positive IgM antibodies anti-leptospira. The patient was treated by i.v. ceftazidime for 14 days with favorable evolution (cytolysis, hyperbilirubinemia, thrombocytopenia, and renal failure resolved over the next few days).

**Conclusions.** Weil's syndrome is a rare, but a very severe form of leptospirosis, with high risk of death without treatment. It is characterized by multisystem dysfunction and can present with high fever, significant jaundice, renal failure, hepatic necrosis, pulmonary involvement, cardiovascular collapse, neurologic changes and hemorrhagic diathesis.

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## THE AEROBIC CAPACITY AND ITS DETERMINANTS IN A SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) COHORT

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**Introduction.** The SLE patients often complain of decreased exercise capacity even if the heart or respiratory damage was ruled out. The cardiopulmonary exercise testing (CPET) accurately assesses the oxygen consumption and the impaired steps in its utilization. Therefore we determined the exercise capacity and the factors which influence it in a SLE cohort by CPET.

**Method.** 31 patients without history of heart disease, in follow at Rheumatology Clinic Cluj-Napoca performed a CPET on a cycloergometer (General Electric - Cortex 3.7 integrated system) using a 10W/min "ramp" protocol. The peakVO<sub>2</sub>, ventilatory threshold (VT1), CO<sub>2</sub> ventilatory equivalent (VE/VCO<sub>2</sub>), pulse oxygen, respiratory exchange ratio (RER) and circulatory power (CircP) were determined. The patients were also assessed by cardiac ultrasound.

**Results.** Among SLE patients, 28 (90.3%) were women, mean age 42.7±10.6 years. The left ventricular ejection fraction (LVEF) was normal (61±7.5%). They registered a decreased peak VO<sub>2</sub> of 18 ml/Kg/min, (72% of predicted VO<sub>2</sub>Max, 85% being considered the normal lower limit). RER>1.1 was reached in 74% of patients. The rest of them (8 patients, 26%) presented fatigue and musculoskeletal pain (4 patients). The anaerobic threshold was at the lower limit (42%), suggesting a deconditioning component of decreased effort capacity. The decreased oxygen pulse (8.7±1.4 ml/beat), in the setting of the normal LVEF, stroke volume and CircP (3137±884 mmHg•mL•min<sup>-1</sup>•kg<sup>-1</sup>) indicates a decreased tissue oxygen extraction. We found a normal mean VE/VCO<sub>2</sub> (33.7), but in all subjects who complained of dyspnea (3 patients) the ratio was above 35, supporting the hyperventilation as the mechanism of dyspnea.

**Conclusion.** Our study showed a decreased functional capacity of SLE patients due to musculoskeletal symptoms, deconditioning and decreased peripheral oxygen extraction.

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## SKIN HYPERPIGMENTATION IN A SYSTEMIC LUPUS ERYTHEMATOSUS PATIENT

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**Introduction.** Systemic lupus erythematosus (SLE) is an autoimmune disease with systemic involvement and diverse clinical manifestations. Skin hyperpigmentation has multiples etiologies and sometimes the differential diagnosis can be difficult.

**Material and methods.** We present the case of a female patient who was diagnosed with SLE with a severe onset with serositis, renal disorder and arthritis 15 years ago. We mention that the patient is also suffering from chronic hepatitis B.

**Results.** In the context of infection with the Hepatitis B virus, the patient had an episode of fulminant hepatitis during immunosuppressive treatment with Ciclosporin A. After that the patient developed erythematous skin lesions with annular forming polycyclic patterns, photosensitivity and anti Ro antibodies led to SCLE (subacute cutaneous lupus erythematosus), therefore the skin manifestation became the main concern. The patient is showing generalized hyperpigmentation and the differential diagnostic may be: an adverse effect to her medications, ochronosis or Addison disease.

**Conclusion.** The purpose of this case report is to illustrate the challenge, which is not only to establish the right diagnosis, but to find the right balance of immunosuppressive treatment in the presence of hepatitis B virus.

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## NEURO-PSYCHIATRIC DAMAGE IN SYSTEMIC LUPUS ERYTHEMATOSUS

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**Introduction.** Neuro-psychiatric lupus is a leading cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE).

The objective of this study was to identify the pattern of neuro-psychiatric manifestations in patients with SLE and monitoring their course after 6 months of treatment. Materials and methods:

**Material and methods.** The study included 26 patients with severe neuropsychiatric SLE who were registered in the Rheumatology Clinic Cluj-Napoca, between 2000-2015. Inclusion criteria were: the diagnosis of SLE, based on clinical manifestations, paraclinical exams and ACR/SLICC criteria and the existence of at least one neurologic or psychiatric episode in the course of the disease, after excluding other causes. Patients were monitored for 6 months using the SELENA-SLEDAI scoring system.

**Results.** In 57.69% of cases, neuropsychiatric manifestations were inaugural symptoms. 50% of patients had at least one related central nervous system (CNS) symptom. 30.77% of subjects had both SNC and peripheral nervous system manifestations. The clinical picture varied widely, most cases (n=7) had cephalgia, polyneuropathy and cognitive dysfunction. Antinuclear antibodies (96.15%) and hypocomplementemia (61.54%) were found in a high percentage. At the onset of NL, 61.54% of the subjects had a high activity score, after 6 months of treatment the majority of them (38.46%) had moderate activity and 23.07% were in remission.

**Conclusion.** Neurological or psychiatric symptoms were mostly encountered at onset of the disease. Although paraclinical exams have a high sensitivity for diagnosing lesions, they are not specific for NL lesions.

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## EXTREME THROMBOCYTOSIS AND BASOPHILIA IN A CASE OF CHRONIC MYELOID LEUKEMIA

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**Introduction.** CML was the first malignant disease found to be consistently associated with a specific cytogenetic abnormality, the Philadelphia chromosome, resulting in the formation of the BCR-ABL fusion oncogene. The most common feature of CML is an elevated WBC count usually  $>25.00 \times 10^3/\mu\text{l}$  and frequently  $>100,00 \times 10^3/\mu\text{l}$ , the WBC differential usually shows granulocytes in all stages of maturation from blasts to mature morphologically normal granulocytes. The platelet count is elevated in 30–50% of patients and is higher than  $1000,00 \times 10^3/\mu\text{l}$  in a small percentage of patients with CML. Excessive thrombocytosis like that are seen in essential thrombocythemia (ET) are uncommon (described as Ph1 positive ET in the past). Basophils are constantly elevated but only 10–15% of patients have  $\geq 7\%$  basophils in the peripheral blood. In contrast to mastocytosis, hyperhistaminemia is uncommon. Elevated basophil count is a treasure of accelerated phase but excessive basophilia is a very rare condition at diagnosis and is suggestive for basophilic leukemia. Extreme basophilia at presentation impose a differential diagnosis with rare chronic or acute basophilic leukemia.

**Material and methods.** We present the case of 30 years old female who was referred to our department for anaemias syndrome.

**Results.** Clinical: pallor, no organomegalies. Blood count cell showed: anaemia, Hb=10 g/dl, leucocytosis  $66,00 \times 10^3/\mu\text{l}$ , extreme thrombocytosis  $3600,00 \times 10^3/\mu\text{l}$  and on peripheral blood surprising an elevated percent of 66% of mature basophils, the lack of immature granulocytes. JAK2V617F mutation is not detected. BCR-ABL transcripts: major molecular response, cytogenetic analysis: 100% presence of Ph chromosome. With the diagnosis of MCL can start a treatment with Hydroxiurea, then dasatinib 100 mg/day. The result is a spectacular one, a normal blood count cell after one month.

**Conclusion.** Presentation with extreme thrombocytosis and basophilia in MCL is exceptional rare representing a diagnosis provocation: MCL, ET or basophilic leukemia.

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## PARAOXONASE-1 ACTIVITIES IN RELATION TO ANTI-OXIDIZED LDL ANTIBODIES IN PATIENTS WITH ABDOMINAL OBESITY

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**Introduction.** It is demonstrated the fact that there is a direct correlation between obesity and oxidative stress markers. Paraoxonase 1 (PON1) is an enzyme with multiple benefic activities against oxidative stress and multiple interindividual variations, determined by punctiform mutations in the gene encoding the enzyme. Our study is the first in Romania to analyse the activities of PON1 in relation with the serum level of anti-oxidized LDL antibodies, in a population of subjects with abdominal obesity.

**Material and methods.** A number of 88 subjects with abdominal obesity admitted for multidisciplinary evaluation and 46 subjects with normal abdominal circumference were included in the study. For each patient we measured clinical parameters that might influence the activities of PON1. The PON1 activities (lactonase, paraoxonase and arylesterase) were measured using spectrophotometric methods. Anti-oxidized LDL antibodies were dosed using specific kits according to the protocol. Statistical analysis was performed using MedCalc software (version 12.5.0.0).

**Results.** We did not find a significant difference between obese patients and non-obese patients regarding age and sex. Our study revealed the fact that PON1 activities were not influenced by the sex of the patients. Among PON1 activities, only the paraoxonase activity was inversely correlated with age ( $p=0.05$ ). Abdominal circumference influenced only the variation of arylesterase activity ( $R^2=6.5\%$ ,  $p=0.003$ ). We didn't find a correlation between the values of the anti-oxidized LDL antibodies and enzymatic activities of PON1 for the study groups.

**Conclusions.** Serum level of anti-oxidized LDL antibodies is not correlated with PON-1 activities in patients with abdominal obesity.

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## THE ANTIOXIDANT DEFENSE OF PRETERM NEWBORNS

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**Introduction.** The antioxidant defense of preterm newborns, intra and extracellular, is poor, depending on their gestational age. The most important enzymatic antioxidant are the ceruloplasmin and transferrin. Proton donors evaluate the non- enzymatic antioxidative defense capacity. Both enzymatic and non –enzymatic antioxidant defense are less developed in newborns than at adults and especially at preterm neonates with different pathology.

The aim of our study was to evaluate the antioxidant defense capacity of preterm newborns with different pathology.

**Material and methods.** We conducted a prospective non–randomized study. In the study group we included 52 preterm neonates. For each of them we measured the ceruloplasmin in the first and third day of life. At 20 newborns from the study group we measured the proton donor capacity as well. The control group consisted of 13 healthy term newborns. In control group we performed one measurement on the first day of life. We used the spectrophotometric Ravin method for ceruloplasmin determination and for proton donors measurement we used the Hatano method.

**Results.** In the study group all preterm newborns presented different forms of respiratory distress, asphyxia and intraventricular hemorrhage. The ceruloplasmin value in the study group was higher in the third day of life than on the first day. The proton donor capacity had the same behavior. The severity of respiratory distress had a significant influence on the antioxidant defense capacity. In the control group the proton donor capacity level was higher than at study on first and third day of life.

**Conclusion.** The antioxidant defense at preterm newborns with different pathology generating oxidative stress is poor than at term newborns. The study of antioxidant defense in different neonatal circumstances could help to implement antioxidant treatment and avoid as much possible the oxidative stress of preterm neonates.

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## METABOLIC SYNDROME IN FEMALE PATIENTS WITH SCHIZOPHRENIA

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**Introduction.** High prevalence of metabolic syndrome has been reported in patients with schizophrenia. The etiology of metabolic syndrome in schizophrenia is multifactorial. Factors that contribute to the development of physical and biochemical abnormalities due to this syndrome are: sedentary lifestyle, antipsychotic medication and mental disorder related issues, including negative symptoms. Implementation of the necessary screening assessments of the metabolic parameters and referral for treatment of the pathological anthropometric and metabolic measurements can improve the health of patients with schizophrenia. The objectives of this study are to estimate the frequency of metabolic syndrome and to examine the association between metabolic syndrome and psychiatric symptoms in female patients with schizophrenia.

**Material and methods.** Forty two female patients diagnosed with schizophrenia were recruited from psychiatric out-patient clinic. Patients underwent one assessment for metabolic syndrome parameters which included anthropometrical measurements, blood pressure, lipid profile, fasting plasma glucose level. The presence of the metabolic syndrome was determined using the International Diabetes Federation criteria. The Positive and Negative Syndrome Scale was used for characterizing symptoms.

**Results.** Mean duration of illness was 13.93 years and mean duration of education was 13.15 years. Metabolic syndrome was found in 48% of the patients (36% with hypertriglyceridemia, 86% with high waist circumference, 59% with low HDL cholesterol, 23% with high fasting glucose). 16% of patients were treated with Olanzapine, 11% with Quetiapine, 11% with Aripiprazole, 7% with Clozapine.

**Conclusion.** Metabolic syndrome is common in female patients with schizophrenia. Systematically assessment of the various components of metabolic syndrome and adequate treatment should help to reduce the cardiovascular risk and mortality in patients with schizophrenia.

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## TYPE 2 CYTOKINES MODULATE THE EXPRESSION OF CALCITRIOL RECEPTOR VDR IN BRONCHIAL EPITHELIAL CELLS

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**Background.** 1,25-dihydroxy vitamin D3/1,25(OH)2D3/calcitriol, acting via its receptor VDR, modulates the levels of enzymes involved in its own metabolism.

We recently found that bronchial epithelial cells, the main host cells for viruses, constitutively express VDR and that type 2 cytokines decrease VDR expression. Since VDR is the main determinant of calcitriol function, these data suggest that treatment with calcitriol would be less effective in diseases with increased levels of type 2 cytokines including parasitoses, asthma, lymphoma.

The objectives of our study were to investigate in vitro the effect of calcitriol on VDR in respiratory bronchial epithelial cells exposed or not to type 2 cytokines

**Methods.** BEAS-2B cells (a bronchial epithelial cell line) and human primary bronchial epithelial cells (HPBEC) infected with rhinoviruses (RV1B or RV16) in the absence/presence of IL-13 were treated with calcitriol 100nM for up to 72h. VDR and virus gene expression were determined by qRT-PCR and cytokines RANTES, IL-8 and IL-6 were measured by ELISA.

**Results.** Type 2 cytokines IL-4 and IL-13 decreased VDR expression in BEAS-2B and HPBE cells. Calcitriol decreased VDR expression in BEAS-2B cells and this effect was enhanced by IL-13, at 48h.

Calcitriol significantly decreased expression of VDR in HPBEC in a dose-response manner.

**Conclusions.** As previously shown in other cell types, we found that calcitriol regulates its own metabolism in respiratory epithelial cells, by down-regulating VDR receptor (involved in the modulation of its metabolic enzymes). Calcitriol down-regulation of VDR expression was increased in a type 2 cytokine environment.

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## COMORBID DISORDERS IN DEMENTIA

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**Introduction.** In 2015 WHO estimated a number of 46.8 million cases of dementia worldwide, with over 818 billion US\$ costs for treating and caring. These costs could be reduced and the life quality improved by early management and prevention of the associated diseases.

The aim of this study was to determine the type and prevalence of the main psychiatric and somatic disorders comorbid with dementia.

**Material and methods.** The sample consisted of 98 participants, mean age  $70.9 \pm 8.5$  years, admitted in the 3rd Psychiatric Clinic of the Cluj County Emergency University Hospital between 01.01.2013 and 31.12.2014. The diagnosis data was collected from the electronic archive of the hospital.

**Results.** We found that the most frequent somatic diseases are the cardio-vascular ones, with a prevalence of 46%, followed by the endocrine, nutritional and metabolic diseases (32%), respectively by the neurological ones (18%). As far as psychiatric comorbidities are concerned, the mental organic disorders are the most frequently associated (44% prevalence), followed by the ones due to psychoactive substance use (17%), and mood disorders (16%).

**Conclusion.** 3 out of 4 patients have at least one comorbid somatic or psychiatric disorder.

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## THE CONSEQUENCES OF FAMILY DISINTEGRATION DUE TO WORKFORCE MIGRATION ON THE MENTAL HEALTH OF ADOLESCENTS

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**Introduction.** Family disintegration is likely to increase the risk of adolescents for developing mental health problems through increased exposure to stressful life events (due to a reduction in control), and through impairing resilience promoting mechanisms (i.e. control, instrumental support and emotional support). Therefore, we hypothesized that adolescents who have parents working abroad would be at significantly higher risk for mental health problems than their peers. Our aim was to determine whether adolescents who have at least one parent working abroad register significantly higher incidence of internalizing problems, externalizing problems, substance misuse problems and sensation seeking/delinquent behaviors.

**Material and methods.** The Romanian sub-sample of the Saving and Empowering Young Lives in Europe (SEYLE) project was used to test our hypothesis. It consisted of 1143 adolescents with a mean age of  $15.02 \pm 0.37$  years (65.4% females), recruited through a cluster-randomized sampling protocol, from the general population. The Strengths and Difficulties Questionnaire (SDQ) was used to screen for internalizing and externalizing problems. Substance misuse and sensation-seeking/delinquent behaviors (i.e. physical risk-taking and sexual behavior) were screened through SEYLE-specific questions.

**Results.** Adolescents that have at least one parent working abroad are at significantly higher risk for developing externalizing problems, i.e. conduct problems and/or hyperactivity,  $RR=1.33$  [95% CI 1.03-1.73],  $p=0.036$  – Chi square test, but not for internalizing problems, substance abuse or sensation seeking/delinquent behavior. When stratified by gender, this relationship remained true only for adolescent girls  $RR=1.45$  [95% CI 1.08-1.93],  $p=0.018$  – Chi square test.

**Conclusion.** Family disintegration due to workforce migration is a risk factor for the development of conduct problems and/or hyperactivity, i.e. externalizing problems, in adolescent girls.

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## BREAKING DOWN THE CONSTRUCT OF NEGATIVE SYMPTOMS OF SCHIZOPHRENIA

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**Introduction.** Negative symptoms of schizophrenia are normally described as a unitary construct and are considered to be a separable domain of pathology from other symptoms of schizophrenia. However recent studies suggest, due to the heterogeneity of these symptoms, that they are actually made of 2 separable domains: Diminished Expression (DE) and Avolition-Apathy (AA). The aim of this study was to assess the factor structure of negative symptoms in out-patients diagnosed with schizophrenia and to examine the relationship of these factors with global functioning sociodemographic characteristics and clinical variables.

**Material and methods.** The participants were recruited from the Outpatient Psychiatric Clinic, a sample of 40 consecutive subjects treated, whom met the criteria for schizophrenia according to ICD-10 and were stable from the point of view of the symptoms for at least 3 month. The patients were evaluated using the Positive and Negative Symptoms Scale (PANSS), Negative Symptoms Assessment-16 items (NSA-16), Global Assessment of Functioning (GAF), Clinical Global Impression-Severity Scale (CGI-s) and were interviewed to assess sociodemographic characteristics. Statistical analyses were performed the Statistical Package for the Social Sciences (SPSS) 12.0 for Windows.

**Results.** A two-factor structure was found: a group with predominantly AA symptoms and another with predominantly DE profile. AA factor consisted of avolition, anhedonia, associativity and the ED factor consisted of alogia and blunted affect. AA and DE negative symptoms subgroups significantly differed on clinically relevant external validator, such as measures of functional outcome, clinical severity, gender, duration of education, age of onset.

**Conclusions.** Our findings suggest that the different subdomains of negative symptoms can be identified within the broader diagnosis of schizophrenia and that these subgroups should be analyzed as separate and distinct domains.

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## EVALUATING THE EFFICACY OF CHELATION THERAPY. STUDY ON A GROUP OF PATIENTS FROM NORTH-WEST OF ROMANIA.

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**The goals** of our study is to analyze the results of Deferasirox treatment of a group of adult patients diagnosed and treated in Hematology Departments of Nord-West Romanian hospitals (Cluj, Maramures, Satu-Mare and Salaj Counties).

**Methods.** We have done a retrospective, transversal study including all the patients with myelodysplastic syndromes (MDS), thalassemia and other anemias that received blood transfusion and chelator treatment.

**Results.** We included in the study 35 patients treated with Deferasirox in the NW region of Romania. The diagnosis included MDS, thalassemia and other anemias.

MDS patients were treated with erythropoietin, low dose chemotherapy, epigenetic treatment, blood transfusions and bethatalasemic patients were transfused. The baseline value of serum ferritin was between 1075 and 6187 microg/l (median- 3631 and mean- 2321). Deferasirox dose that was administered to the patient was 20-30 mg/kg. There was a significant reduction in serum feritin from baseline for all the patients.

Digestive adverse events appeared in three cases (two cases of diarrhea and one case of digestive hemorrhagic episode) and Deferasirox was restarted after treating the adverse effect. In three cases, treatment was temporarily stopped because low ferritin level (under 500 microg/l). Packed red blood cell transfusions were administered after starting Deferasirox treatment (0-3 units/months, median- 1.5 units/months, mean 1.3 units/months). Two patients died during Deferasirox treatment because of main disease or its complications.

**Conclusions.** Analyzing our small group of 35 patient, serum feritin levels decreased after Deferasirox treatment, which proves the efficacy of the drug. Adverse reactions that determined a temporary stop of the treatment were mild/medium short time digestive reactions (diarrhea and digestive bleeding), so we can consider the chelator treatment safe.

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## EARLY OSTEOARTHRITIS IN ADULT HYPOPHOSPHATASIA - A CASE SERIES

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**Background.** Hypophosphatasia is a rare inherited disorder characterized by defective mineralization of bone and teeth. Adult hypophosphatasia is the most frequent and has a wide spectrum of clinical manifestation (1, 2).

**Objectives.** The aim of this paper was to retrospectively identify the cases of adult hypophosphatasia and their musculoskeletal features.

**Methods.** The database and charts of patients seen between 2000 and 2014 in our tertiary rheumatological referral center were reviewed. Low alkaline phosphatase was confirmed on at least two separate occasions. The patients with secondary causes of hypophosphatasia were excluded from the study. The fracture site(s) were recorded, as well as the common risk factors for osteoporosis and the concomitant diseases. Standard knee radiographs were taken and graded according to Kellgren and Lawrence score. DXA was performed in all six patients and revealed osteopenia on two patients and osteoporosis on three patients.

**Results.** Six patients diagnosed with adult hypophosphatasia were identified. All patients were females with the mean age  $48 \pm 4.3$  years. Serum alkaline phosphatase values were low in all patients. All the patients had severe periodontal disease leading to early teeth loss. Stress fractures were noted in all patients, at different sites (metatarsal bones- 5, costal-2, sacrum-1, pubic bone-1). Two patients also had radius fractures. Quite unexpected, 4/6 patients had early osteoarthritis at multiple and different sites other than the fractures, despite long-term control of the concomitant inflammatory disease (normal ESR and CRP, no joint effusion). Bilateral knee involvement was the most prominent with early onset (under 45 years) in all cases. The Kellgren-Lawrence score was 2 for each knee in 3 patients and 1 for left knee and two for right knee in one patient. Chondrocalcinosis was not seen in our series.

**Conclusions.** Early osteoarthritis may be a feature of hypophosphatasia.

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## SERUM AND SALIVARY MARKERS IN IRRITABLE BOWEL SYNDROME

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**Introduction.** Irritable bowel syndrome (IBS) is a functional disorder which affects about 20% of the population and is the result of interaction of genetic predisposition and environmental factors.

The aim of this study is to highlight the possible correlation between occupational stress and the occurrence of IBS. According to the Rome III criteria, the syndrome is defined as recurrent abdominal pain or discomfort for at least 3 days per month, during the previous 3 months, associated with two or more of the following symptoms: improvement with defecation, onset associated with a change in the frequency of stool.

**Methods.** Serum IL-6 and salivary cortisol were measured in IBS patients and healthy controls. Stress was assessed by the standard questionnaires.

**Results.** Stress is encountered in higher amount in IBS compared to the matched control group. Salivary cortisol levels were not higher in IBS patients as compared to the healthy controls. Serum IL-6 levels tended to be higher in patients relative to controls. The biological markers of stress are correlated with the severity of the symptoms measured with the severity symptoms score, also with sleep disorders.

**Conclusions.** Psychological stress, assessed by specific questionnaires, was related with the presence of IBS and high levels of IL-6 but no relation between salivary cortisol and the presence of IBS was found.

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## A GENETIC ASSOCIATION STUDY ON CARDIAC REACTIVITY TO STRESS

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**Introduction.** Considering that heart rate variability (HRV) changes have been observed in both cardiovascular and emotional disorders, it has been argued that autonomic dysfunctions are a key mechanism underlying their comorbidity. Recent research indicated that individual differences in HRV are partially genetic and their heritability may be shared with emotional disorders.

**Material and methods.** The present study focused on the relations between two genetic variations and cardiac reactivity to stress, namely the Val66Met single nucleotide polymorphism in the brain-derived neurotrophic factor (BDNF) gene and a haplotype based on two functionally related polymorphisms in the serotonin transporter gene promoter (5-HTTLPR). These polymorphisms were previously associated with emotional vulnerability and HRV differences in healthy volunteers. In this study, we investigated time (i.e., RR intervals) and frequency domain (i.e., LF, HF, LF/HF) measures of HRV during mental stress in a sample of healthy young volunteers (N=247).

**Results.** The main results indicated a significant genetic association between 5-HTTLPR and RR intervals, but only in men. Male carriers of the low-expressing alleles of 5-HTTLPR showed blunted cardiac reactivity to stress, that is, a lower magnitude of RR intervals changes from rest to stress.

**Conclusions.** Considering that 5-HTTLPR did not influence vagal (HF) or sympathovagal (LF, LF/HF) measures of HRV, this study suggests that the physiological mechanisms underlying the influence of this genetic polymorphism on blunted cardiac reactivity to stress, as reflected by changes in RR intervals, do not involve differences in the autonomic control of the heart.

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## THE IMPORTANCE OF A FEW GENOTYPES WITH MEDICAL IMPLICATIONS; OUR EXPERIENCE IN IDENTIFICATIONS OF THE ALLELES FOR 5-HTTLR, BDNF, COMT AND ZNF804A GENETIC REGIONS

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**Introduction.** The rapid growth of human genetics creates opportunities for studies of disease associations. Given the number of identifiable genetic markers and the clinical outcomes to which these may be linked, the testing and validation of hypotheses in genetic epidemiology is a task of unprecedented scale.

**Material and methods.** In our genetic association studies, we have investigated more than 1100 healthy volunteers and genotyped for different polymorphisms.

**Results.** 5-HTTLPR (ins/del, rs25531) is known to be implicated in neuropsychiatric disorders, in idiopathic pulmonary arterial hypertension, in chronic fatigue syndrome, in IBS, or was analyzed worldwide for personality traits, pharmacogenetics and molecular neuroimaging studies. In our genetic association studies, the 5-HTTLPR genotyping protocol included the ins/del 43 bp and SNP rs25531 polymorphisms; based on these, 5-HTTLPR is triallelic, with two low-expressing alleles (S and LG), compared to the LA allele. In our 1196 samples, the genotypes distribution was in Hardy-Weinberg equilibrium (HWE):  $\chi^2=2.95$ ,  $p>0.05$ .

BDNF Val66Met region is known to be involved in anxiety related disorders, bipolar disorder, OCD; our genotyping procedure was performed for a total of 1142 samples, the Met allele is the rare and low-expressing one. The genotypes were in HWE ( $\chi^2=0.15$ ,  $p>0.05$ ).

COMT Val158Met is known as a candidate region for modulating dopamine levels and function in the cortex, and is implicated in neuropsychiatric disorders. In our association studies Val158Met genotyping was performed for 1133 samples; the genotypes were in HWE ( $\chi^2=0.8$ ,  $p>0.05$ ).

ZNF804A (rs1344706) polymorphism is known has been studied in schizophrenia, bipolar disorder or in pharmacogenetics drug efficacy studies; in our studies, genotyping was performed for a total of 214 samples, and the genotypes were in HWE ( $\chi^2=0.6$ ,  $p>0.05$ ).

**Conclusions.** Our genotyping protocols identified alleles for the four genes using techniques based on PCR-RFLP, ARMS-PCR procedures. A systematic approach may assist in estimating population-wide effects of genetic risk factors in human diseases.

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## DIAGNOSTIC AND THERAPY INSIGHTS: INBORN ERRORS OF METABOLISM, FOCUS ON TREATABLE DISORDERS, PHENYLKETONURIA BEING JUST A PARADIGM

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**Introduction.** Inborn errors of metabolism (IEMs) are genetic disorders characterized by dysfunction of an enzyme or other proteins involved in cellular metabolism. In most cases, IEMs affect the brain, and the first clinical symptoms present in infancy, or, in an unknown proportion of cases, they appear in adolescence or adulthood.

**Material and methods.** We focus on treatable IEMs, presenting acutely or chronically, that can be diagnosed mainly in neuro-psychiatric department. To make our presentation usable, we subdivide these types of disorders into sections according to the physiopathology (Saudubray's classification): a) intoxications (we exemplify the importance of correct treatment in adults with a neurometabolic disease, phenylketonuria being just a paradigm), b) defects of energy metabolism/ metabolism of complex molecules, c) defects in neurotransmitters and vitamins metabolism. Regarding the clinical picture, they could present as: acute encephalopathies/ strokes, ataxia, psychiatric disorders, movement disorders, peripheral neuropathies, spastic paraparesis, cerebellar epilepsy and leukoencephalopathies.

**Results.** We summarize the major biological disturbances and biomarkers identified with advanced biochemical techniques; we outline our experience in few disorders identified in a modern metabolomics approach, based on urinary NMR spectroscopy (Bruker Avance 400 MHz). The <sup>1</sup>H-NMR spectra were recorded with NOESY water presaturation pulse sequence, the limit of detection in the spectral region for different metabolites (for creatinine of 1 mmol/l) being around 100 mmol/mol creatinine.

**Conclusions.** IEMs can affect many organs (liver, kidneys, heart, muscles) but in most cases they involve the nervous system. Our aim is to classify the clinical and biochemical features of the main metabolic treatable diseases (outlining our possibilities of diagnostics through urinary NMR spectroscopy), helping the clinicians avoid overlooking a treatable metabolic disease.

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## CLINICAL AND IMAGING CORRELATION OF HEPATOCELLULAR CARCINOMA IN A GROUP OF PATIENTS – A RETROSPECTIVE STUDY

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**Introduction.** Hepatocellular carcinoma (HCC) is one of the most lethal cancers, that has a slowly increasing prevalence nowadays. In most cases, diagnosis is accomplished in late stages, when only palliative management is available. Our aim was to assess the peculiarities of diagnosis and also the complications in a group of patients diagnosed with HCC.

**Material and methods.** We performed a retrospective analyze of 50 patients diagnosed with HCC between 2007 and 2014, using imagistic methods and in some of the cases percutaneous liver biopsies. HCC diagnosis was established using classical criteria according to EASL and later EASL-EORTC diagnostic guidelines.

**Results.** Out of the total number of subjects included, 70% were diagnosed as cirrhotics. Most common etiologies were – viral (C – 32% and B – 20%), followed by alcoholic liver disease (18%) and other etiologies – NASH, autoimmunity and others (30%). Tumor sizes were up to 19 cm, all tumors though being larger than 2 cm, and mostly multicentric (46%). Patient presented portal vein thrombosis in larger proportion according to dimensions of the tumors (42% for tumors between 2-5 cm and 47% for tumors larger than 5 cm).

**Conclusions.** In our study only 70% of the patients with HCC were cirrhotics, a lower proportion in comparison with data from literature. They were unfortunately diagnosed in later stages, with a high incidence of portal vein thrombosis (BLCL stage C). The main outcome is the need of better screening strategies, preferably according to Japanese Society of Hepatology guidelines, in order to achieve a much frequent diagnosis of earlier stages (BLCL 0/A) with significantly better surviving odds.

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## ANTITHROMBOTIC THERAPY IN PATIENTS WITH ATRIAL FIBRILLATION

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**Introduction.** Atrial fibrillation is the most frequent arrhythmia. It increases the risk of thromboembolic events. Selection of antithrombotic agents includes antiplatelets, vitamin K antagonists and newer anticoagulants.

**Material and methods.** We conducted a retrospective observational study with patients diagnosed with atrial fibrillation, admitted between January 2013-September 2014 in Cardiology Department of The Rehabilitation Hospital Cluj-Napoca.

**Results.** A total of 1868 patients were enrolled. A number of 534 (28.58%) received aspirin, 54 (2.89%) aspirin+clopidogrel, 842 (45.07%) acenocumarol, 93 (4.97%) NOAC, 270 (14.45%) acenocumarol + aspirin. and 75 (4.01%) no antithrombotic treatment. The percentage of stroke and major haemorrhages was 3.75 and 4.12 for aspirin group, 1.85 and 5.56 for aspirin+clopidogrel group, 1.78 and 4.39 for acenocumarol group, 1.08 and 3.23 for NOAC group, 0.37 and 5.93 for acenocumarol+aspirin group, 5.33 and 1.33 for no antithrombotic treatment group respectively.

**Conclusion.** Association of classic oral anticoagulant + antiplatelet conferred the lowest risk of stroke, but the highest risk of hemorrhage. NOAC's provided the best balance between benefits and risks.

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## CLINICAL PRESENTATION AND OUTCOMES IN PATIENTS WITH LEFT VENTRICULAR NON-COMPACTION

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**Introduction.** Left ventricular non-compaction (LVNC) is a distinct cardiomyopathy with a fascinating myocardial phenotype having a spongy appearance. Because of the increasingly accessibility of the new imaging diagnostic methods, LVNC has gained lately attention and awareness from the physicians. The aim of this paper is to assess the correlation between the clinical presentation and imaging appearance of patients, therapeutic management and outcomes.

**Material and methods.** We retrospectively reviewed 15 patients aged 20 to 70 diagnosed with LVNC at Nicolae Stancioiu Heart Institute Cluj-Napoca and followed-up from 2010 to 2014. Echocardiography was the first choice of diagnostic modality, confirmed in some cases by cardiac magnetic resonance imaging (CMR), using the European society of cardiology's diagnostic criteria for LVNC.

**Results.** The spectrum of initial clinic presentation of the patients was wide and non-specific, 56.2% of them presented signs of heart failure symptoms, 18.75% arrhythmias or intraventricular conduction delay and 25% were asymptomatic or paucisymptomatic. Pathologies found associated most frequently were: left bundle branch block in 37.50% of patients, echocardiographic dilated left ventricle, pericardial effusion and severe arrhythmias. Echocardiography and CMR were used to evaluate the enlargement of the non-compacted myocardium, the presence of fibrosis or apical thrombus. All patients underwent the standard heart failure therapy, anticoagulation therapy, one needed cardiac resynchronization therapy and three of them an implantable cardioverter-defibrillator with satisfying overall outcomes in all patients: reduced symptoms, no thromboembolic events or new arrhythmias occurred.

**Conclusion.** The study brings in attention the heterogeneous clinical presentations of patients with LVNC, correlates the severity of symptoms with the enlargement of non-compacted area and proves the importance of adequate therapeutic management in patients outcomes.

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# RELATIONSHIP BETWEEN AORTIC VALVE CALCIFICATION TYPE AND THE PROGRESSION RATE OF AORTIC STENOSIS IN PATIENTS WITH CHRONIC RENAL FAILURE AND CHRONIC HEMODIALYSIS

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**Introduction.** The progression of degenerative aortic stenosis in dialysis patients is known to be faster than in the general population, but without fully clarifying the determinants. Assuming that calcifications of the commissures have a much greater impact on the morphology of the aortic orifice than calcifications of cusps we wanted to clarify whether the degree and type of valvular calcification influence the rate of progression of aortic degenerative stenosis.

**Material and methods.** A prospective study was conducted on a total of 17 patients with chronic renal failure and chronic hemodialysis diagnosed with degenerative aortic stenosis or aortosclerosis. Patients underwent echocardiographic examination of the aortic valve. Correlations were made between the aortic orifice physical parameters (the degree and type of valvular calcification) and the rate of progression of degenerative aortic stenosis (rate reduction of the aortic orifice area -  $\Delta\text{aria}\%$ , and increasing of peak aortic velocity -  $\Delta\text{Vmax}$ ).

**Results.**  $\Delta\text{aria}\%$  was strongly correlated with the number of calcific commissures ( $p<0.0001$ ,  $r=0.809$ ) and with total valve calcifications ( $p<0.0001$ ,  $r=0.756$ ) and did not correlate with the number of calcific leaflets.  $\Delta\text{Vmax}$  was strongly correlated with the number of calcific commissures ( $p=0.024$ ,  $r=0.544$ ) and with total valve calcifications ( $p=0.018$ ,  $r=0.564$ ) and did not correlate with the number of calcific leaflets.

**Conclusion.** The rate of progression of degenerative aortic stenosis in patients with chronic renal failure receiving hemodialysis was correlated with the presence of calcifications in valve commissures and with the degree of valvular calcification and was not correlated with the location of the calcifications in the aortic cusps.

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## DIAGNOSTIC DIFFICULTIES IN A CASE OF AUTOIMMUNE DISEASES ASSOCIATION

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**Introduction.** Autoimmune diseases have an unpredictable development potential. Association of autoimmune diseases in the same patient, which happened frequently in recent years, may have a bad prognosis.

**Material and method.** We tried to identify the etiology of hepatic cytolysis syndrome at a 6-year-old girl. The patient did not present troublesome symptoms. The patient presented to the doctor for an external facial paralysis at which point the hepatic cytolysis syndrome was highlighted.

**Results.** Laboratory investigations ruled out viral hepatitis HBV, HAV, HCV, HDV, CMV, and EBV. Autoimmune hepatitis was initially ruled out by lack of inflammatory syndrome, Ac-ANA negative, and p-ANCA negative. Transglutaminase antibodies were negative. From 6 to 10 years the patient was investigated by medical services in Italy, but etiology has not been elucidated. At 10 years old the patient back in the in Cluj-Napoca, Romania and new investigations were made. Transglutaminase antibodies are currently weak positive. Abdominal ultrasonography shows the presence of liver cirrhosis. Parents refused liver and duodenal biopsy, investigations conducted afterward in Italy. Liver biopsy indicates the presence of autoimmune hepatitis in cirrhotic stage with fibrosis degree IV. Duodenal biopsy shows the presence of celiac disease stage 3C Marsh. Patient starts the gluten-free diet. Drug therapy includes cortisone and Imuran. Hepatic cytolysis syndrome evolution is favorable. At 12 years old the patient returns for investigation, at which point can be detected the presence of an autoimmune thyroiditis.

**Conclusions.** The negative serology makes early diagnosis difficult. The combination of three autoimmune diseases in the same patient can lead to a bad prognosis. Association between celiac disease and autoimmune hepatitis can lead to negative serology for both diseases.

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## THE QUALITY OF LIFE - AN INDICATOR OF FAIR TREATMENT OF ALLERGIC RHINITIS IN ADOLESCENTS

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**Background.** The current concept of quality of life reported to health status is described by patients in relation to personal expectations. Quality of life is measured using individual questionnaires. These questionnaires are multidimensional and cover multiple aspects such as physical condition, emotional, social and cognitive status.

**Material and method.** The study was performed on a total of 42 adolescents (aged between 12 and 18 years) admitted to a university children's hospital in the period between 1 January 2013 and 31st December 2014. We performed a prospective observational study. Medical records contain written consent of the parents regarding investigations and therapy. To assess quality of life we used five symptoms score and visual analog scale. Statistical processing was performed by Student's t-test.

**Results.** Regarding living conditions 20% of parents indicated that they have pets (dog or cat). Depending on severity score, patients were divided into two groups: 26% of patients with mild persistent allergic rhinitis and 74% of cases with moderate-severe persistent allergic rhinitis. After the first week of treatment, 80% of the 31 patients with moderate-severe persistent allergic rhinitis recognized a net improvement of symptoms, with a good quality of life without affecting daily activities and sleep. 10% of the 31 patients with moderate-severe persistent allergic rhinitis continued to maintain the source of allergens (cats and dogs) in the living environment. 7% of patients have not regularly administered treatment, being without family support.

**Conclusions.** Patients' quality of life depends on the time of diagnosis, the promptitude of establishing treatment and allergen avoidance. Moderate-severe persistent allergic rhinitis significantly affects the quality of life. The quality of life is more affected as well as the total score of symptoms is higher.

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## DIAGNOSTIC PROBLEMS OF THROMBOTIC THROMBOCYTOPENIC PURPURA IN A PATIENT WITHOUT THE CHARACTERISTIC CLINICAL PRESENTATION

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Thrombotic Thrombocytopenic Purpura (TTP) is a disease characterized by a congenital or acquired deficiency of the protein von Willebrand factor cleavage called ADAMTS13, which is manifested by the "pentad": microangiopathic hemolytic anemia, thrombocytopenia, neurological manifestations, renal failure and fever.

Our patient, female, aged 33 years old, without significant personal disease history came to the hematological service accusing asthenia, fatigue, bruising to the legs and chest in the absence of trauma. On admission with easily influenced general state, skin and scleral jaundice. Paraclinically normochromic normocytic anemia (Hb=7.5 g/dL), marked reticulocytosis (281.9‰) with schistocytes on the blood picture, severe thrombocytopenia (12,000/mm<sup>3</sup>). In the biochemical evidence LDH=3047 U/L and significant mixed hyperbilirubinemia with predominantly indirect bilirubin.

In the absence of clinical features specific to TTP (neurological signs, fever and renal failure), a differential diagnosis with paroxysmal nocturnal hemoglobinuria (test HAM negative), a neoplastic cause (negative markers and non-suggestive imaging), with collagen diseases (nonspecific immunological profile), valvular heart disease (echocardiography in normal relations) with infectious causes (negative markers) and Evans syndrome (negative immunological tests), disseminated intravascular coagulation was made. ADAMTS13 activity assay revealed a downturn of this protein, the presence of anti-ADAMTS13 antibodies and ADAMTS13 antigen decline confirmed the diagnosis of TTP. Further therapy by plasmapheresis was initiated with the normalization of the hematological profile.

PTT clinical expression may be less suggestive, with the consequence of delaying specific treatment for a disease whose mortality rate is about 90% without treatment.

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## CAFFEINE-INDUCED BEHAVIORAL CHANGES AND OXIDATIVE STRESS IN OVARECTOMIZED RATS

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**Introduction.** Menopause triggers many pathological reactions, being associated with enhanced oxidative stress and behavioral changes. Caffeine was claimed to have antioxidant and neuroprotective effects. The aim of this study was to evaluate the effects of caffeine (6 mg/kg b.w.) administration on oxidative stress and ovariectomy induced behavioral changes, in female rats.

**Material and methods.** The effects of subchronic and chronic caffeine administration were evaluated in two experimental subsets. Caffeine and vehicle were orally administered for 21 (subset 1), respectively for 42 days (subset 2). Two groups underwent sham-surgery and then received vehicle (CON) or caffeine (CAF). The other two groups underwent bilateral ovariectomy and vehicle (OVX) or caffeine (OVX+CAF) were administered. At the end, behavioral tests were performed and oxidative stress biomarkers were assessed in serum and brain tissue homogenate.

**Results.** The subchronic administration of caffeine decreased lipid peroxidation and improved antioxidant defense in blood and brain ( $p=0.01$ ). Furthermore, the enhanced nitric oxide level in brain after ovariectomy was significantly reduced in subchronic treatment ( $p=0.03$ ). The chronic administration decreased the oxidative stress in blood ( $p<0.01$ ). In brain, non-enzymatic antioxidant parameters were improved ( $p<0.01$ ), the activities of antioxidant enzymes were reduced ( $p<0.01$ ) and peroxidation of lipids ( $p=0.01$ ) and nitric oxide synthesis significantly increased ( $p=0.02$ ). The subchronic administration reversed the diminished locomotor activity ( $p<0.001$ ), but no change was observed in chronic treatment ( $p>0.05$ ). Moreover, the chronic caffeine administration had significant anxiolytic effects sustained by increased time spent in open arms ( $p<0.001$ ).

**Conclusions.** The administration of low doses of caffeine for an appropriate period of time may be a new therapeutic approach to control oxidative stress, locomotor activity and anxiety in menopause.

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## KNOWLEDGE PRODUCTION DURING PHD GRADUATE STUDIES IN MEDICINE: A CASE STUDY

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**Introduction.** Article publication rate may be used to define a scholar's merit or to measure the research proficiency of a graduate student. Our aim was to assess the scientific output of articles published in journals with impact factor during PhD studies, as reflected in medical PhD theses defended in 2010 and 2013 in a Romanian higher education institution.

**Material and methods.** Free full-text PhD theses were acquired from the institution's library. Data for each thesis-related article was collected from the thesis, and checked on Web of Science and journal websites. All articles published or accepted for publication during PhD candidature were included in the analysis. The articles related to 81 theses defended in 2010 and 77 theses defended in 2013 were analyzed.

**Results.** Only 18.45% (n=151) articles from 2010 were published in Thomson ISI journals with impact factor, a significantly smaller percent age than the 34.17% (n=240) articles from 2013 (Z-statistic: p-value <0.001). The doctoral student was first author in 67.86% (2010) and 79.27% (2013) respectively, of the articles published in ISI journals with impact factor. The median of impact factor of articles from 2010 was 1.90 (IQR 1.30-3.05), while for 2013 was 1.25 (IQR 0.67-2.26). Articles from theses defended in 2013 exhibited a decreasing rank trend (% Q1:Q2:Q3:Q4; 2010 - 25.00:28.57:29.27:25.00 %; 2013 - 13.41: 18.29: 29.27: 39.02 %), without any significant difference from those in 2010 (p-value >0.15). The 21.43% of articles from 2010 published in Romanian ISI journals with impact factor was significantly smaller than the 48.78% of those from 2013 (p-value <0.01).

**Conclusion.** Medical PhD students proved to have increased their thesis-related articles in ISI journals with impact factor over time, the publication in Romanian journals increased as well, while the journals' ranking suffered no statistically significant modification.

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## STUDY OF APOPTOSIS AND ANGIOGENESIS FOLLOWING MESO-PORPHYRIN MEDIATED PHOTODYNAMIC THERAPY ON IN VITRO MELANOMA MODELS

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**Introduction.** Based on previous data, photodynamic therapy (PDT) shows promise as an adjuvant therapy in melanoma, especially in advanced cases. Melanoma vasculature is involved in neoangiogenesis, inflammation, that lead to further invasion, migration and metastasis.

**Aims.** To study the effects of PDT mediated by a derivative of tetraphenylporphyrin: meso-5,10,15,20-tetrakis (4-hydroxyphenyl) porphyrin (THOPP) on apoptosis, angiogenesis and inflammation on melanoma models in vitro.

**Material and methods.** Contact co-cultures of human endothelial cells (HUVEC) with three melanoma cell lines, respectively: human radial growth phase WM35, metastatic M1-15 and murin B16-F10 were established. Cells were then irradiated with 630 nm wave length, dose 6 J/cm<sup>2</sup> (Philips LED lamp). Cytotoxicity was measured by colorimetry. Flow cytometry with Annexin V-FITC/Propidium iodide markers, active caspase 3 protein levels (ELISA) were determined for cell death assessment. Measurements of sICAM1, VEGF, TNF $\alpha$  (ELISA) and NF $\kappa$ B (Western Blot) were used for angiogenesis and inflammation evaluation.

**Results.** THOPP was an efficient photosensitizer, in all melanomas, without dark toxicity. The mechanism of cell death induction was mainly apoptosis. sICAM1, a marker of cell activation was increased following PDT in WM35 and M1-15 co-cultures, while in B16-F10 it remained unchanged. TNF $\alpha$  was increased following PDT in B16-F10 and M1-15, but not in WM35. PDT decreased NF $\kappa$ B in all co-cultures, while the active form, pNF $\kappa$ B was only decreased in B16-F10. Angiogenesis marker VEGF was increased in all co-cultures, compared to melanoma cultures alone. PDT decreased VEGF production by almost 5x in all co-cultures, with a maximum of almost 19 x decrease in B16-F10.

**Conclusion.** PDT mediated by THOPP was efficient in inducing melanoma cell killing and inhibited angiogenesis. The best results in what concerns tumor cell killing and angiogenesis inhibition were obtained in B16-F10 murin pigmented melanoma.

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## THE EFFECT OF NEW ANTIEPILEPTIC DRUGS ON ANXIETY IN RATS

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**Introduction.** The anticonvulsants are a diverse group of drugs used in the treatment of epileptic seizures. This paper aims to study the possible effect of levetiracetam and pregabalin on anxiety and spontaneous behavior of animals.

**Materials and methods.** 60 Wistar Bratislava rats included in the study were randomized to form 6 homogenous groups of 10 rats. We had a control group groups treated with saline (0.5 ml/100 g), 2 groups treated with levetiracetam (5 mg/kg, 50 mg/kg), 2 groups treated with pregabalin (15 mg/kg, 30 mg/kg) and one group treated with a standard anxiolytic drug, diazepam (1 mg/kg). Two simple tests were chosen to assess a potential anxiolytic effect, open field test (OF) and elevated plus maze test (EPM). The data were statistically analyzed, with a significance at  $p < 0.05$ .

**Results.** Pregabalin 15 mg/kg significantly increased the number of entries in all parts of EPM, compared to saline and diazepam groups. There is a significant difference between pregabalin doses regarding the entries into the open and center parts of EPM. Levetiracetam has a significant increased number of entries in all parts of EPM, especially at highest dose. In the OF test levetiracetam reduces exploratory tendency at high dose compared to control and diazepam. In EPM pregabalin both doses and levetiracetam the highest one significantly increase the motility compared to control group. In OF test only pregabalin 30 mg/kg increases the motility compared to control group. Levetiracetam produces a decrease exploration in both doses compared with control group.

**Conclusion.** Pregabalin 15 mg/kg causes an anxiolytic effects in experimental animals. Pregabalin 30 mg/kg dose has a sedative effect. Levetiracetam 5 mg/kg and 50 mg/kg have anxiolytic effect. Pregabalin anxiolytic effect is dose dependent. The anxiolytic effect of levetiracetam is inconstant in EPM and OF tests.

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## CHOOSING THE RIGHT MEDICAL CAREER - A PILOT STUDY

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**Introduction.** The constant progress in medical sciences contributes to a rapid and sustainable development of medicine. Given this conditions, new medical specialties emerge, new subspecialties appear. Nowadays, choosing the right medical career can be even harder than choosing to study medicine at all. Due to the numerous specialties and opportunities, graduate medical students face one of their most important decisions - what medical field would they work in? Up to 20% of graduate medical students have not considered choosing a medical specialty at all. Others simply choose specialties that appear very attractive, and mainly based on what people will think of them. Results: All these being stated, we conducted a pilot study which aimed at identifying the main reasons why students choose a specialty, what personality traits appear to be more common when choosing a certain medical specialty, and we developed a program to help medical students to better understand the importance of choosing wisely their future profession, in order to become competitive and empathic physicians.

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## GREAT VALUES OF OLD MEDICAL BOOKS AT THE “IULIU HAȚIEGANU” UNIVERSITY OF MEDICINE AND PHARMACY

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**Introduction.** The existence of numerous medical books published in the 17th and 18th centuries in the History of Medicine collections of the Department of Practical skills - Humanities represents a very valuable heritage, not only by the Romanian specific criteria, but also by the international recognition.

**Study Material.** Examination of more than 1,500 old medical books in the History of Medicine collection allowed us to make an assessment of their importance and value. Beside their remarkable iconography, these volumes have a significant value as medico-historical source of documentation.

**Results.** The collection contains numerous books in original editions, which represents a valuable treasure. Hippocrates and Galen's works gives a symbolic value for this collection. This series of old medical book contains volumes from the Renaissance, elaborated by: Paracelsus, Andreas Vesalius, Ambroise Paré, Jean Fernel etc. It also includes volumes written by William Harvey, Marcello Malpighi, Jan Baptista van Helmont, Thomas Sydenham etc. Besides these books, there are volumes of the eighteenth century, written by: Carl von Linné, Albrecht von Haller, Pierre Fauchard etc. The fact that these books are written in 8 languages: Latin, Ancient Greek, old French, old German etc. represents a treasure also from a linguistic point of view. The appearance of these volumes, as well as the variety of their sizes – from 11/7 cm format up to 80/50 cm, the various types of illustrations, paper and bookbinding led to the conclusion that these books are precious jewels.

**Conclusion.** The presence of a medical priceless treasure of old books at the Iuliu Hațieganu University of Medicine and Pharmacy requires a thorough care in the preservation and the restoration of the entire collection, as well as for its scientific and exposing valuing.

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## CONSEQUENCES OF INTRACEREBROVENTRICULAR ADMINISTRATION OF ENALAPRIL ON CARDIAC ARRHYTHMIAS BY CENTRAL MECHANISM

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**Introduction.** Cardiovascular regulation occurs at the central level and involves the cortex, diencephalon structures, brainstem and spinal cord. It demonstrated the possibility of cardiac arrhythmias by electrical or chemical stimulation of the hypothalamic nuclei or areas around the lateral cerebral ventricles. In this paper, we investigated the effects of enalapril on central arrhythmogenesis. For this purpose we injected intracerebroventricularly (icvt) enalapril in different doses, randomly selected. I watched whether or not the administration of enalapril affects production of cardiac arrhythmias produced by intracerebroventricular injection of sodium glutamate.

**Material and method.** Experiments were performed acutely on white rats, Wistar, weighing 100-180 g range, coming within Biobase "Iuliu Hatieganu" in Cluj-Napoca. Experimental animals were anesthetized with ethyl urethane, 1.25 g/kg given intraperitoneally. Enalapril and sodium glutamate - arrhythmogenic substance was administered the lateral cerebral ventricles through with Kovacs after stereotaxic device's coordinates from the Atlas Szentagothai. The animals were maintained on artificial respiration.

**Results.** We followed heart rate and index arrhythmogenic. The data were statistically analyzed by the Wilcoxon test ( $p \leq 0.05$ ). Sodium glutamate injected icvt causes cardiac arrhythmias and ventricular extrasystoles type bradyarrhythmias. Administered at 25 micrograms/10 microL significantly decreases heart rate and confers protection against arrhythmias caused by sodium glutamate. Doses 12.5 and 6.25 micrograms/10 microL produce ventricular premature beats decreased heart rate but reappear after administration of sodium glutamate.

**Conclusions.** High dose of Enalapril, significantly decreases heart rate and gives protection against cardiac arrhythmias produced by the central administration of sodium glutamate.

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## ULTRASTRUCTURAL CHARACTERIZATION OF POLLEN PARTICLES: POTENTIAL USES IN CRIMINAL INVESTIGATIONS

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**Introduction.** In criminal investigations, the Locard exchange principle (“Every contact leaves a trace”) holds that the perpetrator of a crime will bring into and/or take out of the crime scene traces that can be used as forensic evidence. Demonstrating correlations between suspect, victim and the crime scene may provide important clues for on-going investigations. Crime scene investigation therefore includes thorough examination of microscopic materials (microtraces). This study highlights potential uses of pollen microtraces in forensic investigations.

**Materials and methods.** Pollen particles from nine plant species were collected in the geographical area surrounding the city of Cluj-Napoca. Identification and ultra-structural characterization of the pollen particles was performed using a Jeol JSM 25S scanning electron microscope (Jeol, Japan). Morphometric analysis was performed with a Cell<sup>^</sup>D software (Olympus Soft Imaging Solutions GmbH, Germany).

**Results and discussion.** Ultra-structural analysis exhibited species-depending differences concerning shape and dimensions of the pollen particles. It was noted that all particles were larger than 10 micrometers. Such particles are generally being detained in the nasal mucosa, this facilitating their harvesting and consequent uses in the forensic investigation. Different plant species release pollen granules from the stamens at different times during the day and the varying number of particles collected from the crime scene can provide useful information concerning the timeframe in which the crime occurred.

**Conclusions.** Identification of pollen particles on the crime scene may provide additional indications to help forensic investigations.

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# STUDENTS' LIFESTYLE AND BEHAVIORS: THE USE OF SCREENS AND THE INTERNET

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**Introduction.** The inappropriate use of the computer systems can cause serious immediate or delayed health consequences. The aim of this study was the description of the manner of the screens and Internet use among students in order to set up an appropriate prevention program.

**Material and methods.** We used the questions regarding the use of screens and the internet from the online questionnaire of the "Evaluation of the students' lifestyle and behaviors" (ESTICOST) study. The survey is addressed to students of "Iuliu Hatieganu" University of Medicine and Pharmacy being invited to participate voluntarily. The database was in Excel and the statistics in Epiinfo.

**Results.** In 2015, the first year of the study, 81 students filled the questionnaire. In this group of students, 72.84% [CI95%: 61.81-82.13] were female, with the age between 19 and 26 years old, 43.21% [CI95%: 32.24-54.69] of them living in couple. The use of screens and Internet for at least 32.1% [CI95%: 22.15-43.4] of students has led to neglecting the household chores and for 6.17% [CI95%: 2.03-13.82] were affected their grades or school work. The amount of sleep was seriously affected for 19.75% [CI95%: 11.73-30.09] of students. the depression was induced for 3.7% [CI95%: 0.77-10.44] of them and 11.11% [CI95%: 5.21-20.05] feel a boring, empty and joyless life without internet. Trying to reduce the time spend on internet was exercised by only 9.88% [CI95%: 4.36-18.54] of students and without success.

**Conclusion.** the excessive use of screens and Internet affect the student's educational performances, caused sleep disorders, sometimes depression but few of students try to change their style of screen and internet use.

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## EXERCISE TRAINING ASSOCIATED WITH QUERCETIN RESTORES DIABETES-INDUCED VASCULAR DAMAGE IN RAT ARTERIES

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**Background.** Diabetes is an endocrine-metabolic disorder associated with increased risk of cardiovascular diseases due to vascular dysfunctions caused by an impairment of endothelium-dependent relaxation (EDR). Quercetin is a natural flavonoid with multiple pharmacological effects including reducing oxidative stress and improvement vascular function. Exercise training has the effect in restoring endothelial function to diabetics by inhibiting inflammation and oxidative/nitrosative stress and by restoring NO bioavailability in vessels wall. Objectives: The aim of the present study was to investigate the synergistic effects of exercise training and Quercetin in restoring diabetes-induced vascular damage in rat arteries.

**Materials and methods.** Diabetic rats that performed exercise training were subjected to a swimming training program (1 hour/day, 5 days/week, 4 weeks). The diabetic rats received Quercetin (30 mg/kg body weight/day) for 4 weeks. At the end of the study, were performed Echo-Doppler evaluation of carotid arteries and thoracic aortic rings were isolated from all experimental rats. The effect of exercise training associated with Quercetin on carotid arteries elasticity and EDR in response to acetylcholine in isolated phenylephrine-precontracted aortic segments in the presence of indometacin was studied.

**Results.** In sedentary untreated diabetic rats EDR was significantly decreased - maximal relaxation (% of KCl) of aorta and cause morphological and functional changes in carotid arteries. Exercise training associated with Quercetin restored normal EDR in aortic segments and elasticity of carotid arteries from diabetic rats.

**Conclusion.** These findings suggest that moderate exercise training in association with Quercetin treatment protects vascular endothelial function in diabetic rats. Acknowledgement: This research was supported under the contract funded by the University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania, through the internal grant numbered 1494/8/28.01.2014.

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## ELECTRONIC CIGARETTE USE AMONG ROMANIAN ADOLESCENTS

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**Introduction.** Electronic cigarettes are devices that deliver to the lung vapors usually containing nicotine and other chemicals. Electronic cigarettes (e-cigarettes) are promoted as an alternative to traditional tobacco cigarette smoking. However, scientific studies about e-cigarette safety and efficacy remain limited. Moreover, a public health concern is that the use of these products may increase the risk of non-smokers developing nicotine dependence and of current smokers maintaining their dependence.

**Material and methods.** This study has two objectives. First, it aims at assessing awareness, opinions and practices regarding electronic cigarettes (e-cigarettes) use among senior high school students from Romania. Second, the study will investigate the factors associated with experimentation with e-cigarettes among the study sample.

A cross-sectional study was conducted using anonymous questionnaires in May 2013 in two big towns from Romania. It involved 342 senior high school students aged 16-18.

**Results.** The majority of the students (93.9%) reported having heard about e-cigarettes. A percentage of 52.3% of the smokers, 29.2% of the ex-smokers and 8.7% of the non-smokers tried e-cigarettes at least once during their lifetime; 7.8% of the smokers and 4.6% of the ex-smokers used e-cigarettes in the last month, but not the non-smokers.

The results of the bivariate correlation show that, among smokers, e-cigarette experimentation was associated with lower participation in health education activities about it, having parents and siblings using e-cigarettes and smoking higher numbers of cigarettes/day. Among ex-smokers and non-smokers e-cigarettes experimentation was associated with intention to use e-cigarettes in the next year and with having peers using e-cigarettes.

**Conclusion.** The study underlines that health education programs and regulatory interventions addressing e-cigarettes are needed in Romania.

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## MEDICATION ERRORS IN THE TREATMENT OF PATIENTS HOSPITALIZED IN NON ICU DEPARTMENT. CAUSES. THE CLINICAL PHARMACOLOGIST'S ROLE

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**Introduction.** The medication errors can have the negative consequences either on the patients or on the costs in health system. The factors which generate costs can be the medication errors: inadequate dose (F1), untreated problem (F2), drug-drug interactions (F3), useless drug (F4), wrong administration route (F5).

**Aims.** Our aims were to identify the possible causes which lead to the appearance of these errors and to distinguish a pattern of association between causes and factors.

**Material and methods.** A clinical pharmacologist evaluated the drug therapies for 98 patients hospitalized into a non – ICU gastroenterology department of Emergency County Hospital Cluj Napoca, Romania. We tried to identify the causes which led to appearance of the medication errors for these patients.

These causes can appear: before making a decision linked to the patient's drug therapy (C2), at the same time with decision-making for recommendation of the drug therapy (C4) or after therapeutic decision-making (C1,C3).

**Results.** The pattern watched in work hypothesis is confirmed: The absence C1, C2 and C3 is associated with the absence of F1, F2, F4 respective the presence C1, C2, C3 is associated with the presence F1, F2, F4.

**Conclusion.** We can affirm, as a consequence of the obtained results: the producing of the errors (F1-F4) is in direct casual link with the kind of communication of the physician with patient, medical team and monitoring the patient. So, there is a direct causal relation between the cause of error and factor of the error. The clinical pharmacologist, by watching some rules of drug prescription, communication rules and respecting these by the members of the medical team, can reduce significantly the drug errors situations determined by the studied causes.

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## EVALUATION OF HAIR REGROWTH EFFECT OF MINOXIDIL 2% BY USING A CROSS-SECTION TRICHOMETER IN A RAT MODEL OF ALOPECIA

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**Introduction.** Minoxidil 2% and 5% are, so far, the only FDA (United States Food and Drug Administration) approved topical treatments of alopecia. There are no standardized assessment methods of hair regrowth in vivo and there is a great need of an accurate quantitative method, such as the cross-section trichometer.

Our study has investigated the hair regrowth effect induced by two different regimens of Minoxidil 2%, topical application (daily usage versus 3 times/week). The purpose of our research was to evaluate hair regrowth by different noninvasive methods and to assess the correlations between the cross-section trichometer (CST) and other investigation methods applied: Macroscopic (MA), Trichoscopic (TR) and Hair weight (HW).

**Material and methods.** 24 Wistar female rats were assigned into three groups, following hair removal on both sides of the spine. The right patch served as test area and the left functioned as control.

**Results.** All the evaluation methods applied proved that a longer exposure to Minoxidil 2% by daily application (Group I) induced better hair regrowth effect than the 3 times/week regimen (Group II), and both produced a significant effect in contrast to Group III (control). Our results suggest a good/excellent correlation between the MA and TR aspect of hair regrowth in the tested animals ( $p < 0.01$ ). We did not notice any statistical significant correlation between MA and CST, and between HW determination and CST respectively ( $p > 0.05$ ).

**Conclusion.** Our study shows the superior effect of Minoxidil daily regimen also indicating that CST evaluation provides a new way of measuring hair regrowth in a quantitative manner by determining the hair mass in the studied area.

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## THE CYTOTOXICITY PROFILE OF GRAPHENE-BASED NANOMATERIALS ON HUMAN DENTAL FOLLICLE STEM CELLS. IN VITRO STUDIES

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**Introduction.** Graphene and graphene derivative nanoplatelets represent a new generation of nanomaterials with unique physico-chemical properties and high potential for use in dental composite materials. The objective of this study was to assess the cytotoxic potential of graphene nanoplatelets on a line of dental fibroblasts.

**Material and methods.** Graphene-oxide (GO) and its most encountered derivatives, thermally reduced graphene oxide (TRGO) and nitrogen-doped graphene (N-Gr), were synthesized and structurally characterized by spectroscopic techniques, like Raman and <sup>13</sup>C MAS solid state NMR. Several biological effects: cytotoxicity (MTS and Cell Titer blue), oxidative stress induction (DCDHF-DA and malondialdehyde levels), and cellular and mitochondrial membrane alterations) induced by such graphene-based materials on human dental follicle stem cells, were investigated..

**Results.** Graphene oxide shows the lowest cytotoxic effect, followed by the nitrogen-doped graphene, while thermally reduced graphene oxide exhibits high cytotoxic effects. Graphene oxide induces oxidative stress without causing cell membrane damage. Nitrogen-doped graphene shows a slight antioxidant activity; however, at high doses (20 and 40 µg/ml) it causes membrane damage.

**In conclusion** both graphene oxide and nitrogen-doped graphene seem to be valuable candidates for usage in dental nanocomposites.

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## PREVENTION OF PERIPHERAL BLOOD SAMPLE HEMOLYSIS IN PEDIATRIC PATIENTS

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**Introduction.** Specimen Peripheral venous blood is daily practice in healthcare units. Hemolysis of the blood sample can lead to inaccurate results and repeat blood collection → pain treatment decisions wrong and the increase of hospitalization days. Hemolysis is identified by determining the serum K.

**Material and methods.** Objectives: Investigating factors that relate to hemolysis among pediatric patients in Pediatrics III, Cluj-Napoca. Research design: transversal correlational study The dependent variable: the serum K The independent variables: age, anatomical location, the time of application of the tourniquet, the technique. Selection Procedure: group was selected from patients with sheets day of Pediatrics III, Cluj, December 2014-March 2015. The characteristics of the document are: age, anatomical location, the time of application of the tourniquet, the technique. Measuring Tools The value of serum potassium. The storage time of tourniquet. Total duration technique.

**Results.** The results of this study showed that significantly influence the age variable amount of potassium, The results of this study showed that the total duration of the technique significantly influences the variable value of potassium. The independent variable called for keeping tourniquet is statistically significant. The results of this study showed that the total duration of the technique significantly influences the variable value of potassium. This analysis statistically enabled the identification of differences between mean scores of potassium value, thus maintaining said variable length tourniquet influences more than the total length harvesting technique.

**Conclusion.** The results show a statistically significant correlation between the amount of potassium and age, the duration of tourniquet and the total time required harvesting technique

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## EVALUATION OF THE SENTINEL LYMPH NODE STATUS IN CUTANEOUS MALIGNANT MELANOMA

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**Introduction.** Malignant melanoma(MM) is one of the most aggressive tumors affecting humans. The assessment of the sentinel lymph node (SLN) status brings valuable information regarding the prognosis of patients and the therapeutic approach. In the current study we assessed the incidence of sentinel MM micrometastasis and the clinical and mainly the pathological risk factors considered predictors of SLN status in MM.

**Material and method.** The study group was comprised of 39 consecutive, retrospectively analyzed, cases diagnosed with cutaneous MM, for which the assessment of SLN was performed in the Pathology Department of the Emergency County Hospital in Cluj-Napoca, between 01.07.2012- 31.12.2014. The cases were processed according to the Department's protocol for assessing SLN status. HE stains and immunohistochemical stains were used to identify MM cells.

**Results.** Patient age ranged between 24 and 76 years with an average age of 54.23 years. Most cases (28) were in T2 and T3 stages. The clinical risk factors patients with the age of 30 years or less had a significant higher risk of having SLN metastasis. When considering pathological risk factors the Breslow index (BI) and the presence of lymphatic emboli (LE) have maintained their prediction capability in the current limited series. The detection rate of micrometastasis in the study group was of 21% (8 cases). The overall incidence of SLN metastasis was 35.89%.

**Discussion.** Several risk factors are currently proposed as predictors of SLN status and these include age, sex and location of the primary MM(clinical) and BI, ulceration, microsatellitosis, angiolymphatic invasion, mitotic rate, tumor infiltrating lymphocytes, and regression(pathological). The limitations of the study regard the study group size and the limited clinical data and the availability of the information regarding the primary tumor.

**Conclusions.** BI and LE maintain their prediction value in the current limited study group.

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## STEM CELLS DIFFERENTIATION INTO INSULIN-SECRETING CELLS USING TWO NANOPOLYOXOTUNGSTATES

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**Introduction.** Finding innovative methods of treatment of diabetes is a challenge as this chronic disease is the third leading cause of mortality in the world. In this in vitro study, two polyoxotungstates were used to quantify stem cells differentiation into insulin-secreting cells.

**Materials and methods.** Ligands  $\text{Na}_9[\text{SbW}_9\text{O}_{33}] \cdot 19.5\text{H}_2\text{O}$  (L1),  $(\text{NH}_4)_{17}\text{Na}[\text{NaSb}_9\text{W}_{21}\text{O}_{86}] \cdot 14\text{H}_2\text{O}$  (HPA-23, aka L2) and the  $\text{K}_{11}\text{H}[(\text{VO})_3(\text{SbW}_9\text{O}_{33})_2] \cdot 27\text{H}_2\text{O} = \text{PM-1002}$  polyoxometalate (POM1) were prepared according to literature data. The butyltin complex  $(\text{NH}_4)_4(\text{NBu}_4)_5[\text{Na}(\text{BuSn})_3\text{Sb}_9\text{W}_{21}\text{O}_{86}] \cdot 17\text{H}_2\text{O}$  (POM2), with a original formula was first prepared and characterized by us. All nanocompounds were analyzed by UV, FT-IR spectroscopy and transmission electron microscopy (TEM). In addition, POM2 was characterized by multinuclear NMR spectroscopy at room temperature. In this in vitro study, human bone marrow adult mesenchymal stem cells (M-MSCs) and human umbilical vein endothelial cells (HUVECs) were used for determining POMs cytotoxicity by MTT assay, while the POMs' capacity to enhance stem cells' differentiation into insulin-secreting cells was tested on human amniotic membrane adult mesenchymal stem cells (A-MSCs).

**Results.** A 9  $\mu\text{g/mL}$  concentration for both POMs (the highest non-toxic dose for M-MSCs by MTT assay) was established for in vitro differentiation experiments. Taking into account the chemical composition of the 2-4 nm POMs, the more potent stimulating effect manifested by POM1 was due to the  $(\text{VO})_2^+$  vanadyl ions in its structure and to the metal's oxidation state ( $\text{V}^{4+}$ ,  $\text{W}^{6+}$ ). In the absence of vanadyl ions POM2 also exhibited significant effects on stem cells differentiation due to the oxidation state of W (VI) and the presence of organometallic butyltin fragments.

**Conclusion.** Based on our in vitro studies we concluded that both polyoxometalates with low dose-dependent toxicity exhibited significant biological activity and actively enhanced differentiation of stem cells into insulin-secreting cells.

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## HYPOGLYCEMIC EFFECTS OF TWO NANOPOLYOXOTUNGSTATES IN DIABETIC RATS

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**Introduction.** The number of patients with diabetes is expected to increase by 2035 to 592 million persons. We aimed to study the effects of two polyoxometalates (POMs) with insulin-mimetic potential in treating streptozotocin-induced diabetic rats.

**Materials and methods.** Two nanopolyoxotungstates,  $K_{11}H[(VO)_3(SbW_9O_{33})_2] \cdot 27H_2O$  (POM1) and  $(NH_4)_4(NBu_4)_5[Na(BuSn)_3Sb_9W_{21}O_{86}] \cdot 17H_2O$  (POM2), were used in the treatment of streptozotocin (STZ)-induced diabetic rats (by intraperitoneal injection of a single STZ dose, 50 mg/kg). Diabetes (blood glucose levels >200 mg/dL) was confirmed 48 h after STZ administration. Fragments of pancreas and liver were collected from the anesthetized rats. Ultrastructural analyses of pancreatic  $\beta$ -cells and hepatocytes were carried out by transmission electron microscopy (TEM). The diameter of secretory vesicles and insulin granules in TEM photographs of pancreatic  $\beta$ -cells were measured using Cell<sup>^</sup>D software.

**Results.** In STZ-induced diabetic rats treated with POMs (orally administered once a day for 3 weeks, up to a cumulative dose of 4 mg/kg bodyweight at the end of the treatments) post-treatment blood glucose levels were significantly reduced compared to untreated diabetic groups (DCGs). Ultrastructural analysis of pancreatic  $\beta$ -cells in the treated STZ-diabetic rats proved that POMs diminish STZ-triggered cellular degeneration, confirmed by the increased number of insulin-containing vesicles compared with the DCG. Ultrastructural aspects of the hepatocytes in the treated groups indicated that both POMs presented hepatoprotective properties.

**Conclusions.** Preventing STZ-triggered apoptosis of pancreatic  $\beta$ -cells and stimulation of insulin synthesis, vanadium-substituted POM1 showed higher hypoglycemic effects compared to POM2. Our polyoxometalates provided promising results as potential biologically active substances in the development of new diabetes drugs.

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## HYPERCHOLESTEROLEMIA AND HYPERTRIGLYCERIDEMIA IN THE PATIENT WITH CRITICAL LIMB ISCHEMIA AND AMPUTATION

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**Introduction.** Despite that advances in re-vascularisation and due to the absence of an agreement on definition of non-salvageable limb, amputation continue to be performed on patients with critical limb ischemia (CLI) even if its rate are declining. The aim of the present study was to assess if the serum cholesterol or triglycerids profile are different on CLI patients with or without amputation.

**Material and methods.** A retrospective study was conducted on CLI subjects hospitalized at Second Surgical Clinic, Clinical County Hospital Cluj-Napoca, from January 2010 to December 2014. There were included in the study all subjects with principal diagnosis as critical lower limb ischemia and their medical charts were reviewed.

**Results.** 634 patients with age from 32 to 95 years old (median = 66 years) were included in the study. A significantly higher percentage of subjects were male (M:F=79%:21%; p-value < 0.0001). Amputation was performed on 108 patients (17%). A significantly higher percentage of patients without amputation (72%) were smokers compared with patients with amputation (56%, p=0.0019). No significant differences were observed between percentage of CLI patients with (A+) and without (A-) amputation in regards hypercholesterolemia defined as values higher than 200mg/dL (37%:39%, p=0.6955) or hypertriglyceridemia defined as values higher than 150 mg/dL (36%:34%, p=0.6926). The median value of cholesterol was 213 mg/dL on subjects with amputation and 200 mg/dL on subjects without amputation. The median value of hypertriglycerides was 147 mg/dL on CLI subjects with amputation and of 152 mg/dL CLI subjects without amputation. No significant differences were identified between subjects with and without amputation, neither in regards of cholesterolemia (p=0.5105) nor in regards with trygliceridemia (p=0.9708).

**Conclusion.** Our study showed no differences in serum cholesterol or thryglyceridemia profile on critical limb ischemia subjects with and without amputation.

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## mHEALTH & mEDUCATION: EVIDENCE-BASED MEDICAL Apps

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**Introduction.** Development of knowledge and decision-science technology led to change the way that health professionals access, process, and use medical information. Moreover, mobile devices are more frequently used as support of both medical education and practice. The aim of this study was to identify and summarize the characteristics of medicinal apps that follow evidence-based medicine concepts.

**Methods.** The search for ‘evidence-based medicine app’ was conducted on Google on 28 October 2015. The first three pages of search results were used to identify and to summarize the EBM apps. The main feature collected was the aim (clinical /educational), the operation system (e.g. Android, IOS, Windows), the costs, and the users classification.

**Results.** 35 apps were included in the analysis. The majority of apps can be used for free (free:paid=77%:21%; p-value<0.001). 40% of the apps were ranked as 4 (out of 5) while 28% as zero. 62.5% apps rating with zero were paid apps. The apps with the highest rank were: iTriage - Symptoms, Doctors, Diseases, MedPage Today, ACC Guideline Clinical App, Daily Rounds, GoodRx, Figure 1 - Medical Image Sharing for Healthcare Professionals, Prognosis, and Prognosis: Cardiology. A high percentage of investigated apps comprise both diagnosis and treatment features (diagnosis:treatment=40%:54%; p=0.09). Apps providing prediction features were identified in a lower % (prediction:treatment=27%:54%; p=0.002). A significantly higher percentage of identified apps are clinical compared to educational apps (clinical:education=68.5%:8.5%; p<0.001). The most widely used operating systems are IOS and Android (both with the same percentage, 82%). Despite of this percentage, IOS is highly recommended for medical apps rather than Android. Windows is also used for 45% of applications but it is less recommended (Windows:IOS=45%:82%; p<0.001).

**Conclusion.** The most EBM apps are used for assistance of clinical practice in regards of both diagnosis and treatment.

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## SOCIAL MEDIA AS SOURCE OF MEDICAL INFORMATION FOR HEALTHCARE STUDENTS

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**Introduction.** The scope of the research was a more detailed understanding of the influence of social media and the importance of student's usage of social media context in relation to medical information. The research aimed to increase the understanding of social media and the impact on medical information use, informing policy and practice while highlighting gaps in the literature and areas for further research.

**Methods.** The search of PubMed database was performed in October 2015, using terms to identify peer-reviewed research in which social media technologies were an important feature for health occupations, premedical, pharmacy, nursing or medical students. A systematic approach was used to retrieve papers and extract relevant data.

**Results.** There were 435 initially identified studies involving social media, healthcare information and medical students subject headings (MeSH) terminology. Filtering for free full text articles, remained 118 results. Of these studies, 85 were excluded because they were not specific to medical students, or did not involve social media. There were identified 21 interventional and 12 observational studies. The majority of the studies in this systematic review were interventional studies that either assessed the outcomes of online discussion groups or teaching methods through social media. The majority of studies center on using social media as a teaching method, how students use it and the resulting implications on their education. The largest number of original research studies was identified as being published in 2013. Facebook, Podcasts, Multiplayer virtual worlds, Blogs, Twitter, and mixed social media platforms were identified as being used by medical students.

**Conclusion.** Social media is used as a tool of information for students mainly as the means for engaging and communicating with students.

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## MOOD STABILIZERS - A CHANCE FOR CUTTING COSTS FOR DEPRESSION

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Depression ranks third in the top diseases that generate costs, accounting for over 4% of the total expenses for illnesses. According to the forecasts of the World Health Organization, by 2030 depression will occupy the first place in terms of costs occurred by the disease. Thus, depression cannot be neglected, and it must be considered a real public health problem. If we add that almost one third of the patients diagnosed with depression do not respond optimally to specific antidepressant treatment, the picture is bleak. This paper attempts to identify possible new means to achieve combined management of depression through the mood stabilizer as an adjunctive medication for antidepressant therapy, with focus on new approaches to the phenomenology of depression. Given the fact that depression is commonly associated with irritability and personality disorders, mood stabilizers can contribute to improving the therapeutic success of these comorbidities and thus reduce the burden of disease with beneficial consequences in terms of public health.

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## WEB 2.0 TOOLS AS SUPPORT FOR PROBLEM BASED LEARNING

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**Introduction.** Problem Based Learning (PBL) is one of the alternative methods for the traditional medical education. This method has its advantages and limitation, but its added value is well recognized. PBL helps students to develop team working and collaboration. It is useful to have an IT solution to support the PBL process.

**Material and Methods.** We try to identify a Web 2.0 application which offers the facilities necessary for PBL specific: security, collaborative environment, availability, and easy to use. The application must be documented so the users could have a user guide. A PBL scenario was developed and a demo PBL session had place. The participants were students enrolled in first year at Faculty of Medicine, and the PBL topic was biostatistics.

**Results.** For its qualities, we use Moodle as support for PBL process. The implementation is available at the web address <http://web.umfcluj.ro/moodle/> The user manual was developed, and the PBL session was a success. The students had no problem using the on-line interface and they declared that it is a viable option to be used as support for PBL sessions.

**Conclusion.** Moodle seems to be an appropriate solution to be used as support for PBL. It is very important to recognize that Moodle is just an instrument which facilitates learning, but it is not a guarantee. The success of the PBL method lies in the involvement of the students, in the skills of the facilitator and in the quality of the scenario.

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## MUC-1 –GOLD NANOPARTICLES –THE ROAD FROM IDEA TO PROTOTYPE FOR IMMUNOPROPHYLAXIS OF COLON CANCER VACCINE

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**Introduction.** The usage of nanoparticle-based delivery systems using tumor associated antigens has become intensively researched lately. Mucin-1 (MUC-1) glycoprotein has been demonstrated to be over expressed and aberrantly glycosylated in more than 70% of colon cancers. However, it has been shown that tumor associated antigens remain often undetected by the immune system, due to their low immunogenicity. We have therefore reasoned that binding MUC-1 to gold nanoparticles could result in better antigen presentation and consecutive anti-cancer immune activation. The chosen type of nanomaterial has already demonstrated efficiency as basic structure of anti-cancer vaccine construct.

**Results.** We hereby present the MUC-1-GNP synthesis, characterization, along with the already fulfilled testing steps (in vitro, in vivo) for our construct.

**Conclusions.** Results represent encouraging premises for future optimization of vaccine formulation.

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## ADVANCED ENDOMETRIOSIS AND ASSOCIATED CHANGES IN SERUM LEVELS OF INTERFERONS, CHEMOKINES AND GROWTH FACTORS

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**Introduction.** Endometriosis is a common gynecological disease, characterized by ectopic deposits of endometrial tissue outside of the uterine cavity, being associated with pelvic pain and infertility. There is a controversy regarding pathophysiology of endometriosis and it seems that pro-angiogenic growth factors and chemokines might be involved, but their role is not completely understood. The objective of the current study was to evaluate the serum concentration of main growth factors, chemokines and interferons in patients with advanced endometriosis compared to healthy controls.

**Material and Methods.** A total of 157 women were included and divided into two study groups (Group 1 – endometriosis; Group 2 – healthy women). Serum levels of VEGF, G-CSF, GM-CSF, b-FGF, EGF, HGF, IFN- $\alpha$ , IFN- $\gamma$ , MCP-1, MIP-1 $\alpha$ , MIP-1 $\beta$ , RANTES, eotaxin, IL-8, MIG, IP-10, and IL-17 were measured with Human Multiplex Cytokine Panels.

**Results.** VEGF serum levels were significantly lower in women with endometriosis compared to controls ( $1.924 \pm 0.145$ , respectively  $1.806 \pm 0.078$  pg/mL). Serum levels of GM-CSF, b-FGF, EGF, and HGF did not differ significantly between patients with endometriosis and healthy controls. G-CSF had a very low detection rate. Also, serum levels of IFN- $\gamma$ , MCP-1 and IL-8 were significantly higher (mean 14.03, 57.24, and 534.24, compared to 0.58, 20.51, and 259.82), and serum levels of IP-10 and Eotaxin were significantly lower in women with endometriosis compared to controls, (mean 1.15 and 1.01, compared to 3.90 and 3.22).

**Conclusion.** The present study showed that VEGF serum levels are significantly lower in patients with advanced endometriosis compared to healthy controls. At the same time, women with advanced endometriosis have elevated IFN- $\gamma$ , MCP-1 and IL-8 levels, and lower serum levels of IP-10 and Eotaxin indicating an unbalanced immune activity in endometriosis.

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## EXPRESSION OF TWO PUTATIVE CANCER STEM CELL MARKERS IN OVARIAN CANCER

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**Introduction.** Cancer stem cell hypothesis might reflect the heterogeneity of ovarian cancer and explain the still high recurrence rates and poor long-term survival. Nestin and CD133 are regarded as potential markers of cancer stem cells and related to poor prognosis in various cancer sites. Since few studies have focused on their role in ovarian cancer, we aimed to investigate their predictive value and association with neoangiogenesis.

**Material and methods.** The study included 102 patients diagnosed with serous ovarian carcinoma between 2006-2011, treated with primary cytoreductive surgery and adjuvant platinum-based chemotherapy. Immunohistochemical analysis for Nestin and CD133 was performed on primary tumor samples. Based on staining intensity and the percentage of positive tumor cells, semiquantitative histoscores of nestin and CD133 expression have been considered. Endothelial expression of nestin in tumor vessels has been also quantified and CD34 immunostaining was used to assess intratumoral microvessel density (MVD).

**Results.** No positive association was detected between overexpression of nestin or CD133 in tumor cells and clinicopathological factors (FIGO stage, serum CA125, peritoneal carcinomatosis, tumor grade). Their level of expression did not prove to significantly influence survival (median progression-free survival time = 23 vs. 24 months,  $p=0.34$ ; median overall survival time = 55 vs 62 months,  $p=0.15$ ). Nestin positivity in endothelial cells of tumor microvessels significantly correlated with CD34-determined MVD ( $r=0.68$ ,  $p<0.001$ ).

**Conclusion.** The role of cancer stem cell markers in ovarian cancer is still questionable. Coexpression of several markers in parallel might better define their predictive value. Nestin might be regarded as an endothelial marker to mirror angiogenesis. Further investigation is justified in order to better clarify the role of these biomarkers.

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## THE ROLE OF STRUCTURAL EQUATIONS IN UNCOVERING THE COGNITIVE PROCESSES OF PATIENTS WITH OVARIAN CANCER

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**Introduction.** Studies suggest that knowledge of the expectations and preferences regarding treatment management and doctor patient communication are crucial for the treatment decision making process, yet only limited data is available for ovarian cancer patients. We present here the local results for the Romanian patients as part of an international study.

**Material and methods.** Structural equation modelling (SEM) was used to analyze the underlying relationships between Physician's Evaluation by the Patient (PEP), Result of Therapy (RT), Need for Changes in Treatment (NCT) and Patient's Desire to be informed (PD). Anonymous participation was open to all patients diagnosed and treated for ovarian cancer at our institution within a three year period.

**Results.** A total of 108 patients participated. Half of the patients knew their stage of disease, 103 patients underwent surgery, 91 patients had chemotherapy and 51 patients were in the recurrent setting. Our constructed path model based on previous studies and our local experience demonstrated a good fit of data (CFI>0.9, TLI>0.9). There was a significant positive effect of PEP on RT and a significant negative effect of PEP on NCT. The final model explained >80% of the NCT variance.

**Conclusion.** Although significant, this study requires validation on a larger scale. Our data has emphasized the physician's role as the most important person for a patient diagnosed with ovarian cancer during her treatment and afterwards. More that his role as a reliable source of information, coping with the disease alongside with his patients represents another important issue.

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## RESULTS AFTER CYTOREDUCTIVE SURGERY WITH PERITONECTOMY PROCEDURES AND SYSTEMIC CHEMOTHERAPY IN THE TREATMENT OF PERITONEAL SURFACE MALIGNANCIES

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**Introduction.** The implementation of Hyperthermic Intraperitoneal Chemotherapy treatment in Romania should be adapted to the existing particularities. The access of patients is limited, due to high costs of the device and disposables.

**Material and methods.** We presented the results of a prospective non-randomized study (2013-2015), in a consecutive series of 49 patients, with Peritoneal Surface Malignancies (PSM), who underwent Cytoreductive Surgery (CRS) with Peritonectomies procedures and systemic chemotherapy. The presented study had followed assessment of the postoperative treatment risks and survival.

**Results.** The recorded morbidity was 32%, with 24% grade I and II NIC-CTC. Concerning causes, the most representative were superficial "Site Surgical Infections", prolonged ileus, and pleurisy. Concerning causes of the major complications (8% grade III and IV), intraabdominal abscess and digestive fistulas were involved in 72%. The recorded mortality was 4%. The postoperative mortality causes were represented exclusively by cardiac and pulmonary events. Up to the end of the study, 19 patients had died. The 36 month actuarial survival rate was 61%. Using the Kaplan and Meier method, the overall median survival was 516 days (17 months). Among different variables tested, using univariate analysis, the PSM type and "Completeness of Cytoreduction Score" (CCS) had a significant positive effect on survival. In the multivariate analysis model, CCS was the only variable significantly associated with the survival. At baseline 41 patients (84%) had ascites. After the treatment of PSM, ascites decreased significantly and only 2 patients (4%) had ascites.

**Conclusion.** Our study sustains CRS with Peritonectomy procedures and systemic chemotherapy as an important therapeutic resource in PSM. The best indications are PSM from resectable primary tumor, for which the CRS and Peritonectomy procedures lead to sufficient down-staging, with CCS-0 and CCS-1.

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## NEUROENDOCRINE PANCREATIC TUMOR IN A PATIENT WITH TYPE I DIABETES

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**Introduction.** Neuroendocrine pancreatic tumors are rare, accounting for 1-2% of all tumors of the pancreas. These tumors occur at all ages with an increased incidence in the age group 30-60 years. In general, these tumors are small (less than 5 cm), asymptomatic, being discovered by chance and having a good prognosis, having a slow evolution.

**Materials and methods.** We present a case of neuroendocrine pancreatic tumor, diagnosed and treated in young patient of 27 years with type I diabetes. At a routine check-up in the context of chronic disease, type I diabetes, the ultrasound reveals a tumor of the pancreatic tail with dimensions of 5 cm in it's longest axis. CT examination confirms the presence of the tumor and exclude other lesions in the abdominal cavity. The diagnosis of neuroendocrine tumor is confirmed by cytological ultrasound guided biopsy.

**Results.** The patient underwent surgery- corporeo-caudal spleno pancreatectomy.

**Conclusion.** Neuroendocrine tumors are treatable when detected early, having a good prognosis.

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## IS THE LENGTH OF THE FEMORAL NECK REDUCED BY THE MODIFIED DUNN PROCEDURE FOR SLIPPED CAPITAL FEMORAL EPIPHYSIS?

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**Introduction.** The treatment of slipped capital femoral epiphysis (SCFE) is controversial and evolving with the development of new surgical techniques and skills. In situ fixation of SCFE has a low surgical risk, but the head-neck offset remains abnormal leading to potential femoroacetabular impingement. Modified Dunn procedure restores the normal anatomical alignment of the proximal femur, but the risk of avascular necrosis and chondrolysis is increased.

**Material and methods.** This is a single centre, retrospective study, comparing the outcomes of in situ pinning and modified Dunn procedure. Between 2011 and 2014, 9 children (9 hips) underwent the modified Dunn procedure and 10 children (10 hips) pinning in situ for stable and unstable SCFE. Mean age of the patients was 12.7 years with a median follow-up of 18 months.

**Results.** The Southwick angle improved from 68° (64–71.5°) to 9° (7.5–13.5°) ( $p < 0.001$ ), head – neck offset from -4 mm to 4.2 mm ( $p < 0.001$ ), while the length of the femoral neck didn't change significantly ( $p = 0.09$ ) in the modified Dunn procedure group. In the pinning in situ group, the Southwick angle and head – neck offset slightly improved: 34° (23–46°) to 32.5° (21–44°),  $p = 0.02$  and, respectively, -4.7 mm to -4.3 mm,  $p = 0.04$ . Postoperative clinical outcomes were slightly better in the modified Dunn procedure group (8 hips out of 9 had good and excellent results according to the Heyman and Herndorn classification) compared to the pinning in situ group (8 good and excellent results out of 10 hips) ( $p = 0.04$ ). Within our series the positive impingement test at the latest follow-up was not correlated with the type of surgical procedure ( $p = 0.48$ ).

**Conclusions.** Radiographic parameters of the proximal femur assessed in our study improved in all hips that underwent modified Dunn procedure. This technique allows restoration of the anatomic configuration of the proximal femur with improvement of the slip angle and head-neck offset.

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## JOINT HYPERLAXITY: A PREDICTOR FOR DECREASED RISK FOR RECURRENCE IN CLUBFOOT

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**Introduction.** The purpose of this study was to evaluate prospectively the role of joint hyperlaxity, as an intrinsic risk factor for clubfoot relapse. Our goals were (1) to evaluate the Beighton scores of children with relapsed clubfeet treated in our institution with the Ponseti method, (2) to compare them with a control group treated by the same method without relapse, and (3) to determine if the joint hyperlaxity, evaluated by the Beighton score, has an influence in predicting the relapses in clubfoot.

**Material and methods.** Out of 382 children treated for clubfoot deformity with the Ponseti method, 23 children with relapses were included in a group named Relapse group. From the same initial group, 19 children treated for clubfoot deformity, without relapses at the latest follow-up, were included in the Control group. From the children presenting to our outpatient clinics in the same period, we created a third group (Normal group), that included 20 children, without any congenital or acquired foot orthopedic condition. The patient characteristics, including the Beighton score at the time of latest follow-up, were studied in relation to the risk of recurrence.

**Results.** The Beighton score were significantly lower in the Relapse group comparing with the other groups ( $p=0.032$ ). Joint hyperlaxity was the factor most related to the risk of recurrence with an odds ratio of 5.28 ( $p=0.018$ ). With the numbers available, no significant relationship was found between gender, race, parental marital status, or parental income and the risk of recurrence of the clubfoot deformity.

**Conclusion.** Joint hyperlaxity is associated with a reduced risk for recurrence in clubfoot deformity. The identification of children with increased joint laxity will allow tailoring a specific brace regime comparing to those with normal or decreased joint motion.

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## SURGICAL TREATMENT OF ENDOMETRIAL CANCER IN CLUJ-NAPOCA – AN ONCOLOGY STUDY OF 1794 PATIENTS

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**Introduction.** Endometrial cancer is the most common gynecologic malignancy in developed countries. The adequate surgical staging proposed by FIGO (International Federation for Gynaecology and Obstetrics) advocates lymphadenectomy; however, it does not establish the indications, the type and the extent of lymphadenectomy, thus generating multiple controversies.

**Material and method.** Retrospective, analytical study of patients treated for endometrial cancer in the Oncological Institute "Prof. Dr. Ion Chiricuța" Cluj-Napoca (IOCN) between January 2008 and December 2014.

**Results.** During the 7-year period of the study, 1794 new cases of endometrial cancer were registered in the territorial malignity register of IOCN and treated in various departments of our institute.

**Conclusions.** We recommend treating endometrial cancer in tertiary centers by surgeons or gynaecologists-oncologists with experience in extensive peritoneal and retroperitoneal surgery.

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## MAPPING AND IDENTIFYING SENTINEL LYMPH NODE (SN) IN UTERINE CANCER USING METHYLENE BLUE 1%

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**Introduction.** Uterine cancer, the most frequent gynecological cancer, is frequently diagnosed in early stages (stage I), in 80-90% of the cases the disease is limited to the uterus. Developing a reliable charting method, identifying and evaluating sentinel lymph nodes (SN) will limit the side-effects in radical lymphadenectomy surgeries and will bring the necessary prognosis information.

**Material and methods.** This is a randomized prospective study that consists, up until now, of 21 patients treated in The Oncology Institute Cluj-Napoca. The mapping agent is methylene blue 1%. The injection is realized at the cervix and uterine fundus. After identifying and excising SN, a complete lymphadenectomy of the lymphatic basin was performed.

**Results.** The identification rate was 71% (15 cases). In 33% of the cases, SN has been identified unilaterally, and in 67% bilaterally. The most frequent location of SN was external iliac (73%) followed by internal iliac (47%), common iliac (13%) and parametrial (7%). In 2 cases SN was positive and the rest of the excised lymph nodes negative. There were no cases of false negative SN. Most of the cases in which SN has not been identified (5 of 21) have been in the first 10 cases. In the next 11 procedures there was only one case where SN has not been identified.

**Conclusion.** Using methylene blue 1% in mapping and identifying SN in uterine body cancer is a reliable method. Special attention has to be given to injecting: both quantity of tracer and site and means of injection. The current study shall be continued up to a number of 40-50 cases for a better evaluation of the technique.

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## PRIMARY MALIGNANT MELANOMA OF THE UTERINE CERVIX: A CASE REPORT

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**Introduction.** Malignant melanoma of the uterine cervix is a rare neoplasm with poor prognosis. Diagnosis is confirmed by immunohistochemical methods and by exclusion of other primary sites of melanoma.

**Material and methods.** In this paper, we are reporting a case of a 64-year-old patient with a malignant melanoma of the uterine cervix. Diagnosis was made by histological and immunohistochemical method.

**Results.** Chest and abdominopelvic computed tomography and MRI scanning were normal. A radical hysterectomy with bilateral salpingo-oophorectomy and pelvic lymphadenectomy was performed. After performing combined radical surgery and chemotherapy, complete remission of the tumor was achieved. The patient has been followed for 12 months. She is well without any symptoms or signs of recurrence.

**Conclusions.** Malignant melanoma is usually misdiagnosed specially in the chronic form. The immunohistochemical study is useful for definite diagnosis. Treatment is not well established, and the disease histogenesis has been controversial for a long time.

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## CHALLENGES OF WIDE EXCISIONS FOR BIG DIMENSIONS SOFT TISSUE SARCOMAS

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**Introduction.** The poster aims at presenting the surgical challenges of wide excisions for big dimensions soft tissue sarcomas.

**Material and methods.** The poster uses iconography from author's personal experience to emphasize some challenges met during the surgical treatment of these cases.

**Results.** Using careful dissections of the neuro-vascular pedicles, rotated flaps and skin grafts almost every challenge can be overcome.

**Conclusions.** Most important challenges of this kind of excisions are:

- neuro-vascular pedicles identification and dissection
- ensuring oncologic safe resection margins (or at least clear margins)
- postexcisional wound closure.

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## THE ROLE OF ITERATIVE SURGERY IN OVARIAN CANCER. RESULTS OF A COMPREHENSIVE CANCER CENTER

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**Introduction.** Unfortunately the majority of patients with ovarian cancer will have relapse of their disease after frontline surgery and chemotherapy. Despite the evidence for primary surgery, secondary cytoreductive surgery (SCS) in ovarian cancer is less well defined.

**Material and methods.** Patients treated within our institution over a ten year period that were in accordance with our study criteria (advanced stage, serous ovarian cancer, complete patient file) were included with respect to the role of SCS and its impact on progression free survival, overall survival and comorbidities.

**Results.** Over 160 patients diagnosed in advanced stage disease (89% FIGO III, 11% FIGO IV) were selected. At relapse, more than one third underwent SCS followed by platinum based chemotherapy and the rest of the study cohort medical treatment only. Peritoneal carcinomatosis during the first surgical intervention significantly associated with the same intraoperative aspect at relapse ( $p < 0.05$ ). Furthermore, the presence of ascites at relapse was a significant statistical indicator of peritoneal carcinomatosis. Patients that underwent SCS and platinum based chemotherapy had a better OS in comparison with the medical group only ( $p < 0.05$ ), without any differences in comorbidities or admission period.

**Conclusion.** Our results together with the existing body of data support this therapeutic intention, only if a complete debulking can be achieved. Before establishing the definitive role of SCS, results are still awaited from the three ongoing randomized trials.

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## NOVEL TRANSMEMBRANE BIOMARKERS IN THE OVARIAN CANCER MICROENVIRONMENT: ADAM12 AND CDCP1

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**Introduction.** The tumor microenvironment and its vasculature is different from normal vasculature regarding gene and protein expression. Recent evidence regarding the ovarian tumor microenvironment show that ovarian cancer biomarkers can derive from immature blood vessels. Transmembrane localised proteins such as ADAM12 and CDCP1 have been identified and could play an important role within this unique microenvironment.

**Material and methods.** ADAM12 and CDCP1 expression were semiquantitatively evaluated by IHC using staining intensity and the percentage of positive cells. Their expression on tumor stroma and blood vessels was also analyzed. The microvessel density was considered an important baseline factor and evaluated using previously described techniques with an anti-CD34 antibody. Clinical, temporal and biological parameters were correlated with our pathological data.

**Results.** 102 patients diagnosed and treated for ovarian cancer at our institution during a 5 year period were selected. All patients had histologically confirmed serous ovarian cancer and received as a first line treatment complete surgical debulking followed by platinum combination chemotherapy. 83% of patients were diagnosed with advanced stage disease (FIGO stage III/IV). A similar percentage of patients had unfavorable histological grade (G2-3). Regarding CDCP1 expression, 40% of cases exhibited strong tumor cell staining. Analyzing ADAM12, >50% of samples exhibited positive tumor islets. During follow-up, 78 patients relapsed and 55 died. Cases that exhibited positive CDCP1 or ADAM12 microvessels had a significantly elevated MVD ( $p < 0.05$ ). A high CDCP1 or ADAM12 tumor score was significantly associated with a shorter OS ( $p < 0.05$ ).

**Conclusion.** Our results indicate that ADAM12 and CDCP1 might play an important role within the tumor microenvironment in part by neoangiogenesis and further studies should be implemented to uncover their negative prognostic value.

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## VISCERAL FAT THROUGH ADIPOKINES - RISK FACTOR FOR ENDOMETRIAL CANCER

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**Introduction.** In endometrial cancer, visceral obesity, as a risk factor, is associated with a chronic inflammatory process, confirmed by the elevation of serum inflammatory markers in obese patients. The study principal aim is to evaluate the correlation between intra-abdominal fat, assessed by ultrasonography, and the systemic level of leptin and adiponectin, in patients with endometrial cancer.

**Material and methods.** The study is a case-control analysis including 2 groups of patients: group I – 50 patients diagnosed with endometrial cancer, group II – 50 patients without gynecological pathology. The diagnosis of endometrial cancer was made following histopathological examination that evaluated the tissue material obtained through endometrial biopsy. These patients underwent ultrasound and computer tomography examination by which intraperitoneal fat was determined. Leptin and adiponectin levels were determined for each patient.

**Results.** In patients diagnosed with endometrial cancer, the intra-abdominal fat area evaluated by ultrasound ( $251.37 \pm 59.78 \text{ cm}^2$ ) was significantly larger ( $p < 0.0001$ ) compared to the control group ( $159.14 \pm 42.5 \text{ cm}^2$ ).

Adiponectin was significantly elevated ( $p < 0.0001$ ) in the control group ( $11045.68 \pm 4920.93 \text{ ng/ml}$ ) compared to the endometrial cancer group ( $7374.17 \pm 4701.35 \text{ ng/ml}$ ).

The plasmatic level of leptin in the endometrial cancer group ( $40675.50 \pm 27912.73 \text{ pg/ml}$ ) was significantly elevated ( $p < 0.0001$ ) compared to the control group ( $17103.79 \pm 12002.64 \text{ pg/ml}$ ).

**Conclusion.** 1. The plasmatic level of leptin and adiponectin have a linear positive and negative correlation with the visceral fat. 2. Determination of intra-peritoneal fat in association with the adipokines level may be a predictive factor for endometrial cancer.

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## RISK FACTORS FOR NECROSIS AND GANGRENE IN PATIENTS WITH CRITICAL LIMB ISCHEMIA

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**Introduction.** Every year one in thousand people are diagnosed with critical limb ischemia (CLI) in Europe, while one quarter dies within a year due to late diagnosis. The aim of the present study was to identify the risk factors for necrosis and respectively amputation on subjects with CLI.

**Material and methods.** A three years retrospective study was conducted on subjects with CLI hospitalized at the Second Surgical Clinic, Clinical County Hospital Cluj-Napoca from December 2010 to December 2012. There were included in the study all subjects with principal diagnosis as critical lower limb ischemia and data from their medical charts were collected. Binary logistic regression analysis was used to identify risk factors for necrosis and respectively gangrene among collected variables.

**Results.** Four hundred and forty one patients with mean age of  $66.22 \pm 10.40$  years, with a significantly higher percentage of male (M:F = 19.05%:80.95%,  $p < 0.0001$ ) were included in the study. Necrosis was observed in 32.43% of cases (95%CI [28.12–36.96]) while gangrene was observed on 22.45% of cases (95%CI [18.60–26.76]). A significantly higher percentage of patients with necrosis had type II diabetes compared to patients without necrosis (44.06% vs. 29.87%,  $p = 0.004$ ) while the smoking was more frequent on patients without necrosis (70.13% vs. 60.84%,  $p = 0.056$ ). Neither type II diabetes nor smoking proved to be risk factors for necrosis (diabetes: OR=0.58 [0.38-0.88]; smoking: OR=1.34 [0.87-2.06]). Hypercholesterolemia was present on 43.43% of patients with gangrene and on 56.14% of patients without gangrene ( $p = 0.025$ ). Hypercholesterolemia proved a risk factor for gangrene (OR=1.98 [1.24–3.16]).

**Conclusion.** Despite the fact that the patients with necrosis had in significantly higher percent type II diabetes and comprise a significantly higher percentage of smokers compared to non-smokers, regression analysis did not identify any significant risk factor.

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## ANTI VEGF THERAPY IN RETINAL VEIN OBSTRUCTION

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**Introduction.** Retinal vein obstruction (RVO) it's characterized by variable degree of vision loss and complications which can worsen the visual functional rehabilitation. In this issue we try to assess the benefits of the association of anti VEGF therapy in RVO, especially in the resorption of macular oedema and in preventing neovascular glaucoma (NVG).

**Material and methods.** Retrospective study of 35 patients whit RVO treated with LASER photocoagulation and anti VEGF (avastin). We followed the level of visual acuity (VA), OCT evaluation of macular thickness and the incidence of NVG.

**Results.** 4 patients presented at the end of therapy VA more than 4/50, and central macular thickness was under 265 microns in 14 patients. NVG appeared only in 2 patients.

**Conclusion.** Anti VEGF therapy is useful in RVO therapy in preventing the NVG and in reducing the macular oedema. By thus, antiVEGF therapy offers a better visual outcome for the patients with RVO.

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## PERCUTANEOUS VERSUS OPEN SURGERY IN ACUTE TRAUMATIC ACHILLES TENDON RUPTURE

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**Introduction.** Traumatic Achilles tendon rupture is seen more and more frequently in current orthopedic practice. The lesions appear at a hypovascularized area of the tendon located 2-7 cm proximal to the calcaneal insertion. Alterations in the structure of collagen fibers are related with age, physical activity, different metabolic diseases or clinical conditions. Aims: The main goal of the present article is to compare the clinical and the functional results of the percutaneous vs open surgery in the acute traumatic Achilles tendon rupture.

**Material and Methods.** The study was conducted on 23 patients admitted to the Orthopedics and Traumatology Clinic of Cluj-Napoca between January 2011 - June 2015. The elapsed time before surgery, the treatment options depending on lesion's location, technical difficulties, costs, postoperative care, the average healing time, complications were analyzed. The patients followed the same rehabilitation protocol.

**Results.** In most of the cases, the classical methods of Achilles tendon reconstruction were used (77%). The complications rate was about 8% (iterative rupture, delay in scarring, deep venous thrombosis. No clinical or functional difference was noted between the patients treated percutaneous vs open surgery.

**Conclusions.** The percutaneous surgical techniques are a viable alternative for the acute ruptures of Achilles tendon, the classic intervention has clear indications in lesions diagnosed late, in the iterative tendon ruptures.

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## R0 RESECTION IS THE MAJOR PROGNOSTIC FACTOR FOR OVERALL AND DISEASE-FREE SURVIVAL AFTER PELVIC EXENTERATION FOR RECTAL CANCER

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**Introduction.** We reviewed our experience with pelvic exenteration for rectal cancer to identify the prognostic factors for long-term survival.

**Material and methods.** Between 2000 and 2014, 42 patients (males, 22; median age, 58 years) undergoing total pelvic exenteration for rectal cancer were identified from a prospective database. Overall survival and disease-free survival analysis was performed using the Kaplan Meyer method. Clinicopathologic variables were evaluated as prognostic indicators of long-term survival by log-rank test and multivariate Cox regression.

**Results.** Indications for surgery were recurrent rectal cancer in 16 and primary rectal cancer in 26 patients. At the time of pelvic exenteration 26 patients had total, 16 posterior exenteration and 12 required sacrectomy; 30 patients below and 12 above levator ani exenteration (8 coloanal anastomosis). On pathology 12 patients were T3 and 30 T4; 21 N0, 11 N1 and 10 N2; R0 resection was achieved in 37 patients (88 percent). Perioperative mortality after pelvic exenteration was 2.4 percent and major complications rate 31 percent. Median follow-up was 36 (2–176) months. Median disease-specific survival was 33 (17-54) months (CI 95%) and overall survival 62 (45-76) months (CI 95%) Univariate analysis identified three factors associated with decreased survival: recurrent rectal cancer, positive surgical margins, and N1-N2. On multivariate analysis only positive surgical margin was an independent predictor of unfavorable outcome ( $P < 0.001$ ).

**Conclusions.** Total pelvic exenteration can be performed safely in selected patients with rectal cancer and can result in significantly prolonged survival. R0 resection remains the major prognostic factor.

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## IMPACT OF SOY ISOFLAVONES ON BREAST CANCER CELLS: CYTOTOXICITY AND EXOMETABOLOME ANALYSIS

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**Introduction.** Multiple efforts to identify efficient chemopreventive agents for breast cancer have focused on soy isoflavones. Despite remarkable progress, the understanding of soy isoflavones mechanisms remains challenging especially due to their dose-dependent effect and the tumor heterogeneity. The aim of this study was to evaluate the viability of different breast cancer cells exposed to soy isoflavones and to assess their metabolic state using an exometabolome profiling approach.

**Material and methods.** MCF-7 (Estrogen responsive) and MDA-MB-231 (Estrogen nonresponsive) breast cancer cells were exposed to increased concentrations of genistein, daidzein and a soy seed extract (Glycine max) for 72h. Cell viability and proliferation were evaluated using tetrazole-MTT assay. For extracellular metabolite analysis, the cell culture supernatant was analyzed using <sup>1</sup>H-NMR spectroscopy. Visualization and multivariate statistical analysis was performed using MetaboAnalyst 3.0.

**Results.** For MCF-7 cells, low concentration of isoflavones (<3.5 ug/mL genistein, <8.93 ug/mL daidzein and <81.42 ug/mL soy extract) stimulated cell growth while higher concentrations caused an inhibitory effect. For MDA-MB-231 cells, only an inhibitory effect was observed. Following <sup>1</sup>H-NMR analysis, 32 extracellular metabolites were identified and relatively quantified to the area of the internal standard. The inhibitory effect of all isoflavones was associated with higher extracellular levels of hydroxyproline, threonine and asparagine and lower levels of pyruvate and serine.

**Conclusion.** Soy isoflavones induced a biphasic growth effect on estrogen responsive cells, probably due to their structural resemblance to 17- $\beta$ -estradiol. Both cell lines exhibited specific exometabolome profiles. Upon soy isoflavone exposure, substantial changes in aminoacids uptake were observed, suggesting that isoflavone inhibitory effects were caused, most probably, by an alteration of protein biosynthesis.

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## EVALUATION OF PLASMONIC AND HYPERTHERMIA PROPERTIES OF CORE-SHELL IRON OXIDE-GOLD NANOPARTICLES

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**Introduction.** We report a novel two-stage synthesis method of magneto-plasmonic nanoparticles (MP-NPs). The hydrophobic iron oxide magnetic NPs (MNPs), prepared by thermal decomposition technique, have been transferred into water phase and capped with citrate groups. Under ultraviolet illumination, the gold ions are reduced preferentially on the magnetic NPs generating the formation of a gold shell surrounding the magnetic core.

**Materials and methods.** Iron(III) acetylacetonate dissolved in a mixture of oleic acid, oleylamine and octylether and heated at 300°C for 1h give rise to MNPs. Water transfer has been achieved by treating the MNPs with aqueous solution of tetramethylammonium hydroxide. The gold shell has been deposited by reduction of tetrachloroauric (III) acid under ultra-violet (UV) irradiation by the citrate groups attached to the surface of MNPs. The NPs have been analyzed by TEM, UV-VIS, DLS and SERS and magnetic heating system.

**Results.** The MNPs have a spherical shape with the mean diameter between 7 and 20 nm depending on the magnetic precursor/surfactant-solvent ratio. Upon gold coating, the UV-VIS absorption spectra revealed the existence of a strong plasmonic peak, which red-shifts as a function of the shell thickness surrounding the magnetic core. The MP-NPs display a core-shell architecture of different geometries. The plasmonic properties of MP-NPs are evidenced by the detection of SER spectra of methylene blue, 5-fluorouracil and doxorubicin. The hyperthermia performance of MNPs in organic solvent, increases with their mean diameter and is little affected by both the water transfer and gold coating.

**Conclusion.** A facile method to synthesize magneto-plasmonic NPs has been achieved together with their morphological, optical and hyperthermia characterization.

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## CORE-SHELL MULTIFUNCTIONAL NANOOBJECTS FOR TARGETED DRUG DELIVERY APPLICATIONS

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**Introduction.** The design of multifunctional nanocarriers for targeted drug delivery applications has recently emerged as a very hot research topic. Among them, plasmonic nanoliposomes seems to hold a great promise since they possess the capacity to combine the drug delivery properties of the liposomes with the plasmonic properties of noble metal nanoparticles. In this letter we report the successful synthesis and characterization of a new class of very stable multifunctional nanoobjects having a core-shell architecture. The nanoobjects core is represented by cationic liposomes whereas the shell is formed through a uniform decoration of the core with PEGylated gold nanoparticles (PEGAuNPs).

**Materials and methods.** The plasmonic liposomal nanocarriers were prepared by taking advantage of the electrostatic interactions between the small unilamellar cationic liposomes and the negatively charged pegylated gold nanoparticles synthesized using an original method developed in our laboratory. Once synthesized the multifunctional nanocarriers have been investigated by means of UV-Vis absorption spectroscopy, Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), Zeta Potential Measurements and Surface Enhanced Raman Spectroscopy (SERS).

**Results.** The TEM images revealed the attachment of AuNPs onto the outer surface of the cationic liposomes which was also confirmed by DLS and UV-Vis data. The plasmonic properties of the complexes have been evaluated by SERS. It is shown that PEG molecules, decorating the nanoparticles outer surface, mediated their interaction with the liposomes thus enabling the acquisition for the first time of the liposomes SER spectra in aqueous environment. In this way the plasmonic properties of the multifunctional nanoobjects has been experimentally proven.

**Conclusion.** The successful synthesis and characterization of a new class of multifunctional nanoobjects possessing a liposomal core decorated with plasmonic nanoparticles is reported.

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## COMPARATIVE STUDIES ON POLYPHENOLIC COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SCHISANDRA CHINENSIS LEAVES AND FRUITS

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**Introduction.** *Schisandra chinensis* (Turcz.) Baill) is a new pharmacopoeial species, introduced into the European phytotherapy just a few years ago, due to its traditional uses in Traditional Chinese Medicine. Its fruits have been used for thousands of years as an astringent, sedative, adaptogenic and tonic agent to treat chronic coughs, spontaneous sweating, palpitation and spermatorrhea. The aim of this paper was to evaluate the antioxidant and antimicrobial activities and the polyphenolic content of *S. chinensis* leaves and fruits.

**Material and methods.** The identification and quantification of phenolic compounds was performed by LC-UV-MS techniques and the antioxidant activity was determined by several electron-transfer assays. The antimicrobial tests were performed by a disk diffusion method.

**Results.** The main flavonoid from the leaves was isoquercitrin ( $2486.18 \pm 5.72$   $\mu\text{g/g}$  plant material), followed by quercitrin ( $1645.14 \pm 2.12$   $\mu\text{g/g}$  plant material). Regarding the fruit composition, the dominant compound was rutin ( $13.02 \pm 0.21$   $\mu\text{g/g}$  plant material), but comparing with the leaves, fruits can be considered a poor source of phenolic compounds. The antioxidant assays revealed a higher antioxidant activity for the leaves extract, in correlation with the phenolic composition. *S. chinensis* leaves extract showed efficient activities against targeted bacteria, being more active than the fruits extract.

**Conclusion.** The results suggest the leaves of *S. chinensis* as a valuable source of bioactive compounds with significant antioxidant and antimicrobial activities.

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## PHENOLIC CONTENT, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF VERATRUM ALBUM L. (MELANTHIACEAE)

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**Introduction.** *Veratrum album* L. (Melanthiaceae), known as white hellebore, is distributed in Europe and parts of Asia. The roots and the rhizomes of the species have been traditionally used to treat scabies and chronic malaria. Phytochemical and pharmacological studies have shown that the main active components of the species are the alkaloids, known for their antihypertensive activity. The aim of this work was to investigate the polyphenolic profile and to evaluate the antioxidant and antimicrobial activities of plant ethanolic extracts.

**Material and methods.** The vegetal extracts from the aerial parts were obtained by ultrasonication, in 70% ethanol. The total phenolic content was determined using UV-VIS spectrophotometric methods. Identification and quantification of polyphenols was performed using a LC-UV-MS method and the antioxidant activity was determined by TEAC and EPR assays. The modified micro dilution technique was used to evaluate the antimicrobial activity.

**Results.** The chlorogenic acid was the dominant compound (150.80  $\mu\text{g/g d.w.v.p.}$ ) whereas flavonoids as quercitrin (50.84  $\mu\text{g/g d.w.v.p.}$ ) were found at low concentrations. The ethanolic extracts of the studied species showed moderate antioxidant activity by TEAC and EPR assays. All tested microorganisms were susceptible to the plant extracts with minimum inhibitory concentration (MIC) about 0.0078 mg/ml and minimum bactericidal concentration (MBC) ranging between 0.015 – 0.062 mg/ml.

**Conclusion.** The present study provides original data concerning the phenolic composition and bioactivities of the *V. album* species.

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## EFFECT OF PASTURE DIET ON MILK FATTY ACID, VITAMIN A, AND CHOLESTEROL CONCENTRATIONS IN CARPATHIAN GOATS

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**Introduction.** A way to improve the nutritional quality of goat milk fat could be the supplementation of the goat diet with sources rich in polyunsaturated fatty acids (FA), such as pasture. Many studies have reported the influence of these diets. Nevertheless, the effect of grazing on the milk fat composition of Carpathian goat breed is little known. Accordingly, the aim of this study was to evaluate the effect of pasture diet on milk FA, vitamin A and cholesterol concentrations in Carpathian goats.

**Material and methods.** Samples of bulk milk were collected from two Carpathian goat farms, distinguished by their feeding system: pasture diet, characterized by 70% grasses and 30% legumes, and indoor diet, based on alfalfa hay and concentrate in a 60:40 ratio. Samples were collected at fortnightly intervals, throughout the lactation period of animals (April to September). Milk FA and cholesterol concentrations were determined by gas chromatography, while milk vitamin A concentration was determined by HPLC.

**Results.** Compared to indoor diet, the pasture diet increased the concentrations of cis-9,trans-11-conjugated linoleic acid ( $P<0.001$ ), 18:3 n-3 ( $P<0.01$ ), and total polyunsaturated FA ( $P<0.05$ ) in milk fat, but decreased 14:0 ( $P<0.05$ ) and 16:0 ( $P<0.01$ ) milk fat levels. Also, when compared to indoor diet, pasture diet increased vitamin A ( $P<0.01$ ) and decreased cholesterol ( $P<0.001$ ) concentrations in milk fat.

**Conclusion.** The results of the present study suggest that pasture diet can be used to improve the nutritional quality of milk fat in Carpathian goats.

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## OCCURRENCE OF TRICHOTHECENS A AND B IN WHEAT AND FLOUR SAMPLES FROM ROMANIA

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**Introduction.** Food safety is a priority of the European Commission, which has established maximum or recommended levels for some mycotoxins. Regarding trichothecens A and B in unprocessed wheat and wheat flour, maximum levels (ML) have been set only for deoxynivalenol (1750 µg/kg and 750 µg/kg, respectively), and indicative levels have been set for the sum of HT-2 and T-2 toxins (100 µg/kg and 50 µg/kg, respectively) (2006/1881/CE; 2013/165/UE).

**Material and methods.** In this study, 31 Romanian wheat samples and 35 flour samples collected from Romanian supermarkets in 2014 were analyzed to evaluate the presence of deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-AcDON), 3-acetyldeoxynivalenol (3-AcDON), nivalenol (NIV), diacetoxyscirpenol (DAS), neosolaniol (NEO), HT-2 and T-2 toxins. Analysis was performed using liquid chromatography coupled to tandem mass spectrometry. Limits of quantification ranged between 1.6 and 313 µg/kg.

**Results.** For the 66 analyzed samples, analytical results showed that some investigated samples contained DON and NEO. DON was detected in the 26% and 3% of wheat and flour analyzed samples, respectively. NEO occurred in one flour sample. Regarding the values detected, one wheat sample was above ML of DON. Knowing the mean values for the detected mycotoxins and the wheat and products supply quantity for Romanian population according to FAOSTAT (365 g/capita/day), the dietary intake for each mycotoxin was estimated: for DON 0.57 µg/kg body weight/day and for NEO 3.5 pg/kg body weight/day.

**Conclusion.** Due to DON, contamination, further research is necessary to confirm their presence in food and also to estimate the dietary intake for Romanian population.

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## ELECTROCHEMICAL DETECTION OF B-LACTAM ANTIBIOTICS

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**Introduction.** Antibacterial drugs have revolutionized the treatment of infectious diseases, but there are different problems associated with the use of antibiotics: antibiotic overuse, infection control, surveillance for resistance, antibiotic use in animals and crops, environmental contamination with antibiotics. The use and misuse have resulted in the development and spread of antibiotic resistance, a major health problem for the modern world, each year in the European Union alone, over 25 000 people dying from infections caused by antibiotic-resistant bacteria. For these reasons, there is a need for developing new analytical sensors, capable to detect selectively low concentrations of antibiotics from different matrices.

**Material and methods.** The purpose of this study was the development of an electrochemical sensor for the analysis of different penicillins and cephalosporins. For the detection of these  $\beta$ -lactam antibiotics we employed the peak obtained by the electrochemical oxidation of the  $\beta$ -lactam ring. Several electrode materials were used and the best results were obtained with the boron-doped diamond electrode.

**Results.** We investigated the influence on the electrochemical signal of the structure of each molecule of antibiotic and the results showed that the lateral chain plays an important role on the oxidation peak.

**Conclusion.** The analytical method was optimized in terms of analysis conditions (pH, pre-treatment conditions, electrochemical parameters), selectivity and of sensitivity and it was applied to real samples.

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## ASSESSMENT OF PERFUME INGREDIENTS WITH APHRODISIAC POTENTIAL BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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**Introduction.** Studies performed in the last years have been focusing mainly on the analysis of perfumes in terms of potentially allergenic substances and in a lesser extent in terms of flavors that can attract consumers. Companies are interested in using those flavors which consumers like and make them buy the perfume. A special attention was dedicated to perfume ingredients with aphrodisiac potential, because they could play a key role in the fragrance choice and buying decision process. In the present study the composition of some of the best-selling perfumes of recent years has been evaluated, focusing on the quantitative assessment of potential aphrodisiac ingredients of these fragrances, such as vanillin, ethylvanillin, trans- and cis-methyl dihydrojasmonate, muskolactone and muscone.

**Material and methods.** Seven samples of different brands (three women's and four men's fragrance) were analyzed by gas chromatography-mass spectrometry. All the other major and minor constituents were assigned based on MS spectra library (Wiley) matching, followed by pattern analysis using chemometric data-mining (PCA). Internal standard method was used for quantitative analysis.

**Results.** Trans-methyl dihydrojasmonate was found in all analyzed fragrances, with the highest concentration found in a perfume for women.

**Conclusion.** Trans-methyl dihydrojasmonate was found in all analyzed fragrances, suggesting in a way that the "winning" blend is the classic aroma of jasmine, both for men and women. Even though perfumes of completely different origin were considered, in principle they tend to own considerable similitudes in terms of the nature and relative percent of constituents.

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## A NOVEL GRAPHENE/ $\beta$ -CYCLODEXTRINE BIOSENSOR FOR DOPAMINE DETECTION

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**Introduction.** Graphene and cyclodextrins have been widely employed for sensors elaboration combining the enhanced sensibility given by the special properties of graphene and cyclodextrins. These sensors were applied for a wide range of biological molecules detection, among which dopamine, an important neurotransmitter, can be mentioned. A graphene/ $\beta$ -cyclodextrin biosensor with tyrosinase was developed for dopamine detection.

**Material and methods.** In order to improve the thermal, mechanical and electrical properties, the graphene oxide was reduced with ascorbic acid, a simple green method to avoid the environmentally harmful reducing agents. The glassy carbon electrode was modified with reduced graphene oxide (RGO),  $\beta$ -cyclodextrin ( $\beta$ -CD) and polyethylenimine (PEI) by using layer by layer method. The obtained nanocomposite was then characterized by Raman and FTIR spectroscopy, optical microscopy and electrochemical impedance spectroscopy.

**Results.** The electrochemical behavior of dopamine was investigated by electrochemical methods on electrodes modified through multiple possible combinations during the optimization process and the best results were obtained on the electrode modified with 1 layer of RGO, 1 layer of  $\beta$ -CD and 1 layer of PEI.

**Conclusion.** This nanoplatform was used to immobilize the tyrosinase obtaining a biosensor applied for the dopamine determination from pharmaceutical products, serum and urine samples with good recoveries, enhanced sensitivity and good selectivity.

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## NANOSTRUCTURED PLATFORMS BASED ON GRAPHENE-POLYPYRROLE COMPOSITE FOR IMMUNOSENSOR FABRICATION

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**Introduction.** The entrapment of functionalized graphene in polypyrrole (PPy) conductive layers improves their engineering features. The association of graphene-PPy composite with nanosphere lithography generated a hybrid polystyrene-graphene-PPy patterned platform, with a further enhancement of their characteristics: an improved mechanical stability and electron transfer rate, combined with an increase of the active surface area.

**Material and methods.** The substrate was modified with N-hydroxysuccinimide activated carboxylic functional groups, for the controlled immobilization of antiparacetamol antibody in order to elaborate an immunosensor for paracetamol detection.

**Results.** The behavior of the nanostructured hybrid platform was tested by imagistic, impedimetric and electrochemical quartz crystal microbalance experiments proving the successful patterning of the composite material and the biomolecule's immobilization. The sensor allowed the specific detection of paracetamol on a linear range of 0.1 to 25  $\mu\text{M}$  on the unstructured platform.

**Conclusion.** The analytical signal was improved on the patterned material in comparison with the unstructured one, proving the superior nature of the structured surface.

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## GRAPHENE OXIDE BASED IMMUNOSENSOR FOR THE ACETAMINOPHEN DETECTION

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**Introduction.** Immunosensors are high sensitive biosensors that are based on the interaction between an antigen and its complementary antibody. Graphene is another carbon based 2D material that shows great perspectives in electrochemical biosensors development.

**Material and methods.** A novel label-free immunosensor for the selective detection of acetaminophen (APAP) was developed by modifying a graphite based screen-printed electrode with graphene oxide after the functionalization with N-hydroxysuccinimide (NHS) in the presence of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC). The graphene oxide sheets were suspended in a NHS/EDC mixture and the succinimide moieties reacted with the graphene oxide carboxylic groups in the presence of EDC forming esters with groups that are easy to remove. This step activates the graphene oxide in order to react with the amine groups from anti-acetaminophen antibody (antiAPAP Ab) within amides bonds.

**Results.** The modified graphene was deposited onto graphite based screen-printed electrodes via a layer-by-layer method. The obtained nanostructured platform was used for antiAPAPAb immobilization during the immunosensor development which was successfully applied for acetaminophen detection with minimal interference of dosage forms excipients and serum components.

**Conclusion.** The above described immunosensor was applied with good results for the determination of acetaminophen in synthetic and real samples by using square wave voltammetry with a limit of detection of 0.17  $\mu\text{M}$ .

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## MODIFIED GRAPHENE OXIDE BASED APTASENSOR FOR THE MUCIN 1 DETECTION

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**Introduction.** Mucin 1 (MUC1, CA 15-3) as cancer biomarkers has been more extensively studied, being involved in tumor proliferation and metastasis, in chemoresistance and alteration of drug metabolism. Their overexpression has been associated with breast, ovarian, pancreatic, liver, colon or lung cancer.

**Material and Methods.** An electrochemical aptasensor based on modified graphene oxide screen printed electrodes (SPE) was developed for the indirect detection of MUC 1 through the signal obtained after the methylene blue (MB) electrochemical reduction. The graphene oxide was functionalized with succinimide moieties in order to activate it for the reaction with amine groups from the MUC1 aptamer (5'-GCAGTTGATCCTTTGGATACCC TGGTTTTTTTTTTTTTTT-3' with NH<sub>2</sub> terminal group) within amides bonds. The nanostructured platform was obtained via layer-by-layer deposition of graphene oxide onto the graphite based SPE.

**Results.** After the complete optimization and characterization, the platform was incubated with aptamer and then the MB was used as label, in order to obtain the electrochemical signal. Moreover, the MB interacted directly with MUC1 aptamer, being stable, simple, cost-effective, and avoiding external modification of the biomolecules. After the incubation with MUC 1 protein, the aptasensor was successfully tested in spiked samples using electrochemical impedance spectroscopy and differential pulse voltammetry.

**Conclusion.** An electrochemical aptasensor for Mucin 1 detection was elaborated and a limit of detection of 0.6 ng/mL was obtained.

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## INNOVATIVE CARBON NANOTUBES AND GRAPHENE BASED MODIFIED ELECTRODES AS PLATFORMS FOR BIOSENSORS CONSTRUCTION: ELECTROCHEMICAL AND SPECTRAL CHARACTERIZATION

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**Introduction.** Graphene and carbon nanotubes (CNTs) are allotropic forms of carbon consisting of sp<sup>2</sup> hybridized atoms arranged in 2D sheets, rolled up to form tubes. Their unique properties generated their broad use in electrochemical sensing and bio-sensing.

**Material and methods.** The incorporation of single and multi-wall CNTs into layer by layer (LBL) films containing enzymes enhances the electron diffusion through the films, the electrochemical surface area, reduces the distance between the active site of enzyme and the electrode surface, provide a homogeneous porous composite film that facilitates substrate transfer and increases the response of the sensor. Other functionalization procedure was used in order to activate carboxylic groups from graphene oxide in order to react with the amine groups from biocomponent within amides bonds. The functionalized graphene sheets were deposited onto graphite based screen-printed electrodes via a LBL method.

**Results.** The structural features of the films deposited onto electrodes were characterized by FTIR, Raman spectroscopy and optical microscopy. EIS experiments were used to characterize the electron transfer properties of the electrode surface during all the modification steps. Cyclic voltammetry determinations were carried out in [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup>, Ru(NH<sub>3</sub>)<sub>6</sub>Cl<sub>3</sub> and Fc(MeOH)<sub>2</sub> solutions, in multisweep conditions and were used for qualitative characterization of the accumulation/rejection phenomena and also the mass transport through the various films.

**Conclusion.** The obtained nanostructured platforms were used for horse radish peroxidase and anti-acetaminophen antibody immobilization during the bio/immunosensors development that was successfully applied for acetaminophen detection.

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## PREDICTING CHROMATOGRAPHIC BEHAVIOR OF SOME CHIRAL $\beta$ -BLOCKERS FROM MOLECULAR STRUCTURE BY QSAR/QSPR ANALYSIS

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**Introduction.** The chiral HPLC separation parameters of a series of fourteen  $\beta$ -blockers on four polysaccharide-based CSPs were evaluated through computational studies using the MOE software. Several semi-empirical mathematical models were built and refined by PLS and O2PLS multivariate data analysis correlating chromatographic data with the set of molecular descriptors.

**Material and methods.** The structures were preoptimized using PM6 method and the resulting geometries were further refined by LMD conformational search, MMFF94x force field and standard settings in MOE; the lowest energy conformations were chosen to calculate 340 descriptors. O2PLS was performed using the software Simca.

**Results.** The models for retention time revealed the importance of certain descriptors shaping retention and enantioselectivity of the studied enantiomers. Most of the variables that have a significant impact on modeling the retention times describe H-bond donors and acceptors, which may imply that the enantiomer and chiral selector complex interact through hydrogen bonding, possibly between the polar carbamate groups (carbonyl and NH groups) and the atoms with these properties.

**Conclusion.** The best approach for data modeling was obtained using log-transformation, with the resulting models showing relatively good predictive abilities. Nevertheless, the predictive power of the models could be further improved by expanding the number of observations on the entire range of  $\beta$ -blockers as well as by feeding into the model 3D descriptors derived from the used CSPs.

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## MOLECULARLY IMPRINTED POLYMER BASED ELECTROCHEMICAL SENSOR FOR THE SENSITIVE DETECTION OF PESTICIDES

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**Introduction.** The extensive use of pesticides has raised serious public concerns, due to their possible harmful effects on human health, environment and food safety. Glyphosate is a widely used organophosphoric pesticide that accumulates in water and crop commodities. Although it has been considered as toxicologically harmless it was proven it affects the cell cycle regulation. Molecularly imprinted polymers are promising materials continually being used in sensor fields as biomimetic elements. Lately, considerable attention has been given to microporous metal-organic frameworks as highly sensitive and selective platforms for the detection of analytes.

**Material and methods.** In the present study, a highly selective electrochemical sensor was developed for the detection of glyphosate based on molecularly imprinting microporous-metal-organic framework. Glyphosate was imprinted in a polythioaniline-gold nanoparticles film to create specific cavities in the polymer network for selective stereo chemical recognition.

**Results.** The sensitivity of the sensor could be enhanced by an increase in the electrochemical conductivity through the use of gold nanoparticles. The molecularly imprinted sensor was characterized by linear sweep voltammetry. Several experimental parameters have been optimized. A linear response was obtained in the range of 5.9 fM to 5.9 nM. Selectivity studies were carried out towards the binding of a similar molecule, aminomethylphosphonic acid, and the sensor exhibited excellent selectivity.

**Conclusion.** A MIP sensor for the detection of glyphosate has been developed with a wide linear range, high sensitivity and good reproducibility for glyphosate detection applicable for tap water samples.

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## GAS-CHROMATOGRAPHIC SEPARATION OF FATTY ACIDS IN SERUM FROM ADRENOLEUKODYSTROPHY PATIENT

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**Introduction.** Fatty acids are most commonly separated by gas-chromatography, after derivatization to their methyl esters (FAME). Certain fatty acids (long-chain fatty acids) have been identified to have higher profiles in patients with peroxisomal disorders (e.g. adrenoleukodystrophy) than in healthy volunteers. The aim of this study was to develop and optimize an efficient method for the separation of fatty acids in the serum of a patient diagnosed with adrenoleukodystrophy, with a focus on long and very long chain fatty acids, with the possibility of identification of certain fatty acids as biomarkers for the disease.

**Material and methods.** For this purpose, we have developed the separation method in three stages: derivatization, extraction and separation, which were all optimized. The fatty acids were derivatized with methanol in acidic, non-aqueous media (70°C, 3 hours), then the methyl esters were extracted twice in hexane and the solution was concentrated under a stream of nitrogen. The samples were analyzed using a GC-MS system, through a HP-5ms column (30 m, 0.25 mm, 0.25  $\mu$ m), in a gradient temperature program. The mass detector was set in SIM mode.

**Results.** Results showed a good separation in terms of resolution of the fatty acids methyl esters. Considering the higher retention of very long chain fatty acids methyl esters, the long analysis times were hard to optimize. The temperature gradient was focused on the resolution of all FAME, but especially it was focused on increasing the resolution of very long FAME, which would be quantified.

**Conclusion.** In conclusion, we have developed and optimized the gas-chromatographic separation of fatty acids obtained from the serum of a patient with adrenoleukodystrophy, after their derivatization to their methyl esters.

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## NEW HYDRAZONES BEARING THIAZOLE SCAFFOLD AS ANTIMICROBIAL AND ANTIOXIDANT AGENTS

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**Introduction.** Microbial resistance is more than decades, a threat to the effectiveness of common therapy. In the case of patients at risk, this can involve the extension of the disease or even death. In this context of the alarming development of the microbial resistance, the discovery of new effective substances is required urgently. Oxidative stress is involved in the onset and progression of over 100 diseases. Antioxidants are molecules capable of interacting with free radicals and stopping their chain reactions before essential vital molecules are damaged. In new drug development studies, combination of different pharmacophores may lead to new compounds with higher biological activity. Therefore the combination of thiazole- and hydrazone-type compounds might provide new effective drugs for the treatment of multidrug resistant microbial infections and for diminishing oxidative processes.

**Material and methods.** New series of hydrazones were synthesized, in good yields, by reacting 4-methyl-2-(4-(trifluoromethyl)phenyl)thiazole-5-carbohydrazide with differently substituted benzaldehyde. The resulting compounds were characterized via elemental analysis, physico-chemical and spectral data. An antimicrobial screening was done, using Gram (+), Gram (-) bacteria and one fungal strain. 2,2-Diphenyl-1-picrylhydrazide assay was used to test the antioxidant properties of the compounds.

**Results.** Tested molecules displayed moderate-to-good growth inhibition activity. Monohydroxy, para-fluorine and 2,4-dichlorine derivatives exhibited better free-radical scavenging ability than the other investigated molecules.

**Conclusion.** The obtained results suggest that the new hydrazones bearing thiazole scaffold may be considered for further investigation and optimization, in designing antimicrobial and antioxidant drugs.

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## BIOLOGICAL EVALUATION AND MOLECULAR DOCKING OF SOME NOVEL CHROMENYL-DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS

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**Introduction.** Various thiosemicarbazones (TSCs) and their heterocyclic thiadiazoline (TDZ) derivatives showed to possess important biological effects, such as antimicrobial, antioxidant, anti-HIV-1 and anticancer. In addition, chromenyl derivatives exhibit a wide range of pharmacological activity.

Molecular docking is a process that predicts the conformation of a ligand within the active site of a receptor and finds the low-energy binding modes between them.

Based on the above mentioned and in continuation of our research on nitrogen and sulfur containing compounds, we investigated some previously reported chromenyl-TSCs as well as their corresponding TDZs for their antimicrobial properties. The molecular docking study of all the compounds has been done for a better understanding of the drug-receptor interactions.

**Material and methods.** MIC and MBC/MFC ( $\mu\text{g/mL}$ ) values of these compounds were evaluated against bacterial and fungal strains. Spectinomycin, Moxifloxacin and Fluconazole were used as reference drugs.

All the compounds were further subjected to molecular docking, against four targets that were chosen based on the specific mechanism of action of the reference drugs used in the antimicrobial screening.

Docked ligand conformations were analyzed in terms of binding affinity and hydrogen bonding between ligands (compounds) and the four receptor proteins.

**Results.** All the compounds exhibited great antimicrobial effects. Most of them were more active than the used reference drugs. In silico studies supported the antimicrobial properties of these compounds and revealed that all the investigated compounds showed good binding energies towards the four receptor protein targets.

**Conclusion.** All the compounds tested exhibited excellent antimicrobial activities, most of them being more active and showing better binding energies to the targets than used reference drugs, compounds bearing fluorine atoms in their structures having the best affinity.

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## NEW THIAZOLIN-4-ONES: CHEMICAL SYNTHESIS AND ANTIMICROBIAL POTENTIAL EVALUATION

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**Introduction.** Based on the proven biological potential of the compounds with thiazolin-4-one core in their structure and as a continuation to our increased interest in the chemistry of thiazole and its derivatives, we report herein the synthesis of new thiazolin-4-ones diversely substituted in positions 2 and 5, with structures that include in addition to the thiazolin-4-one ring, other heterocycles with known biological potential like the thiazole and chromone. The synthesized compounds were evaluated for their biological potential regarding the antibacterial and antifungal properties.

**Material and methods.** New 2-(alkyl/aryl-amino)-5-arylidene-thiazol-4(5H)-ones were synthesized starting from various thiourea derivatives, by condensation with  $\alpha$ -ethyl-bromoacetate/monochloroacetic acid, followed by modulation in the fifth position of the thiazolin-4-one ring by condensation with several aromatic aldehydes. The structural elucidation of the synthesized compounds was based on CHNS quantitative elemental analysis and spectroscopic data (MS, FTIR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR). Their antimicrobial activity was assessed in vitro against standardized bacterial and fungal strains, as MIC and MBC/MFC, using the broth microdilution method.

**Results.** The CHNS quantitative elemental analysis results and spectral analysis data were in accordance with the assumed structures. The antimicrobial screening results revealed that all the synthesized compounds have antimicrobial properties. All the compounds presented good antibacterial activity, similar or superior to the Spectinomycin, Gentamycin and Moxifloxacin used as references, while most of them were more active than Fluconazole and Ketoconazole used as reference antimycotic against *Candida* sp.

**Conclusion.** 23 new thiazolin-4-ones were synthesized and investigated for their antimicrobial potential. All compounds presented antibacterial and antifungal properties while some of them showed the most promising antifungal activity.

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## SYNTHESIS OF SOME NEW THIAZOLYL-OXADIAZOLES WITH BIOLOGICAL POTENTIAL

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**Introduction.** Considering that molecules containing oxadiazoles or thiazoles are associated with many types of biological actions, the combination of the two systems in the same molecule could result in compounds with an expanded therapeutic area.

**Material and methods.** 13 thiazolyl-oxadiazoles were obtained by oxidative cyclization:

After the condensation of aldehydes and acyl hydrazine the resulted hydrazide-hydrazone derivatives were subjected to the reaction with potassium carbonate and iodine at room temperature until the conversion was completed.

8 thiazolyl-oxadiazoles were obtained by dehydrative cyclisation, by the one-pot reaction of aryl carboxylic acids with acylhydrazide and POCl<sub>3</sub> as dehydrating agent.

**Results.** The completion of each reaction was monitored by TLC. The structures of the newly synthesized compounds were correlated with the data obtained from <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectrometry.

**Conclusion.** In summary, we have used two different methods for the synthesis of thiazole-1,3,4 – oxadiazole derivatives: an oxidative cyclisation method using K<sub>2</sub>CO<sub>3</sub>/I<sub>2</sub> and a dehydrative cyclisation using phosphorus oxychloride. Mild reaction conditions, environmental friendly catalyst and higher yields make the oxidative cyclisation method superior to the dehydrative cyclisation method.

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## ANTITUMOR ACTIVITY OF NEW Cu(II) COMPLEXES

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**Introduction.** A new N-sulfonamide ligand (HL1= N-(5-(4-methoxyphenyl)-[1,3,4]-thiadiazole-2-yl)-toluenesulfonamid) and two Cu(II) complexes, [Cu(L1)2(py)-2-] (C1) and [Cu(L2)2(py)-2(H2O)-] (C2) (HL2=N-(5-(4-methylphenyl)-[1,3,4]-thiadiazole-2-yl)-benzenesulfonamide) were synthesized.

**Material and methods.** The X-ray crystal structures of the complexes have been determined. In the complex C1, the Cu(II) ion is four-coordinated, forming a CuN4 chromophore and in the complex C2, the Cu(II) ion is five-coordinated, forming a CuN4O chromophore. The ligand acts as monodentate, coordinating the metal ion through a single N thiadiazole atom. The molecules from the reaction medium (pyridine and water) are also involved in the coordination of the Cu(II) ion. The complexes have a square-planar (C1) and a slightly distorted square pyramidal (C2) geometry. The compounds were characterized by FT-IR, electronic, EPR spectroscopic and magnetic methods.

**Results.** The nuclease activity studies of the synthesized complexes confirm their capacity to cleavage the DNA molecule. The cytotoxicity studies were carried out on melanoma cell line WM35 and confirm that both compounds inhibit the growth of these cell lines.

**Conclusion.** They have a higher activity compared to a platinum drug, carboplatin.

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## INVESTIGATION OF THE METAL COMPLEXES - DNA INTERACTIONS. EXPERIMENTAL TECHNIQUES

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**Introduction.** Deoxyribonucleic acid (DNA), as a vector for genetic information in living organisms, is one of the main targets for pharmacologically active molecules, especially for antitumor, antiviral and antibacterial agents. In this context, understanding how metal complexes interact with DNA is of crucial importance, as it represents an important step in the reaction pathways resulting in the complex-initiated cleavage of the nucleic acid.

**Material and methods.** A series of experimental techniques can be employed in the study of metal complexes-DNA interactions: UV-Vis spectroscopy (metal complex titration with DNA and DNA thermal denaturation), competitive binding fluorescence studies and DNA viscosity measurements.

**Results.** Interpretation of the data provided by the employed techniques gives insight regarding the type and intensity of the interaction taking place between the DNA molecule and the metal complex. UV-Vis spectroscopy and fluorescence studies indicate the strength of the interaction, while the viscosity measurements allow us to distinguish between the different possible interaction types: covalent binding (adduct formation via alkylation) or non-covalent binding (intercalation between adjacent base pairs, minor and major groove binding and electrostatic interactions with the sugar-phosphate backbone).

**Conclusion.** Considering that an essential step in exerting its effect is the interaction of a drug with its target, studying the type and strength of metal complexes-DNA interactions from a quantitative as well as qualitative point of view is of outmost importance. The presented techniques provide the necessary information in order to assess the therapeutic potential of such molecules.

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## IN VIVO TOXICITY ASSESSMENT OF SOME RUTHENIUM COMPLEXES IN RODENTS

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**Introduction.** Ruthenium complexes represent a class of transition-metal-based agents with important and diverse therapeutic applications. Over the last few decades, many examples of anticancer ruthenium compounds have been reported, some of which are already into clinical trials, such as the well-known complexes [imiH] trans-[Ru(N-imi)(S-dmso)Cl<sub>4</sub>] (NAMI-A) and [Na]trans-[Ru(N-ind)<sub>2</sub>Cl<sub>4</sub>] (NKP1339). The mechanisms of antineoplastic activity may involve interactions with DNA, but also with non-nuclear targets, such as mitochondria or cell surface, hypoxia-sensitizing, antioxidant or apoptotic activity. In addition, ruthenium has the ability to mimic iron in binding to certain biological molecules reaching high concentrations in fast dividing cancer cells that have higher affinity to iron. In vivo toxicity studies are required to verify relative low toxicity of these compounds and to assess the therapeutic security level.

**Material and methods.** The most effective compound in vitro was selected and tested in vivo in mice and rats and its intraperitoneal toxicity was evaluated. Hematological and histopathological analyses were performed.

**Results.** The symptoms were observed only in case of the highest doses as well as some histopathological changes. The hematological profile was not modified at any doses tested.

**Conclusion.** The ruthenium complex tested can be considered to have low toxicity after intraperitoneal administration in rodents.

**Acknowledgements.** This work was supported by the Swiss Enlargement Contribution in the framework of the Romanian-Swiss Research Program, project number IZERZO-142198/1 and the European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/136893.

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## SYNTHESIS AND CHARACTERIZATION OF NEW BETA-AMINO ACIDS CONTAINING THE THIAZOLE CORE

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**Introduction.** Beta-amino acids are key structural units of natural peptides and their synthetic analogues, with applications in drug research, functional investigations and molecular recognition studies. The thiazole ring is commonly found in diverse naturally occurring and synthetic biologically active compounds, most of them being introduced in therapy for the treatment of inflammation, cancer diseases, bacterial and fungal infections. Considering the wide applicability of heterocyclic amino acids in drug discovery, our aim was the synthesis of new thiazole based beta-amino acids, as key synthons for the further production of unnatural peptide analogues.

**Material and methods.** The Hantzsch condensation of thiobenzamides with 1,3-dichloroacetone followed by Sommelet reaction was applied for the synthesis of a series of thiazolic aldehydes. Their condensation with malonic acid in the presence of ammonium acetate was investigated for the obtention of the corresponding thiazole derived beta-amino acids. The synthesized compounds were purified and characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectrometry.

**Results.** A series of 3-amino-3-(2-arylthiazole-4-yl)propanoic acids were synthesized and physico-chemically characterized starting from thiazolic aldehydes, malonic acid and ammonium acetate. The spectral analysis confirmed the structures of the synthesized compounds.

**Conclusion.** New beta-amino acids containing the thiazole core were synthesized and characterized. Their utility as key-synthetic intermediates will be exploited in further studies.

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## LIPOPHILICITY EVALUATION OF SOME NEW PYRIDIN-3/4-YL-THIAZOLO[3,2-B][1,2,4]TRIAZOLE COMPOUNDS WITH ANTI-INFLAMMATORY ACTIVITY

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**Introduction.** Among polyheterocyclic compounds thiazolo[3,2-b][1,2,4]triazoles are well known for their pharmacological activities. Presently, drug research and design involves structure-property relationships studies. Lipophilicity is a physico-chemical property which influences the pharmacokinetics and the pharmacodynamics of compounds. As a consequence, lipophilicity plays a very important role in drug development. Therefore, the aim of the present study was to evaluate the lipophilicity of some new pyridin-3/4-yl-thiazolo[3,2-b][1,2,4]triazole compounds in order to determine possible structure-lipophilicity relationships.

**Material and methods.** Reversed-phase thin layer chromatography (RP-TLC) was used to determine the lipophilicity parameters. Five different concentrations of methanol-water were used as mobile phase (55%, 60%, 65%, 70%, 75% methanol). Computed log P values were estimated using different software.

**Results.** Different chromatographic lipophilicity parameters were obtained by RP-TLC. All chromatographic lipophilicity parameters were correlated with different computed log P values, good correlation being observed ( $R^2 > 0.83$ ). Lipophilicity chart and lipophilicity space divided the tested compounds into different groups according to their structural similarities.

**Conclusion.** Newly obtained pyridin-3/4-yl-thiazolo[3,2-b][1,2,4]triazole compounds with anti-inflammatory activity were investigated by RP-TLC. All experimental results were correlated with various log P values. PCA analysis distributed the tested compounds into three groups. Compounds possessing good and moderate anti-inflammatory activity were placed in the first two groups.

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## SYNTHESIS AND PHYSICO-CHEMICAL CHARACTERIZATION OF SOME NEW THIAZOLIC CHALCONES

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**Introduction.** Natural and synthetic chalcones represent key structures which can be exploited individually, or as precursors for the synthesis of new compounds with biological potential, from classes of epoxydes, flavones, flavanones or auronones. The thiazole ring is commonly found in the structure of natural and synthetic compounds possessing different biological effects. Based on these considerations, our aim was the synthesis and physico-chemical characterization of new chalcones containing the thiazole core, in order to evaluate their biological potential.

**Material and methods.** The thiazolic chalcones were synthesized by the Claisen-Schmidt condensation of some thiazolic aldehydes with acetophenone derivatives substituted in the ortho- and/or para- positions with hydroxy and methoxy groups. The synthesized compounds were purified and spectrally characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR, IR and MS.

**Results.** It was investigated the influence of the nature and the position of the substituents grafted on the acetophenone rest for the reaction progress. The synthesized chalcones were purified by recrystallization or by column chromatography. The structures of the obtained products were confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR, IR and MS analysis.

**Conclusion.** A series of new thiazolic chalcones were obtained and physico-chemically characterized. Their biological potential will be investigated in further studies.

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## SYNTHESIS AND CHARACTERIZATION OF SOME AMINO-THIADIAZOLE AND MERCAPTO-TRIAZOLE DERIVATIVES AS KEY INTERMEDIATES FOR THE OBTENTION OF NEW POLYHETEROCYCLIC ANTI-INFLAMMATORY AGENTS

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**Introduction.** The 1,3,4-thiadiazole and 1,2,4-triazole rings are associated with anti-inflammatory, analgesic and antioxidant activities. Consequently, our aim was to synthesize new imidazo[2,1-b][1,3,4]thiadiazole and thiazolo[3,2-b][1,2,4]triazole derivatives bearing adequate moieties which could lead to anti-inflammatory and analgesic properties.

**Material and methods.** The condensation of hydrazides with potassium thiocyanate afforded the corresponding 5-aryl-3-mercapto-1,2,4-triazoles, as intermediates for the synthesis of thiazolo[3,2-b][1,2,4]triazoles. The 5-aryl-2-amino-1,3,4-thiadiazoles, key-intermediates for the synthesis of imidazo[2,1-b][1,3,4]thiadiazoles, were synthesized by the condensation of aromatic carboxylic acids with thiosemicarbazide. The condensation of the obtained mercapto-triazoles with  $\alpha$ -halogenocarbonyl compounds afforded the corresponding thiazolo[3,2-b][1,2,4]triazoles, via acyclic thioether intermediates. Imidazo[2,1-b][1,3,4]thiadiazole derivatives were directly obtained by the condensation of amino-thiadiazoles with  $\alpha$ -halogenocarbonyl compounds.

**Results.** A series of amino-thiadiazoles, mercapto-triazoles, thioethers, thiazolo-triazoles and imidazo-thiadiazoles substituted with phenyl, p-chlorophenyl, p-methoxyphenyl, p-bromophenyl and p-trifluoromethylphenyl were synthesized in good yields. In order to investigate the influence of the reaction conditions (temperature, catalyst) for the reaction course in the formation of the polyheterocyclic ring systems, the synthesis was performed at room temperature, in the presence of sodium acetate and at reflux, in acid catalysis. The obtained compounds were characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR and MS.

**Conclusion.** A series of amino-1,3,4-thiadiazole and mercapto-1,2,4-triazole derivatives were synthesized and physico-chemically characterized. Their utility as key-synthetic intermediates was exploited for the synthesis of new imidazo[2,1-b][1,3,4]thiadiazoles and thiazolo[3,2-b][1,2,4]triazoles.

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## FORMULATION DEVELOPMENT AND EFFICACY EVALUATION OF AN ANTI-AGING CREAM CONTAINING HERBAL EXTRACTS

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**Introduction.** The main objectives of the present study were to formulate an anti-age cream based on natural ingredients - Centella asiatica oil, Spilanthes acmella oil and Zingiber officinale extract and to evaluate the characteristics and the efficacy of the emulsion.

**Material and methods.** Centella asiatica oil and Spilanthes acmella oil which stimulate the collagen synthesis and Zingiber officinale extract which reduces elastin fibers' degradation were selected as active ingredients. Formulation 1 (F1) was prepared using glyceryl stearate and Cremofor25 while Formulation 2 (F2) was prepared using glyceryl stearate and AmphisolK as emulsifiers, the other ingredients remaining the same.

The characterization of the formulas was performed by measuring the viscosity, the oil droplet size and the polidispersity index of the emulsions; also the texture analysis (firmness, adhesiveness, consistency and spreadability) was performed using Brookfield texture analyzer CT3.

The anti-aging effect of the F2, which revealed the most suitable texture and stability characteristics, was evaluated. The thickness of the dermis was assessed before and after 6 and 12 weeks of cream application using the scanner DUB-cutis®.

**Results.** The mean diameter of oil drops was  $22.72 \pm 7.93$  mm (F1) and  $10.26 \pm 4.72$  mm (F2) and the polidispersity index was 35.4% and 45.7%, respectively. The mean values for consistency were  $594.7 \pm 10.3$  g (F1) and  $300.5 \pm 14.5$  g (F2), the average values for adhesion were  $15.61 \pm 0.8$  mJ (F1) and  $22.25 \pm 4.3$  mJ (F2) for firmness were  $51.2 \pm 0.8$  g (F1) and  $30.3 \pm 4.3$  g (F2) and the spreadability had values between 72.63 mm<sup>2</sup> (F1) and 73.3 mm<sup>2</sup> (F2). The mean values of the thickness of the dermis increased from  $1454 \pm 287.95$  μm to  $1846.4 \pm 451.74$ .

**Conclusions.** An antiage emulsion with appropriate texture and excellent cosmetic properties was formulated. The tested cream revealed positive effects on increasing the thickness of the dermis after 12 weeks of cream application.

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## OFF-LABEL AND UNLICENSED MEDICATION USE IN HOSPITALIZED CHILDREN - AN OBSERVATIONAL STUDY

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**Introduction.** Off-label medication use is a frequent phenomenon among the therapeutic pediatric approaches, involving both benefits and risks. The objective of this study was to identify the prevalence of this phenomenon at the Pediatrics Clinic III, Cluj- Napoca.

**Material and methods.** This observational prospective study included all pediatric patients consecutively admitted in the Clinic, during a 61 days interval. The demographic information, medical history and treatments prescribed during hospitalization, were recorded. Off-label, unlicensed or contraindicated medication use was identified using the Summary of medical product characteristics available from the National Agency for Medicines and Medical Devices and the information mentioned in the British National Formulary (2014 edition).

**Results.** Respiratory problems were the most frequent reason for hospitalization among the 100 evaluated patients. The average age was 3.9 years old ( $SD \pm 4.02$ ) and 59 (59%) of children were 2 to 11 years old. 31 (88.57%) of children less than 2 years old and 49 (83.05%) of those 2-11 years old, received off-label medications during hospitalization. 42.30% of the 78 prescribed specialties were used off-label, at least once and 14.10% were unlicensed. 76% of all children received at least one off-label medication. We identified 174 instances of off-label use, 25 instances of unlicensed use, 7 instances of contraindicated medications, while 2 patients received 2 medications without any mention of a pediatric use. Ibuprofen, fluticasone and salbutamol were the most frequently recommended as off-label for dose, indication or age. The most frequent reasons for the off-label use, as expressed by the prescribing physicians, were the severe clinical presentation and the lack of the appropriate medications at the hospital's pharmacy.

**Conclusion.** Off-label medication use was frequent in the analyzed group and the results are comparable to those presented in the literature.

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## MARKERS FOR THE VALIDATION OF A HEART FAILURE ANIMAL MODEL

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**Introduction.** For both left ventricular hypertrophy (LVH) and heart failure (HF), high blood pressure is the most prevalent cause. The major objective of the present study was to perform abdominal aortic banding (AAB) in rats, to induce pressure overload in order to mimic high blood pressure and to induce its complications LVH and subsequently HF. With the use of different markers, we characterized the evolution of the cardiac function from normal to LVH and to HF.

**Material and methods.** We used two animal groups: AAB (n=20) and sham (n=10). Echocardiography was performed at four time-points (baseline, 8, 18 and 24 weeks post-operation - PO), whereas plasma and tissue analysis was performed at two time-points (18 and 24 weeks after operation).

**Results.** LVH parameters (heart weight-to-body weight ratio, LVmass, LVmass-index, anterior, posterior and relative wall thickness) showed significant increase in AAB compared to sham rats at 18 weeks PO. LV dimension parameters (LVEDd, LVEDs) increased significantly in the AAB group, suggesting dilation of the LV at 24 weeks PO. LV performance parameters, fractional shortening (FS%) and ejection fraction (EF%), decreased in the AAB group suggesting a transition from LVH (at 18 weeks PO) to decompensated HF (at 24 weeks PO).

Malondialdehyde (MDA) plasma levels at 18 and 24 weeks were higher in AAB, compared to sham rats, whereas at 24 weeks PO, superoxide dismutase (SOD) plasma levels were lower in AAB compared to sham rats.

**Conclusion.** MDA, SOD and echocardiography parameters were used to validate a rat model of pressure-overload. This animal model can be used in the future for pathophysiology and pharmacology studies.

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## CO-PRESCRIBING OF RENIN-ANGIOTENSIN SYSTEM (RAS)-ACTING AGENTS IN CLINICAL PRACTICE

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**Introduction.** Due to concerns that combining RAS-acting agents could increase the risk of hyperkalaemia and renal failure compared with RAS-acting monotherapy we conducted an investigation to describe the extent and patterns of co-prescription of RAS-acting agents in an electronic health record database.

**Material and methods.** The retrospective study included all patients hospitalized during 18 months in 2013-2014 with a prescription of a RAS-acting agent at hospital discharge: angiotensin-converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARBs) or a combination of the two. Variables like demographics, co-morbidities, co-medications and proportion of patients with high creatinine and potassium values before discharge were analyzed.

**Results.** Out of a total number of 10315 patients, 1003 (9.72%) were prescribed an ACEi, 254 (2.46%) an ARB and 24 (0.23%) were co-prescribed an ACEi and an ARB. There were no differences regarding sex and age between these groups. In patients with diabetes mellitus (n=1674) an ACEi, ARB and a combination of the two were prescribed in 192 (11.47%), 71 (4.24%) and 14 (0.83%) respectively. In patients with renal disease (n=609) an ACEi, ARB and a combination of the two were prescribed in 62 (10.18%), 27 (4.43%) and 5 (0.82%) respectively. Creatinine values (as per the last measurement before discharge) higher than 1.7 mg/dL and potassium values higher than 5.5 mmol/L were found in 2.64%, 6.88% and 13.04%, respectively in 1.03%, 2.02% and 0% of patients discharged with an ACEi, ARB and a combination of the two.

**Conclusion.** The co-prescription of (RAS)-acting agents is higher in patients with diabetic and renal disease comparing to overall population, being prescribed in more patients with altered creatinine values compared to patients receiving monotherapy.

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## PREVALENCE AND TREATMENT OF POSTOPERATIVE PAIN

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**Introduction.** Pain is one of the most frequent symptoms reported by patients in postoperative period and could and should be minimized. The present study aimed to evaluate postoperative pain and analgesia used for its relief in oral and maxillofacial surgery.

**Material and methods.** A prospective study was conducted on patients aged over 18 years old, hospitalized in the Clinic of Oral and Maxillofacial Surgery in Cluj-Napoca, Romania, between September 2014 and February 2015. Patients gave informed consent for participation. Patients self-evaluated the intensity of postoperative pain using the Numeric Pain Rating Scale (NRS). Predictive factors for analgesic consumption and pain intensity were also analyzed.

**Results.** The mean age for the 104 patients included was 45.9 years (range: 18-82). Almost half of the patients reported moderate and severe postoperative pain ( $\text{NRS} \geq 4$ ) during the first 24 hours after the intervention. Mean NRS scores for reported pain intensity were: 1.3 before surgery, 2.1 after 1 hr, 2.0 after 4 hrs, 1.8 after 8 hrs, 1.9 after 12 hrs and 1.4 after 24 hrs from the moment of surgery. The main analgesics used were metamizole, ketoprofene and paracetamol. Depression, type of anesthesia and surgery were found to be associated with postoperative analgesic consumption, while presence of co-morbidities and type of surgery with pain intensity.

**Conclusion.** Even if patients received analgesic treatment in the postoperative period, they still reported moderate to severe pain during the 24 hours after the surgery. Metamizole was the analgesic most used for the relief of pain while it is one of the most popular analgesics in Romania. Identifying predictors in patients at risk for postoperative pain could help to assure a better, early and effective pain management.

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## DRUG-DRUG INTERACTIONS OF STATINS IN HOSPITALIZED PATIENTS: RESULTS FROM A PROSPECTIVE OBSERVATIONAL STUDY

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**Introduction.** Statins association with other drugs may enhance the risk of adverse reactions, of which the most frequent are the muscle-related ones due to elevated serum concentration of the statins.

**Aim:** To determine the prevalence of statins potential drug-drug interactions (pDDIs) in hospitalized patients that had been prescribed statins before/during hospitalization and to find out how often they are associated with clinical outcomes.

**Patients and methods:** This prospective, non-interventional study performed in two internal medicine departments included patients with statin therapy before/during hospitalization. Data on each patient demographic characteristics, co-morbidities and treatment was collected from medical charts and interviews. We evaluated patients' therapy for pDDIs using Thomson Micromedex Drug Interactions checker and we ranked the identified DDIs accordingly. Each patient with statin treatment before admission was additionally interviewed in order to identify the clinical outcomes.

**Results.** In 109 patients on statin treatment we found 35 pDDIs of statins in 30 (27.5%) patients, most of which in the therapy before admission (27 pDDIs). The pDDIs were moderate (20 pDDIs) and major (15 pDDIs). The drugs most frequently involved in the pDDIs were amiodarone (10 pDDIs) and fenofibrate (6 pDDIs). We identified 12 pDDIs of statins with CYP3A4 inhibitors and 4 pDDIs of statins with colchicine, a CYP3A4 substrate. Two of the patients with pDDIs reported muscle pain, both having additional risk factors for statin induced muscle toxicity. No other pDDIs had clinical outcome.

**Conclusion.** The prevalence of statins' pDDIs was high in our study, mostly in the therapy before admission, with only a small number resulting in clinical outcome (2 cases of muscle pain). Further prospective research on a larger number of patients would allow a more accurate estimation of the pDDIs' prevalence and a causal association with muscular effects.

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## STUDY OF ROSMARINIC ACID IN SOME PLANT EXTRACTS

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**Introduction.** Rosmarinic acid (RA), which is an ester of the caffeic acid, is one of the most significant antioxidant naturally polyphenolic compound in the family Lamiaceae. This study was performed to evaluate the RA and polyphenolic contents in some medicinal plants (*Origanum vulgare*, *Melissa officinalis*, *Rosmarinus officinalis*, *Ocimum basilicum*, *Salvia officinalis* and *Hyssopus officinalis*), and their antioxidant activity.

**Material and methods.** In this study, using HPLC-MS system, quantification of RA was carried out in ethanolic extracts of these medicinal plants. The total polyphenolic content was spectrophotometrically determined. The extracts were screened for antioxidant activities using DPPH, FRAP, hemoglobin ascorbate peroxidase activity inhibition (HAPX) and EPR spectroscopy methods.

**Results.** The ethanolic extracts revealed the presence of the RA in the largest amount in *O. vulgare* (1.24 mg/mL) and in the lowest in *R. officinalis* (0.13 mg/mL). The *O. vulgare* extract exhibited the highest antioxidant capacity in line with the rosmarinic acid and polyphenolic contents.

**Conclusion.** The obtained results may indicate that the rosmarinic acid content could be used as a taxonomic marker and confirm, at the chemical level, differences among species. Furthermore, these medicinal species can be exploited as sources of natural antioxidants linked to their total polyphenolic and RA contents.

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## THE INFLUENCE OF MOLECULAR STRUCTURE MODIFICATIONS ON VIBRATIONAL PROPERTIES OF SOME BETA BLOCKERS: A COMBINED RAMAN AND DFT STUDY

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**Introduction.** We report results of a systematic Raman, SERS and DFT study on four beta-blocking molecules: atenolol, metoprolol, propranolol and, for the first time reported in the literature, on bisoprolol. The choice of these molecules was motivated by the structural similarities between the first three on one hand and by their differences relative to propranolol.

**Material and methods.** All reagent used were analytical All the molecules were purchased from Sigma-Aldrich. Hydroxylammonium chloride and sodium hydroxide were purchased from Merck and VWR, respectively, whereas tri-natriumcitrate-dihydrate, and tetrachloroauric(III) acid trihydrate were from Roth. All reagents were used without further purification.

**Results.** While the phenyl group vibrations dominate the Raman spectrum in the case of atenolol, bisoprolol and metoprolol, the spectrum of propranolol presents high intensity vibrations of the naphthyl group. SERS performed on gold and silver colloids, at various pH conditions, revealed a higher sensitivity for propranolol detection. The pH dependence of the spectrum indicates that the studied beta-blockers attach themselves to the metal nanoparticles in a protonated form. The molecular adsorption geometry on metal nanoparticles surface has been evaluated by using the experimental SER spectra and the quantum chemical calculations.

**Conclusion.** The Raman spectra revealed the major role played by the central aromatic group, the ring vibrations dominating the overall spectra in the 200-2000 cm<sup>-1</sup> region. The replacement of the phenyl ring (existing in ATE, BIS and MET) with a naphthyl one (for PRNL), leads to a dramatic change in the Raman pattern. The SERS results show a higher sensitivity for PRNL as compared to BIS, ATE and MET. The molecular orientation relative to the surface is most probably determined by the oxygen atoms with the central ring having a tilted orientation relative to the nanoparticles surface.

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## STUDY ON THE EUROPEAN MARKET OF MEDICINES

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**Introduction.** Increasingly more and in different situations discussions revolve around marketing and market. We are convinced of this if we follow various economic TV programs, browse through specialized publications and analyze the organization and the operation of companies operating in a market economy. Therefore, marketing and market are important, interesting and worth investigated. The objectives of the present study were to analyze and present the pharmaceutical market in Romania and Europe, especially from the following five European countries, the EU-5 (France, Germany, Italy, UK and Spain). Another objective was to highlight the pharmaceutical market predictions for the coming years.

**Material and methods.** For conducting the study, “desk research” and predictive methods were primarily used, studying the files of pharmaceutical companies, national statistics, government reports, company analysis published data and market studies, as well as information from the media.

**Results.** Highlighting achievements include pharmaceuticals market values, production of medicines in Europe and in Romania in recent years, the major producers and distributors, the best-selling or the most expensive drugs in Romania. Results present the market of medicines in Europe, the medicines distributors and producers, exploring the availability and consumption of certain types of products, such as original and generic medicines or the medicines subject to prescription.

**Conclusion.** Predictions show that by 2018 there will not be major changes in the medicines market, emphasizing that this economy is relatively stable, with no significant fluctuations from year to year.

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## RISK MANAGEMENT IN PREVENTING MEDICATION ERRORS

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**Introduction.** Medication errors represent frequent risks in professional practice. In a community pharmacy, they can be prevented by making and implementing effective risk management plans. The aim of this paper was to create a risk management plan for the most important medication errors encountered in a community pharmacy. Our objectives were to present appropriate tools for the community pharmacist to manage such errors and a practical example of identifying risk minimization strategies.

**Material and methods.** In order to identify the most frequent medication errors, the brainstorming method was used. The errors were organized, based on their relationship, using the affinity diagram. Because of the large number of errors found and of limited resources, a prioritization matrix was used, in order to select the most important ones. For each of them, a risk minimization strategy was proposed.

**Results.** Following the brainstorming, a number of 35 medication errors were identified. Using the affinity diagram, they were grouped into three categories: prescribing, dispensing and administration errors. Within the prioritization matrix, these errors were classified using four criteria: patient safety, relevance for pharmacists, human resources needed and the cost of implementing a risk minimization strategy. Based on the final score, a total of 18 medication errors were selected, for which risk management strategies were proposed.

**Conclusion.** The implementation of a risk management plan, including preventive and corrective actions in the field of medication errors, represents an essential step in addressing this issue and in managing their consequences for patients. The risk management plan can be an important part in the policy of quality assurance in a community pharmacy activity.

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## AN ANALYSIS OF THE FACTORS INFLUENCING THE ANTIMICROBIAL ACTIVITY OF DIFFERENT SILVER WOUND DRESSINGS

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**Introduction.** The wound dressings with antimicrobial properties – as silver dressings - represent the therapeutic strategies used for the management of wound infections and wound healing, with an impact on the cost of health-care. The aim of this study was to compare the in vitro antimicrobial activity of several silver dressings.

**Material and methods.** Five silver dressings were included in the study: Actisorb®Ag, Atrauman®Ag, Mepilex®Ag and PansAg (Velfina). The analysis of the antimicrobial activity was conducted using four clinically relevant pathogens: Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Klebsiella. The antimicrobial activity was evaluated by using a zone-of-inhibition (ZOI) test. Silver release was determined by Inductive Coupled Plasma Mass Spectrometry (ICPMS).

**Results.** The results of the test indicated antimicrobial activity for all the dressings tested, but demonstrated different degrees of efficacy against the pathogens included in the test. The dressings containing silver in the ionic form (Aquacel®Ag) proved to be the most efficient ones, according to the test performed. There was no correlation between the silver content and the antimicrobial activity of the evaluated dressings. There was no relationship between silver release and silver content. The results suggest that the antimicrobial activity of the silver dressings tested is influenced by: (1) the silver's chemical and physical form (metallic, ionic state or salt); (2) the distribution of the silver within the dressing (silver-coated or impregnated); (3) the nature of the dressing's support materials (hydrocolloid, foam, fibrous dressings).

**Conclusion.** The study suggests that the selection of the wound dressings must be based on an assessment of the overall characteristics of the dressings involved, which combines the antimicrobial activity of the silver with their exudate management properties.

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## FORMULATION OPTIMIZATION OF ENOXAPARIN SODIUM LOADED POLYMERIC MICROPARTICLES FOR COLON-SPECIFIC DELIVERY

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**Introduction.** The aim of this study was to investigate the influence of formulation factors on the characteristics of enoxaparin sodium polymeric microparticles for colon-specific delivery, especially the in vitro release, and optimize the preparation conditions.

**Material and methods.** An experimental design with three factors and three levels was used. The variables were Eudragit® FS 30D-Eudragit® RS PO ratio (EFS/ERS), polyvinyl alcohol (PVA) concentration and sodium chloride (NaCl) concentration. The responses were the morphology and size of microparticles, the encapsulation efficiency of enoxaparin in microparticles and the percentages released over 24 hours in environments that simulated the gastric, duodenal and colonic medium. All samples were prepared according to the experimental design matrix consisting of 17 experiments. The particles were obtained by the double emulsion method followed by solvent evaporation.

**Results.** The size of the microparticles was not affected by any of the studied factors. The encapsulation efficiency was positively influenced by the increase of NaCl concentration, the percentages of encapsulated enoxaparin reaching 94% for some formulations. Also, increasing the ratio EFS/ERS ensured a relatively complete release of enoxaparin in the environment simulating the colonic pH. Based on these results, the optimum conditions for the preparation of enoxaparin polymeric microparticles were decided and the optimum formulation was prepared. The results obtained for the optimum formulation in terms of in vitro enoxaparin release in conditions which simulated gastrointestinal transit were good, the microparticles releasing only 9.42% enoxaparin in acidic environment and 15.16% in the medium which simulated the duodenal pH, but allowing the release of up to 89.24% in the medium which simulated the colonic pH.

**Conclusion.** The in vitro release profile of enoxaparin was close to the ideal one, therefore the system is promising for an in vivo study.

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## FORMULATION, PREPARATION AND IN VITRO AND IN VIVO EVALUATION OF KETOPROFEN COMPRESSION-COATED TABLETS FOR COLON-SPECIFIC RELEASE

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**Introduction.** The aim of this study was to formulate and prepare compression-coated tablets for colonic release (CR-tablets), and to evaluate the bioavailability of ketoprofen following the administration of a single dose from minitabets with immediate release (IR-tablets) compared to CR-tablets.

**Material and methods.** CR-tablets were prepared based on time-controlled hydroxypropylmethylcellulose K100M inner compression coat and pH-sensitive Eudragit® L 30D-55 outer film-coating. The clinical bioavailability study consisted of two periods, in which two formulations (IR-tablets and CR-tablets) were administered to 6 volunteers, according to a randomized cross-over design. The pharmacokinetic parameters of ketoprofen were determined using the noncompartmental pharmacokinetic analysis. The apparent cumulative absorption amount of ketoprofen was estimated by plasma profile deconvolution.

**Results.** CR-tablets were able to delay ketoprofen's release. Compared to IR-tablets used as reference, for the CR-tablets the maximum plasma concentration (C<sub>max</sub>) was lower (4920.33±1626.71 ng/mL vs. 9549.50±2156.12 ng/mL for IR-tablets) and the time needed to reach C<sub>max</sub> (T<sub>max</sub>) was 5.33±1.63 h for CR-tablets vs. 1.33±0.88 h for IR-tablets. In vitro-in vivo correlation of the cumulative absorption amount of ketoprofen showed similar values for the two formulations. Also, the lag time presented by CR-tablets both in vitro and in vivo (3-6 h) can be correlated with the colon arrival time mentioned in the literature (3-4 h).

**Conclusion.** The obtained pharmacokinetic parameters and the in vitro-in vivo correlation demonstrated the reliability of the developed pharmaceutical system and the fact that it is able to avoid the release of ketoprofen in the first part of the digestive tract.

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## INVESTIGATING THE INFLUENCE OF FREEZING RATE ON MELOXICAM CRYSTAL CHARACTERISTICS DURING LYOPHILIZATION PROCESS

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**Introduction.** Lyophilization is a drying method recently used to develop fast dissolving oral dosage forms. This study aimed at investigating the effect of different freezing rates applied during freeze-drying, on meloxicam crystal size and stability.

**Material and methods.** Three stabilizers at ratios of 0,25 – 1 – 1,75% were used to suspend micronized meloxicam (PVP K25, PEG 4000 and Poloxamer 188). The suspensions were processed by sonication followed by high pressure homogenization. Using a design of experiments, an optimal suspension was obtained, in terms of average size and size distribution. It was lyophilized with three different freezing rates, of 1°C / minute (fast), 0,5°C / minute (slow) or with thermal treatment.

**Results.** The initial average crystal size was  $4,51 \pm 0,57 \mu\text{m}$  with polydispersity index (PdI) 1. Poloxamer was chosen as stabilizer for the optimal suspension, since it delivered the lowest average size,  $463,5 \pm 9,71 \text{ nm}$  and PdI  $0,312 \pm 0,014$ , while preserving the active substance crystallinity. Significant crystal size increase was noticed only when freeze-drying after thermal treatment, with mannitol as cryoprotectant. Neither fast freezing nor the slow have produced significant size changes during freeze-drying, meaning that no aggregation phenomena occurred. The suspension stability was positively influenced by the freeze-drying process, especially when annealing was applied, the Zeta potential absolute values increased. At all freezing rates, the use of mannitol led to higher Zeta potential values, thus lower stability.

**Conclusion.** The average crystal size was constant before and after the freeze-drying process, despite of the different freezing regimes. On the contrary, freeze-drying process promoted higher suspension stability.

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## EXPERIMENTAL DESIGN METHODOLOGY FOR THE PREPARATION AND FORMULATION OPTIMIZATION OF QUERCETIN-LOADED SOLID LIPID NANOPARTICLES

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**Introduction.** The aim of this study was to optimize the preparation of quercetin-loaded solid lipid nanoparticles (Q-SLN) by investigating the influence of formulation and process parameters on the nanoparticles' characteristics.

**Material and methods.** A D-optimal experimental design was constructed considering the lipid ratio, type and concentration of stabilizer, preparation method and sonication time as independent variables. Q-SLN were prepared by the sonication method using glycerol monostearate and cetyl alcohol as solid lipids. Four stabilizers were evaluated: Tween 80, Poloxamer 188, Poloxamer 407 and Soluplus. The stabilizer was either used as an aqueous solution (method A) or melted alongside the lipids (method B). Q-SLN were characterized in terms of size, polydispersity index (Pdl), Zeta potential and quercetin's encapsulation efficiency (EE).

**Results.** According to the experimental data, the smallest particles were obtained by using Soluplus as stabilizer. Q-SLN prepared with Tween 80 showed higher Pdl values, while the samples that contained Poloxamer 188 were more homogeneous. In addition, the former led to higher percentages of encapsulated quercetin, but the latter gave lower EE values. However, regardless of the stabilizer's type, an increase in the concentration resulted in a decrease in size and increase in EE, respectively. The same effects were observed when increasing the sonication time. Apart from the Zeta potential, the influence of the lipid ratio and preparation method on the Q-SLN characteristics could not be determined. Based on these findings, an optimized formulation was established and prepared. The optimized formula presented a small size of 138,43 nm, good homogeneity and stability indicated by the low Pdl (0,26) and Zeta potential (-37,57 mV) values, and a high EE of 94,52%.

**Conclusion.** The overall data showed that the Q-SLN characteristics were mainly influenced by the concentration of stabilizer and sonication time.

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## FORMULATION OF PREDNISOLONE LOADED LONG-CIRCULATING LIPOSOMES FOLLOWING QUALITY BY DESIGN (QbD) APPROACH

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**Introduction.** Quality by design principles were used to assist the formulation of prednisolone loaded long- circulating liposomes in order to gain a more comprehensive understanding of the preparation process. Risk identification and risk analysis was performed in order to obtain the highest risk factors, which were further on subjected to a D-optimal design of experiments.

**Material and methods.** A 19-run D-optimal experimental design was used to study the impact of the highest risk factors on Y1-prednisolone liposomal concentration ( $\mu\text{g/ml}$ ), Y2-encapsulation efficiency (EE%), Y3-size (nm) and Y4-Polydispersity Index. Partial least squares (PLS) method was employed for data fitting and for calculation of statistical parameters.

**Results.** 4 out 6 studied variables were identified as critical parameters affecting the studied responses. Prednisolone molar concentration and the molar ratio of DPPC to MPEG-2000-DSPE had a positive impact on both Y1 and Y2, while the rotation speed at the formation of the lipid film had a negative impact. Increasing prednisolone molar concentration and DPPC concentration along with a slower rotation speed led to a better encapsulation of the drug.

Y3 and Y4 were highly influenced by prednisolone molar concentration and extrusion temperature. Higher drug concentrations led to a final product with smaller size and more homogenous distribution, due to phospholipid-drug interactions causing a smaller internal-to-external volume ratio of the liposomes. An increase in extrusion temperature determined an increase in lipid bilayer fluidity, the liposomes being more susceptible to size reduction.

The accuracy and robustness of the model was further on confirmed.

**Conclusion.** Using this developed model, we were able to establish a design space for prednisolone liposomes in a laboratory setting, within which preparation variability is minimized.

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## DEVELOPMENT AND VALIDATION OF NIR AND RAMAN SPECTROSCOPIC METHODS FOR FAST CHARACTERIZATION OF TABLETS WITH AMLODIPINE AND VALSARTAN

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**Aim.** The objective of this study was to develop and validate NIR and Raman chemometric methods for fast characterization in terms of API content and crushing strength of fixed-dose combination tablets containing amlodipine and valsartan.

**Materials and methods.** The calibration samples for the content uniformity assay were prepared according to a full factorial experimental design with 2 variables (amlodipine, valsartan) and 5 levels (80-90-100-110-120%) resulting a total of 26 formulations. The NIR spectra recorded in transmittance configuration and the Raman spectra in reflectance configuration were analyzed through PCA in order to select the spectral domains that are most sensitive to the change of the API's concentration. The calibration samples for the tablet crushing strength were prepared by compressing the formulation corresponding to the 100% concentration level at six different forces, covering the range of 45N-240N. In this case both NIR and Raman spectra were recorded in reflectance configuration. The selected spectral regions were pre-processed in order to build the PLS regression models. The validation was performed on fully independent batches prepared in three different days by the same procedure.

**Results.** Using the NIR method, the most suitable calibration algorithm for valsartan was using SNV as a pre-treatment method (PLS=2, RMSECV=2.222 mg) on the 12489–5994 cm<sup>-1</sup> region and for amlodipine using SD+SNV (PLS=4, RMSECV=0.482 mg) on the 9149–7660 cm<sup>-1</sup> region. For the Raman method the best calibration algorithm for valsartan was using SNV (PLS=9, RMSECV=1.924mg) on the 150-1635 cm<sup>-1</sup> region, and for amlodipine MSC (PLS=8, RMSECV=0.443 mg) on the 500-1532 cm<sup>-1</sup>; 1626-1713 cm<sup>-1</sup> spectral regions. The best models for crushing strength assessment were developed without applying any preprocessing method using the entire spectral regions.

The NIR and Raman methods developed for content uniformity and tablet crushing strength estimation were successfully validated, using the accuracy profile approach.

**Conclusion.** The research proves the advantages of vibrational spectroscopic methods as PAT tools, due to their non-destructive nature and the short time of analysis that offers the possibility of rapid quality evaluation of physical and chemical properties of fixed-dose combination tablets.

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## THE PHARMACOKINETICS OF TWO NEW FORMULATIONS OF ERLOTINIB

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**Introduction.** Erlotinib is an inhibitor of tyrosine kinase epidermal growth factor receptor used in the treatment of neoplasm other than lung small cell cancer and pancreatic cancer.

There have been developed two new formulations that will be evaluated in terms of pharmacokinetics and the product with the best results will be proposed to be conducted in a pivotal study and registration as a new generic product on the market, with lower cost.

**Material and methods.** An open, balanced, randomized, crossover clinical study with three periods and three treatments (two test and one reference formulations containing erlotinib hydrochloride equivalent to 150 mg erlotinib) was conducted on 15 in healthy volunteers in fasting condition. 13 subjects completed the study. Washout period was 14 days.

27 blood samples were taken (of 4 ml) during each period. After centrifuging the plasma was separated and stored at -50 °C until analysis. For determination of plasma erlotinib it was used HPLC method coupled with mass spectrometry.

Non-compartmental pharmacokinetic analysis was performed using WinNonlin version 5.2. Calculated pharmacokinetic parameters were C<sub>max</sub>, T<sub>max</sub>, AUC<sub>0-t</sub>, AUC<sub>0-∞</sub>, AUC\_% Extrap, Kel and T<sub>1/2</sub>.

Statistical analysis was performed using SAS version 9.3.1 Type III ANOVA for calculating the least square means.

**Results.** It was evaluated the 90% confidence interval for the ratio of test / reference product averages for the parameters considered: C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub>.

Test Formulation A: C<sub>max</sub>: 109.41% [93.46%-128.09%]; AUC<sub>0-t</sub>: 109.61% [98.53%-121.94%]; AUC<sub>0-∞</sub>: 107.39% [96.22%-119.86%].

Test Formulation B: C<sub>max</sub>: 108.53% [92.71%-127.06%]; AUC<sub>0-t</sub>: 94.19% [84.67%-104.78%]; AUC<sub>0-∞</sub>: 95.19% [84.93%-106.68%].

**Conclusion.** Test Formulation A was the closest to the reference formulation and has been chosen to be tested on an adequate number of volunteers in a pivotal study.

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## A PHARMACOKINETIC DRUG INTERACTION ANALYSIS BETWEEN ATOMOXETINE AND ITS INHIBITOR, BUPROPION. A STUDY ON HEALTHY VOLUNTEERS

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**Introduction.** The analysis of the pharmacokinetic parameters of atomoxetine, a compound which is considered a substrate for CYP2D6 metabolizing enzyme, and of its active metabolite, 4-hydroxyatomoxetine.

**Patients and methods.** An open-label, single-center, non-randomized clinical trial which included 20 healthy volunteers, was assessed. The study comprised 2 periods: the reference period (I), when the subjects were administered a single oral dose of 25 mg atomoxetine and the test period (II) when the volunteers received a single oral dose of 25 mg atomoxetine along with 300 mg bupropion. Before the second period, a 6 day treatment was performed, where the volunteers received an oral daily dose of 150-300 mg bupropion. The pharmacokinetic parameters of atomoxetine and its active metabolite were determined using a non-compartmental method and a statistical analysis (ANOVA).

**Results.** The multiple-dose bupropion administration significantly changed the pharmacokinetic parameters of atomoxetine (ATM) and its metabolite, 4-hydroxyatomoxetine (AMM). The mean peak plasma concentration (C<sub>max</sub>) increased for ATM: 386.09±137.34 vs. 226.43±96.06 ng/ml, meanwhile the AMM concentration decreased: 212.62±145.95 vs. 707.93±269.31 ng/ml. The total areas under the curve from time zero to infinity (AUC<sub>∞</sub>) were also significantly modified: 1583.05±1040.29 before and 8062.64±4162.15 h\*ng/ml after the bupropion administration for ATM; 5754.71±1235.5 before and 3858.89±1221.92 h\*ng/ml after, for AMM respectively.

**Conclusion.** The multiple dose bupropion intake, in healthy volunteers, significantly modifies the atomoxetine and its active metabolite pharmacokinetics.

**Acknowledgments.** This work was supported by CNCS Romania – project PN-II-ID PCE-2011-3-0731. PhD student Todor Ioana acknowledges financial support from a POSDRU grant, no. 159/1.5/S/136893 grant with title: “Parteneriat strategic pentru creșterea calității cercetării științifice din universitățile medicale prin acordarea de burse doctorale și postdoctorale – DocMed.Net\_2.0” [Strategic partnership for increasing scientific research performance in medical universities through doctoral and postdoctoral grants].

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## FORMULATION AND OPTIMIZATION OF PLACEBO ORODISPERSIBLE TABLETS BY EXPERIMENTAL DESIGN APPROACH

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**Introduction.** Orodispersible tablets are solid oral dosage forms developed to improve patient compliance to drug therapy. The aim of this study was the optimization of an orodispersible tablet placebo formulation, prepared by direct compression with superdisintegrants.

**Material and methods.** The study was based on an experimental design (DoE) with 5 factors and 2 variation levels, which resulted in 19 runs. Mixtures of two superdisintegrants were used, croscopolidone (CPV) and sodium starch glycolate (SSG), in variable ratios. Mannitol and microcrystalline cellulose (MCC) were used as fillers. The compression force was selected as technological parameter and varied on 2 levels. The tablets were evaluated for disintegration time, wetting time, water absorption ratio, mechanical strength and friability and in vivo disintegration time.

**Results.** The results showed good fit with the developed models, the ANOVA test confirmed that the response variations were determined by the formulation and technological factors. The in vitro disintegration times ranged between 19.83 s and 47.66 s. Disintegration was favored by the presence of CPV. High percentages of SSG determined slow disintegration and wetting, but high water absorption ratios. High compression forces and SSG ratios determined increased crushing strength. For the optimization, two constraints were applied: the disintegration time was minimized and the crushing strength was maximized. The optimal formulation contained 3.41% SSG, 6.58% CPV, 37.96% mannitol, 14.99% MCC, 35.03% lactose, 1% magnesium stearate and 1% sodium saccharine.

**Conclusion.** Direct compression with superdisintegrants led to the preparation of optimal orodispersible tablets and DoE facilitated the understanding of factor effects.

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## LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY DETERMINATION OF FLUCONAZOLE LEVELS IN HUMAN PLASMA FOR BIOAVAILABILITY STUDIES

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**Introduction.** The aim of this study was to develop a rapid method for the determination of fluconazole plasma concentrations for the study of its bioavailability and therapeutic drug monitoring.

**Material and methods.** A rapid high-throughput liquid chromatography method with tandem mass spectrometry detection (LC-MS/MS) was developed. Separation was performed using a Zorbax SB-C18 column with mobile phase consisting of methanol and 0.1% formic acid in water (45:55, v/v) in isocratic elution, with a flow rate of 0.6 mL/min. Detection of fluconazole was performed in multiple reaction monitoring mode (m/z 219.9 from m/z 306.9) using heated electrospray positive ionization. Prednisone was used as internal standard. A single-step protein precipitation with methanol was used for plasma sample preparation.

**Results.** The method was validated with respect to selectivity, linearity ( $r > 0.9978$ ), intra-day and inter-day precision ( $CV < 7.3\%$ ) and accuracy (bias  $< 13.5\%$ ) over a range of 105 - 5600 ng/mL plasma. The lower limit of quantification (LLOQ) was 105 ng/mL and the recovery was between 100.6-108.2%.

**Conclusion.** The LC-MS/MS method developed and validated stands out through simplicity, sensitivity, selectivity, accuracy and accessibility, essential features for high-throughput methods used in routine analysis. It has wide applicability and can be used for clinical and bioequivalence studies, clinical level monitoring and pharmacokinetics.

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## THE IN VIVO ENDOCRINE DISRUPTIVE POTENTIAL OF BUTYL PARABEN

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**Introduction.** Parabens are used as antimicrobial preservatives in personal care products, pharmaceuticals and as food additives. In vitro studies showed that butyl paraben (BuPB) is a relatively potent endocrine disruptive compound.

**Material and methods.** The endocrine disruptive potential of BuPB was evaluated at three dose levels (corresponding to NOAEL (21 mg/kg bw), 5 x NOAEL (105 mg/kg bw) and 10 x NOAEL (210 mg/kg bw)) using the immature rat uterotrophic assay. The compound was administered by subcutaneous injection to Wistar female rats (17-21 days old) for three consecutive days. Estradiol (10 µg/kg bw) was administered as positive control, while the negative control received sunflower oil. The relative uterus weights were calculated by using uterus (mg)/body weight (g) ratio or the uterus (mg)/brain (mg) ratio.

**Results.** The relative uterus weights (uterus/bw) were significantly increased in all doses when reported to body weight, suggesting a relatively strong endocrine disruptive activity (estrogenic activity) of BuPB on Wistar prepubescent female rats. However when uterus/brain ratios were used only the 10 x NOAEL dose induced statistically significant increase compared to the negative control.

**Conclusion.** In the present study BuPB showed estrogenic activity. However, in vivo data should be interpreted with caution, given that a change in body weight will influence the relative organ weight and therefore it might lead to over- or underestimation of the toxicity of a compound.

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## EVALUATION OF THE (ANTI)ANDROGENIC EFFECT OF BINARY MIXTURES OF SELECTED FOOD ADDITIVES AND COSMETIC PRESERVATIVES ON AN ANDROGEN RESPONSIVE CELL LINE

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**Introduction.** During the last two decades a major concern could be noticed regarding the endocrine disruptive potential of certain xenobiotics, with a possible negative impact on humans and wildlife. Most of the studies focused on evaluating the (anti)estrogenic potential. However the impact on the androgenic system is of similar importance.

**Material and methods.** This study aimed for the investigation of the androgenic/anti-androgenic (A/AA) potential of binary mixtures of the selected compounds and the predictability of the combined effect of mixtures based on dose addition (DA) and response addition (RA). An androgen responsive cell line (MDA-kb2) stably transfected with a luciferase reporter gene was exposed to binary mixtures of the selected compounds (with or without adding dihydrotestosterone (DHT)) for 24 hours, followed by cell lysis and luminescence measurement.

**Results.** No significant luciferase induction could be seen in case of the evaluation of the A potential of the individual compounds or binary mixtures. In case of individual evaluation BP, BHA and BHT behaved as androgen receptor (AR) antagonists. These compounds inhibited the AR dependent DHT (5- $\alpha$ -dihydrotestosterone)-induced luciferase activity in a concentration dependent manner (with IC<sub>50</sub> values of 58.51  $\mu$ M, 172.51  $\mu$ M and 43.18  $\mu$ M, respectively), without affecting cell viability. PG showed no androgenic/anti-androgenic (A/AA) effect.

In case of the BP+BHA, BP+BHT and BHA+BHT mixtures we confirmed that the DA model can predict the experimental effect, with some differences however in terms of correlation strength for the different mixtures. Even though the statistical analysis indicated that theoretically it is possible to predict the effect of the PG+BHA mixture by the DA model, this one has no realistic value (the IC<sub>50</sub> value and Hill equation proposed by Sigma Plot for PG are derived from a forced fitting of a curve to the experimental data).

**Conclusions.** The present study confirms, at least partially, the possibility to predict the effect of binary mixtures of AA compounds by using the dose additivity model.

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## INTERACTIONS OF FLUOXETINE AND NORFLUOXETINE WITH NUCLEAR ESTROGEN RECEPTORS

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**Introduction.** Fluoxetine (FLX), a widely used antidepressant, can induce estrogenic effects in vitro and in vivo, causing reproductive and endocrine toxicity in fish, rodents and humans. In vivo, fluoxetine is demethylated to norfluoxetine (NFLX), an active metabolite for which there are no published data on its endocrine effects.

This study aimed to assess if NFLX can also interact with nuclear estrogen receptors (ERs) and to assess the (anti)estrogenic potential of FLX-NFLX mixtures.

**Material and methods.** The in vitro (anti)estrogenic activity of NFLX and FLX-NFLX equimolar mixtures was assessed using a luciferase reporter gene in the T47D-Kbluc breast cancer cell line. These cells express nuclear ERs that can activate the transcription of the luciferase reporter gene upon binding of ER agonists. The luminescence readout was monitored for cells treated with NFLX, FLX and mixtures of NFLX-Estradiol (E2), FLX-E2, NFLX-FLX, NFLX-FLX-E2. Cell viability was assessed using a resazurin-based assay.

**Results.** Both NFLX and FLX were able to induce a significant increase in luminescence compared to control. In mixtures with 30 pM E2, a significant increase in luminescence was observed at low micromolar FLX and NFLX concentrations compared to E2 alone. At 10  $\mu$ M, both compounds slightly decreased the E2-induced luciferase activity. Equimolar mixtures of FLX and NFLX also elicited an increased response in the presence of E2 compared to the response of E2 alone.

**Conclusion.** FLX and NFLX can induce estrogenic effects in vitro at micromolar concentrations. In mixtures with E2, these compounds can either potentiate or decrease the activity of E2, depending on concentration. Equimolar mixtures of FLX and NFLX can elicit estrogenic responses in vitro and can potentiate the response of E2.

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## HPLC DETERMINATION OF NITRATES AND NITRITES IN VEGETABLES FROM CLUJ-NAPOCA FARMERS MARKET

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**Introduction.** The nitrate and nitrite content is a qualitative aspect of food. European countries imposed legislative limits of nitrate content in food commercialized on their territory. The most important contribution of dietary nitrate intake belongs to vegetables. The aim of this study was to implement a dual determination of nitrate and nitrite from vegetable matrix purchased from Cluj-Napoca Farmers Markets. The data obtained was compared with the legislative limits imposed by the European Union and by Romania in specific laws. Also, the average nitrate content per vegetable product was compared with the latest EFSA report on the nitrate content of European vegetables. The probability of exceeding the nitrate daily limit intake was appraised.

**Material and methods.** 224 vegetable products were purchased from Cluj-Napoca Farmers Market from May to July 2015. It was implemented a HPLC method for dual nitrate and nitrite assay from vegetable products. The nitrate was determined in UV at 222 nm wavelength and the nitrite participated first in Griess reaction to form azo-derivatives that were determined and 520 nm wavelength.

**Results.** Above average nitrate levels imposed by EFSA report were determined for onions, garlic and pepper. The limits were surpassed by 48%. Consuming 900 grams of vegetables from the first 3 Santamaria categories (green beans, peas, tomatoes, garlic, carrots, zucchini, cabbage) the accepted daily nitrate intake is not reached. For vegetables like parsley, kohlrabi, celery, consuming 600 grams exceeds the European limits of nitrate intake. Only by consuming 400 grams of vegetables from the fifth Santamaria category (lettuce, rocket, beetroot and spinach) exceeds the daily nitrate limit 1.5 times.

**Conclusion.** The average nitrate determination per vegetable product respects the legislative limits considered. Vegetables from Santamaria's V category contributed the most at the dietary nitrate intake: rocket, lettuce, spinach.

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## CHANGES IN BODY COMPOSITION DUE TO INDIVIDUALIZED NUTRITION COUNSELING

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**Introduction.** Obesity epidemic around the world represents a serious public health problem, as it significantly increases the risk of chronic diseases such as cardiovascular disease, type-2 diabetes, hypertension, and certain cancers. The main objective of this study was to assess changes in body composition during individual sessions of nutrition and dietetic counseling in overweight and obese patients (BMI  $\geq 25$  kg/m<sup>2</sup>).

**Material and methods.** A total of 30 patients (BMI  $\geq 25$  kg/m<sup>2</sup>) received up to 8 face-to-face individual sessions of nutritional education delivered by a dietitian over a 10 weeks period. During sessions we used the person-centered approach, based on individual needs which involve the patient in goal setting and decision making. Anthropometric and body composition parameters were measured in each counseling session. Body composition was determined by bioelectrical impedance analysis using a body composition analyzer (Tanita BC418-MA).

**Results.** Our study revealed important decrease in body weight (mean 7.32%,  $p < 0.05$ ) and total body fat mass (mean 16.07%,  $p < 0.05$ ) due to individualized nutrition counseling. Furthermore, the patients who were engaged in physical exercise during the counseling period showed a greater reduction in total body fat mass than those who did not (22.64% vs. 11.65%).

**Conclusion.** Our results showed that significant weight loss, especially from fat mass can be achieved by individualized nutritional counseling and physical activity, offering an effective strategy in obesity intervention and prevention. Also, it highlights the influence of dietitians in the healthcare system.

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**CONSUMERS' KNOWLEDGE, INTEREST AND ATTITUDE ON FUNCTIONAL FOOD****LUIA FLOREA<sup>1</sup>, LAURA GAVRILAȘ<sup>1</sup>, LORENA FILIP<sup>1</sup>, ANAMARIA COZMA<sup>1</sup>, ROXANA BANC<sup>1</sup>, OANA STANCIU<sup>1</sup>, DAN ISTRATE<sup>1</sup>, DOINA MIERE<sup>1</sup>****<sup>1</sup>Bromatology, Hygiene, Nutrition, Department 3, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania****<sup>2</sup>Medical Informatics and Biostatistics, Department 12 - Medical Education, Faculty of Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania**

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**Introduction.** The term functional food is widely used as a marketing term, but there is no definition recognized globally. The definition of functional food encompasses a wide variety of diet components believed to reduce the risk of specific disease and promote overall health and well-being. The goal of the present study was to evaluate the knowledge, interest and attitude on functional food among Romanian consumers and to test the hypothesis whether there is a link between higher income and higher education and knowledge, interest and attitude on functional foods.

**Material and methods.** Consumers' knowledge, interest and attitude on functional food was assessed using a 10 multiple choice or one choice questionnaire designed by the research team. Data were collected from an anonymous, self-administered online questionnaire using Google Docs in Romania, distributed on social networking sites (n=265).

**Results.** The study showed that 243 out of 265 consumers recognize functional food as whole foods that provide health benefits. The analysis revealed a significant interaction between high income and knowledge of functional foods ( $p < 0.05$ ). Research suggests, furthermore, that high income consumers were more likely to read food labels than low income consumers.

**Conclusion.** Romanian consumers are aware of the health benefits of functional foods but often confuse functional foods with dietary supplements, diet food, medicine herbs and fortified food. Health professionals could benefit from this results as they play an important role in educating and informing consumers.

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## NUTRITIONAL INTERVENTION IN SOME PATIENTS WITH TEMPOROMANDIBULAR JOINT DYSFUNCTION

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**Introduction.** Highly prevalent among the ages 25-54, frequent in women, temporomandibular joint dysfunction (TMD) affects the temporomandibular joint and its structures, primarily by inflammation. Painful symptoms cause proper feeding to be problematic for chronic TMD patients. The aim of this study was to evaluate the efficacy of a modified Mediterranean diet (soft-Mediterranean diet) in reducing the patients' symptoms and pain levels.

**Material and methods.** 3 patients (A, B, C) with TMD symptoms were selected and asked to write a food diary for 21 days. Information about age, height, weight, physical activity level, food allergies and/or intolerances, other acute or chronic diseases were registered. Patients were asked to write the amount and type of food they ate, type and dosage of medication used, and record the intensity of their pain (1-absent pain, 10-unbearable pain). After 21 days, patients were asked to follow a personalized soft-Mediterranean diet for 21 days and continue writing in the food diary.

**Results.** All patients reported a decrease in TMD symptoms upon following the suggested diet. Although patient A continued taking ibuprofen as a means to treat the symptoms, patient C reported a significant decrease of medication doses and frequency.

**Conclusion.** Due to the numerous anti-inflammatory compounds found within the Mediterranean diet, a modified version of it could bring significant improvement to TMD patients and may contribute to the reduction of TMD pain.

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**STUDY REGARDING NUT CONSUMPTION AMONG THE POPULATION OF ROMANIA**

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**Introduction.** Based on their beneficial effects on human health, the global consumption of nuts has increased significantly in the past ten years. However, little is known about the patterns of nut consumption in Romania. Therefore, the aim of this study was to evaluate the habits regarding nut consumption among the Romanian population.

**Material and methods.** Study was carried out using a questionnaire about nut consumption habits, such as the type and amount of nuts consumed, the frequency of consumption, the reasons for consumption and the purchase place. Research was conducted through community portals and, within 3 months time (from March to May 2015), 310 people (260 females and 50 males, with age ranging between 17 and 55 years) participated in it.

**Results.** Only 3% of the respondents reported not consuming nuts, mainly because they are high in calories or cause allergic reactions. The most preferred among the nut consumers were walnuts, almonds, and cashews, while the least preferred were macadamias, brazil nuts and pecans. About 31% of the respondents reported consuming nuts once per week, followed by 27% eating nuts 2-3 times per week. Most of the participants (59%) reported consuming nuts in the recommended amount of 30 g per serving and mainly raw, for their pleasant taste and for their health benefits. A high proportion (67%) of respondents indicated to purchase the nuts from supermarkets.

**Conclusion.** The majority of respondents consume nuts in an adequate amount per serving, but with a low weekly frequency. Nuts are consumed especially for their pleasant taste and for their health benefits, mainly raw, purchased from supermarkets. Further research, on a larger number of subjects, is needed to confirm the results of the present study.

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## BEER CONSUMPTION PATTERNS AMONG STUDENTS: A STUDY AT FIVE UNIVERSITIES IN CLUJ-NAPOCA

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**Introduction.** Drinking beer is one popular behavior that is adopted by many university students. The aim of this study was to assess the beer consumption patterns among students who attend different universities in Cluj-Napoca.

**Material and methods.** The study was carried out at five universities from March to May in the academic year 2014/2015 and included 100 students of both genders (1:1), with 20 students from each university. All students included in this study had the habit of drinking beer. The students were asked to fill out a questionnaire consisting of 13 questions with respect to beer consumption patterns: amount consumed, frequency of consumption, preferred type, preferred place to consume it, and reasons for consumption.

**Results.** The results showed that nearly 47% of the students drank beer 2-3 times per week, 39% 2-3 times per month, and only 5% every day. With regards to the amount consumed per week, 51% of the respondents drank 2-3 beers, 35% one beer, and only 14% more than 4 beers. Male students reported drinking beer more often and in higher amounts compared to female students. Moreover, students of technical faculties ranked first when it comes to how often they drink and to the amount consumed, respectively. Both male and female students indicated a preference for lager beer and for drinking it in public places, such as bars, pubs, restaurants. Most male students cited mood as the reason for drinking beer, whereas female students reported social reasons such as the entourage.

**Conclusion.** The present study on beer consumption patterns among university students in Cluj-Napoca showed that male students drink beer more frequently and in higher amounts compared to female students. Most students prefer to drink lager beer. When choosing to drink beer, male students are mainly influenced by their mood, while female students by the entourage.

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## CONSUMPTION PATTERNS OF SOFT DRINKS AMONG ADOLESCENTS IN CLUJ-NAPOCA

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**Introduction.** It is well known that nutritional habits which are adopted during childhood and adolescence influence the future health status of an adult. This study aimed to examine the popularity of soft drinks among adolescents from Cluj-Napoca and to identify potential nutritional imbalances in their lifestyle.

**Material and methods.** Participants were 104 adolescents from Cluj-Napoca, aged 14 to 19, who were also pupils in local schools. They completed online a written anonymous questionnaire with minimum information regarding their weight, height and gender. Questions about their preferences, family relationship, sports, lifestyle were included.

**Results.** The adolescents were classified after their estimated fat using body mass index and the results revealed that the vast majority of teenagers have normal weight (55.7%). Soft drinks available on the market are preferred by 65% of the teenagers while homemade products are less consumed. Overall, the teenagers buy their drinks from the supermarkets and consume them most when they go out, and least, at school. 44.6% of adolescents consider the label helpful in identifying the product, 28.1% find important information about the content such as the enormous quantities of sugar or additives, while 27.3% believe it is just a commercial issue. Regarding their lifestyle, the adolescents from Cluj-Napoca are active, they practice a sport 2 or 3 times a week, more than 1 hour. The fact that most teenagers don't have a regular meal time can be considered a negative aspect.

**Conclusion.** Soft drinks are very popular among adolescents in Cluj-Napoca and the consumption is supported by their families, although there is seen a lack of information regarding the content and the long term effects of these beverages in relation to the health. For the years to come, raising awareness and fostering a culture of prevention is fundamental for the well being of the future adults.

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## NUTRITIONAL HABITS IN ROMANIAN BASKETBALL PLAYERS

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**Introduction.** Throughout the last decade a traditional and well-balanced diet has been recommended as the proper nutritional standard for professional players in all types of sports and all levels of performance. The purpose of this study was to examine nutritional habits among Division B basketball players from one Romanian team.

**Material and methods.** The evaluation has been done using a questionnaire and it was submitted to 12 players. The questionnaire contained a preliminary part about age, weight, height, age in sport, number of training hours per week and eighteen items (fourteen one choice, one multiple choice, two open answer and one food frequency table).

**Results.** Most (50%) of the interviewed players perform 10 hours of training per week, but only one has a personal nutritionist. All of them have regular meals at home, and lunch is considered the most substantial meal. Fast-food are consumed rarely (50%) or once per week (42%). There was a significant group (91%) which reported that they eat 2-4 snacks per day and the snacks consist mostly of fruits (39%) or sweets (22%). The most consumed foods are the butter (every day), potatoes, milk, cheese and eggs (4-5 times per week), yoghurt, rice and pasta (two times per week), and beans and mushrooms (once per week). On the other hand, vegetables such as peas, soybeans, lentils, cauliflower or spinach are consumed once a month or less. 83% of interviewed players eat meat twice a day, especially chicken, and regarding water consum the same percentage drink more than 3 liters of water daily.

**Conclusion.** The players interviewed reported good nutritional habits, but further investigations are warranted to gain solid knowledge on what the actual dietary intake of macro/ and micronutrients are among the professional players of today.

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## EVALUATION OF THE KNOWLEDGE OF THE POPULATION ABOUT ATOPIC DERMATITIS AND NUTRITION

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**Introduction.** Atopic dermatitis (AD) is a complex pathology having a strong connection with nutrition. The aim of this work was to evaluate people's knowledge about AD and nutrition and it starts with three hypothesis: (i) people tend to confuse AD with other diseases, (ii) a small number of people know the triggers of AD, (iii) a small number of people know how AD can be prevented.

**Material and methods.** The evaluation has been done in Romania, during 3 months, in 2015, using an online platform. Nineteen questions were submitted to 131 people about income level, age, education, job, number of children, specific knowledge about AD (multiple choice) and relationship between AD and food (false or true questions).

**Results.** The socioeconomic pattern of the population was evaluated through seven questions: sex, age, civil status, children, education, job, life environment. The 101 women and 30 men who answered the questionnaire were 18-50 years old, and 37 (28%) had at least one child. 115 (88%) of those interviewed had higher education (Bachelor or Master Degree). Regarding general aspects about AD, only 24.4% of interviewed people knew that this pathology is different from infantile psoriasis, food allergy, contact dermatitis or others similar pathologies, approximate 60% knew that infants and children up to seven years are vulnerable to AD, and 80.2% recognized food like a cause for AD, followed by genetic factors (47.3%), and house dust mite (44.3%). About food and AD, the results showed that 77.3% of those interviewed knew the negative influence of cow milk in AD, but only 47.3% knew that citrus fruits can prevent AD. On the other hand, 90.1% recognized the high importance of breast milk for infants to prevent AD.

**Conclusion.** The level of people knowledge about AD was moderate, either for housekeepers, workers, or students. More than 80% of interviewed people knew and recognized that between AD and nutrition it is a strong connection.

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## ASSESSING NUTRITIONAL KNOWLEDGE FOR FUTURE EDUCATION STRATEGIES

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**Introduction.** Children's wellbeing and their potential learning ability are improved by adopting and maintaining a healthy lifestyle, including healthy food choices. Worldwide studies have shown important gaps in children's nutritional knowledge and have also shown a correlation between lack of nutritional education and children's food choices, nutritional status and medical condition. Considering the statistics regarding the prevalence of children's diseases and also their unhealthy eating habits, a correlational descriptive research study was made, for the first time, in 2012, in Targu-Mures, to assess the nutritional knowledge in secondary school children.

**Material and methods.** A questionnaire regarding nutritional knowledge, based on school curriculum, was applied to 183 children, 13-15 years of age, including 55% girls and 45% boys, from 4 different schools.

**Results.** The study revealed important gaps in children's nutritional knowledge. From the total of interviewed children, 88% didn't know the role of proteins and glucose, 69% didn't know about the role of vitamins, and 35% didn't know the role of water in organism. The results have also suggested that children are aware of obesity consequences and they are open to receive information and support, to prevent such a condition. The majority of children were interested in school-based nutritional education, including nutrition camps, as an opportunity for developing cooking skills.

**Conclusion.** Considering the results of the study, it is obvious that children must benefit from school-based nutrition education, as a major preventive method. Future studies are necessary for designing the most effective nutrition interventions.

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## EFFECTS OF WHEAT AND LENTIL GERMINATED SEEDS ON PLASMA LIPID PROFILE IN RATS

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**Introduction.** Germinated wheat has demonstrated the ability to neutralize free radicals, lower cholesterolemia and improve immune function, while lentils are known to reduce serum lipids and the risk of colon cancer and type 2 diabetes. The aim of this study was to evaluate the effects of the consumption of wheat and lentil germinated seeds on plasma lipid profile in rats.

**Material and methods.** Forty-four male Wistar rats were divided into 6 groups of 7 or 8 rats each with similar mean body weights. To induce hypercholesterolemia, rats received cholesterol (1.5%) dissolved in sunflower oil and cholic acid (0.375%), added to a standard diet based on food pellets (80 g/d). Two groups of rats received the hypercholesterolemic diet for two weeks and wheat or lentil germinated seeds for the next two weeks. Other two groups received germinated seeds in the first two weeks and hypercholesterolemic diet with germinated seeds for the next two weeks. Germinated seeds were orally administered (80 g/d). The lipids levels in plasma were determined by biochemical analysis.

**Results.** The diet high in cholesterol significantly altered the plasma lipid profile, producing a two-fold increase in total cholesterol, an increase in LDL cholesterol and a reduction in HDL cholesterol. Wheat and lentil germinated seeds failed to prevent lipid alterations in hypercholesterolemic diet conditions. However, lentil germinated seeds were able to maintain a normal cholesterol level, when given a normal diet.

**Conclusion.** In order to obtain the positive effects of wheat and lentil germinated seeds on plasma lipid profile, their consumption should be made along with a low cholesterol diet.

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## IMPROVING THE QUALITY OF LIFE IN CHILDREN WITH OVERWEIGHT OR OBESITY (STRAJA HEALTH CAMP FOR OVERWEIGHT CHILDREN 19 JULY-9 AUGUST 2015)

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**Introduction.** In the last two decades, overweight and obesity in children and adolescents have reached epidemic proportions, becoming a major global nutrition problem. Because of this, the disease was considered the disease of the XXI century. This paper aims to study the effectiveness of multi-factorial program for overweight or obese children in order to improve the quality of life by losing weight.

**Material and methods.** The study group consisted of 28 children (14 girls and 14 boys), aged 8 -17 years. They were included on the grounds of election participation in the camp, all from urban areas. Were measured following anthropometric indices, as follows: measuring the current weight (with a scale), measuring the height (using a taliometer) and determining the percentage of fat (using a scale with bioelectrical impedance). The target group of children were observed during the 3 weeks of camp, being carried out initial, middle and final measurements. Factors that contributed to the final outcome are well established: food plan, daily physical activity and psychological counseling of children.

**Results.** The average weight loss was 4.57 kg representing an average of 6.5% (total weight of the lot of children being 1969,9kg). The average percentage of fat decreased by 3.23%.

**Conclusion.** The multifactorial program of 3 weeks resulted in a mean weight loss of 4.57 kg. This effective program (including components of nutrition, physical and psychological activity) had satisfactory results that improve the lifestyles of children with weight problems (a weight loss of 5% of body weight reduces the risk of long-term complications of obesity).

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## NUTRITION IN HEMODIALYSIS: THE RELATIONSHIP BETWEEN QUALITY OF LIFE AND DIETARY ADHERENCE IN PATIENTS UNDERGOING HEMODIALYSIS

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**Introduction.** Chronic kidney disease has a significant impact on patient quality of life when the renal function progressively deteriorates and the patient undergoes hemodialysis. The aim of this study was to evaluate the relationship between quality of life and dietary adherence in patients undergoing hemodialysis.

**Material and methods.** A total of 64 patients who were regularly undergoing dialysis at Avitum Medical Center, in Cluj-Napoca, were selected for this correlational study, that was conducted over a period of two months (March-April 2015). As evaluation tools were used two questionnaires: SF-36 Questionnaire, assessing the quality of life for patients, and Adherence to Treatment Questionnaire, containing 36 questions, grouped into 5 parts (general information, compliance with dialysis sessions, medication compliance, fluid restrictions and diet). Scores obtained using the two questionnaires, interdialytic weight gain, predialytic serum potassium levels, and predialytic serum phosphate levels over a period of 24 consecutive dialysis sessions were considered as biochemical indicators of dietary and fluid adherence. Data were analyzed by SPSS Ver.13.0.

**Results.** Our study showed no significant relationship between quality of life and fluid adherence (assessed by body average growth index). There is no significant association between quality of life and dietary compliance (assessed by serum phosphate levels and serum potassium levels). However, the present study revealed a significant relationship between treatment adherence, on the whole (medication, compliance with diet and dialysis sessions), and quality of life.

**Conclusion.** Dietary compliance is an essential condition, but not sufficient to ensure improved quality of life for dialysis patients; it must be accompanied by a high adherence to medication therapy and compliance with dialysis sessions to ensure an increased quality of life for these patients.

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## FOOD EDUCATION - A NECESSITY! COMPARISON BETWEEN "WE LOVE EATING" CAMPAIGN - CLUJ-NAPOCA (ROMANIA) AND "JAMIE'S MINISTRY OF FOOD", BRADFORD (ENGLAND)

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**Introduction.** In order to be healthy we have to know what choices we have to make and what kind of products and lifestyle we have to choose. In order to help people to choose the right side, two projects have started. "We Love Eating" campaign is a EU-financed pilot project, and is officially being launched in seven cities across the EU, including Cluj-Napoca. This European initiative is coordinated by P.A.U. Education. Being healthy is important for everyone, but especially for children, pregnant women and older people, which are at crucial points in the life cycle, when healthy habits make a big difference; therefore their habits will represent the main targets of this project. "Jamie's Ministry of Food" project was founded by Jamie Oliver, in 2008, because people from England have stopped cooking from scratch and started eating a lot of fast foods. In England there are a lot of centers where people can attend a cooking class and start making their own dishes.

**Material and methods.** The project has started since September 2014 and was ended on September 2015. "We Love Eating" project is about developing a positive relationship with food: what we eat, where we eat, and with whom we eat.

**Results.** "We Love Eating" project had a very big impact on the people from Cluj-Napoca because they have received a food education, through the main events and workshops. "Jamie's Ministry of Food" had, as well, an educative impact, and after an eight-week cooking class period people were confident to cook at home from scratch and, as a result, their diets were more based on healthier choices.

**Conclusion.** Both projects are made for citizens, in order to improve their lives and to make healthier communities, by promoting the benefits of a healthy lifestyle over a lifetime.

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**inSHAPE EXPERIENCE – SPORT, NUTRITION, PSYCHOLOGY****LAURA GRECU<sup>1</sup>, ILIE DRAGOTA<sup>2</sup>, RAMONA ILEA<sup>3</sup>**<sup>1</sup>**Cabinet of nutritional counseling, Program Coordinator inSHAPE, Cluj-Napoca, Romania**<sup>2</sup>**CSM Câmpia Turzii, Romania**<sup>3</sup>**Individual Cabinet of Clinical Psychology and Psychotherapy, Cluj-Napoca, Romania**

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**Introduction.** The majority of the health conditions associated with an unhealthy lifestyle result either from inadequate education, the absence of an environment that promotes healthy habits or the inability to cope with daily challenges. In order to achieve a healthy lifestyle, improve health and maintain the results in time it is necessary to implement a multidisciplinary approach that addresses all the factors involved.

**Material and methods.** inShape program is divided into three sections: sport, nutrition education and psychological counseling, each of them led by a specialist in that specific area.

**Results.** The benefits achieved by the participants fit into three categories: health, mental and physical. Body composition and appearance, cardiovascular fitness and endurance capacity, sleep and daily energy level, all improved. Regarding nutritional education, participants confirmed the importance of knowing not only what to do, but also why it is important for them to do it. The psychological counseling helped them to set real goals, find motivation, be aware of success, not focus on just failure and understand the importance of their emotions in the process of change.

**Conclusion.** The challenges that showed up along the program and also the different evolution between the participants who chose to attend all activities and those who chose only the activities perceived as necessary confirmed the importance of the multidisciplinary approach in order to obtain a healthy lifestyle and improve health.

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## NUTRITION EDUCATION AMONG PREGNANT WOMEN AND PRESCHOOL CHILDREN

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**Introduction.** Nutrition education is more than just learning about nutrients. It is also about developing habits that will ultimately improve the quality of life. The need for nutrition education among pregnant women and preschool children arises firstly from the fact that nutrition during pregnancy and infancy plays an important role in the development of mother and child and secondly from the fact that obesity and chronic diseases are reaching epidemic levels even in childhood. In order to address these key issues, I developed several nutrition workshops.

**Material and methods.** The target group was comprised of 102 pregnant women and 99 preschool children. Aiming to promote a healthy lifestyle and prevent chronic diseases, a number of nutrition workshops were provided. The length of one session was 2 hours and included a balanced mix of theoretical and practical approaches.

**Results.** Mothers and preschool children have had access to valuable information. Among other things, they learned how to feed themselves properly, how to prepare healthy meals and how to recognize unhealthy foods. At "Clubul Mămicilor"[Mums' Club], mothers learned about the dietary needs of 1-3 year old children and learned how to make healthy and delicious cookies for picky eaters. Future mothers from "Școala Mamei"[Mothers' School] are now more aware about the importance of healthy eating for the development of their baby. Moreover, preschool children had the opportunity to prepare healthy, attractive and tasty meals and so they started eating more fruits and vegetables.

**Conclusion.** The efficiency of these workshops is however proportional not only to the number of participants, but also with the extent to which they are available to an individual in time, combined with personalized counseling. Life-long learning about nutrition should be encouraged in order to maximize the impact that nutritional education has on the public's health and quality of life.

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## THE NONALCOHOLIC FATTY LIVER DISEASE – NOSOLOGICAL FRAMEWORK, RISK FACTORS, EPIDEMIOLOGY AND THERAPEUTIC POSSIBILITIES

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**Introduction.** The nonalcoholic fatty liver disease (NAFLD) is the consequence of complex metabolic disorders that consist mainly in the accumulation of lipids in the liver in a degree that exceeds 5% of the liver's weight. The patho-physiological key is the insulin resistance. This disease is frequently associated with obesity, dyslipidemia, type 2 diabetes and cardiovascular pathology and is considered a hepatic manifestation in the metabolic syndrome.

**Material and methods.** The nonalcoholic fatty liver disease is diagnosed by noninvasive and histological tests in absence of hepatic fat accumulation (alcohol, other hepatic diseases, drugs, obesity, type 2 diabetes). The NAFLD is a complex concept with a spectrum that contains distinct histological, pathogenic, evolution and prognostic entities: hepatic steatosis, nonalcoholic steatohepatitis with different grades of fibrosis, hepatic cirrhosis with complications, especially hepatocellular carcinoma.

**Results.** Worldwide prevalence is between 6.3 and 35%, on average 20% of NAFLD is considered the most frequent hepatic disease in the western countries. The “endemic” nature of obesity registered in some countries determined the rise of NAFLD incidence at an alarming rate becoming a public health problem.

**Conclusion.** Because the pathogenic mechanisms are incompletely elucidated, the therapy is not standardized. The therapy targets are the risk factors (obesity, dyslipidemia, type 2 diabetes, and insulin resistance) and histological improvement: this is realized by lifestyle modification (weight loss, diet, and physical exercises), pharmacological therapy, bariatric surgery, and hepatic transplant.

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## THE APPRAISAL OF NITRATE AND NITRITE INTAKE FROM VEGETABLES CONSIDERING THE SPECIFICS OF THE MOST RECOMMENDED DIETS

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**Introduction.** The nitrate and nitrite content is nowadays an important quality aspect of vegetables. European and international organizations imposed limits of daily human nitrate intake based on toxicity studies in the ‘50 and ‘60. The beneficent role of dietary nitrate intake has been evaluated recently in heart diseases, obesity, osteoporosis and insulin resistance. The aim of this paper was to study the most appreciated diets in the medical field from the point of view of the potential nitrate contribution and to appraise the nitrate intake of these diets for European and Cluj-Napoca citizens.

**Material and methods.** Literature data about EFSA 2008 report of European citizens average nitrate intake from vegetables, diet’s specific vegetable servings, legislative imposed limits of food nitrate content and the practical determination of nitrate content in vegetables collected from Cluj-Napoca Farmers Market was gathered and correlated.

**Results.** The most appreciated diets have an important impact on the nitrate intake because of the outstanding portion of vegetable servings they have: DASH diet 19%, Mediterranean diet 29%, Vegetarian diet 31%, and Mayo diet 12%. The maximum daily nitrate intake of the European citizens is overcome by at least 20% for all caloric levels of the diets considered. The nitrite limit is also surpassed by at least 273%. The average nitrate intake for Cluj-Napoca citizens is at least 4 times smaller than the limit imposed.

**Conclusion.** The Mediterranean and Vegetarian diet contribute the most at the dietary nitrate intake – the vegetal servings of these diets represent more than 25% of the total food groups. These diets overcome the limits of daily human nitrate intake. Cluj-Napoca citizens have a 2.65 times smaller nitrate intake than European citizens.

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## **BODY IMAGE – AN ANALYSIS OF HOW THE BODY IMAGE IS VIEWED BY THE STUDENTS OF CLUJ-NAPOCA AND WHAT ACTIONS ARE THEY WILLING TO TAKE TO IMPROVE IT**

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**Introduction.** Body image is a widespread preoccupation among people of both genders at all ages. One study concluded that 46% and 74.4% of normal-weight men and women thought about their weight or appearance “all of the time” and “frequently”. Body image disorders are mostly influenced by the way the people perceive themselves in the mirror. What they see in the mirror and the way they feel about it is modulated by their age, sex, sexual preferences, races, ethnic group, marital status and their lifestyle. Even if most people know how to stay healthy, millions of them find it difficult to exercise and eat right, or even to decide which to change first. While changing the diet alone can interfere with the establishment of a regular exercise routine, it has been found that changing the exercise and diet at the same time gives a bigger boost than changing them in sequence. The aim of this study was to identify the correlation between body image, lifestyle and health outcomes, more specifically to determine the influence of body image on lifestyle and to assess the actions that students from Cluj-Napoca are willing to take to improve it.

**Material and methods.** Intense study of existing literature and internet data, processed and analyzed by personal view, from which resulted a metatheory necessary for future studies.

**Results.** The results showed that body image and the way that people perceive themselves in a mirror is a major factor for their mental and physical health. For having the best results in improving their body image, people must make diet and exercise changes at the same time.

**Conclusion.** In conclusion, the way that people perceive themselves in a mirror is a very important factor for their health, and their body image can be improved by combining diet and physical activity. Further research is needed to assess the body image perception of the students from Cluj-Napoca and what actions are they willing to take to improve it.

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## HORMETIC RELATIONS IN THE CASE OF BISPHENOL A

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**Introduction.** Studies have shown that bisphenol A (BPA) has negative effects on human organism. Studies regarding endocrine disruptors frequently identify potential unconventional dose-response relations, called NMDR (non-monotone dose-response) relationships, which are hormetic relations. The purpose of present study was to analyze the scientific literature, regarding the effects of bisphenol A, and the methodology for assessing the risk of exposure to BPA.

**Material and methods.** BPA is a component of food packages, baby bottles and a number of non-dietetic products. The high affinity of bisphenol A for ERR- $\gamma$  (estrogen-related receptor) explains the negative effects of BPA exposure.

**Results.** In 2012, Laudet et al. showed that BPA leads to abnormal development of otic vesicle in zebrafish. Laudet demonstrated that ERR- $\gamma$  induces otoliths malformations and this receptor is implicated also in glucidic metabolism of muscles and heart. In 2015, Lagarde et al. reported 30% NMDR relationships for BPA, from a total of 170 NMDR. Lagarde elaborated a methodology for evaluating the plausibility of NMDR relations and applied it in 10 studies regarding BPA, in which NMDR relations were reported. Forty-nine NMDR relations were identified and many effects were reported, including on the reproductive system.

**Conclusion.** Lagarde methodology represents a significant progress in risk assessment of exposure to chemical substances for which were reported hormetic NMDR relations. Worldwide organizations must take into account this methodology for evaluating the risks of BPA exposure, for establishing new TDI (tolerable daily intake) and SML (specific migration limit) values.

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## TOPICAL APPLICATIONS FOR THE PREVENTION OF CARIES AND HYPERSENSITIVITY

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**Introduction.** Dental caries involve a dynamic process with interspersed periods of demineralization and remineralization that remains the most prevalent chronic disease in both children and adults.

As a result of dental caries and tooth surface lesions, dentinal hypersensitivity (DH) is a common clinical condition that it is one of the most painful and least successfully treated chronic problems of the teeth.

Prevention of dental caries and hypersensitivity has always been difficult to tackle. The phenomenon of reversal of incipient or early enamel caries forms an important part of prevention leading to apparent repair of the lesion.

**Aim.** The purpose of this study was to make an analysis of published studies on dentin tubule occlusion and remineralization competence of various toothpastes containing fluoride, CPP-ACP and hydroxyapatite (HAP) as active ingredients.

**Materials and methods.** A search of the international databases for the identification of publications on the subject using the search words “dentin hypersensitivity”, “enamel remineralization” that contained a comparison study with HAP .

**Results and discussions.** All the studies have shown the efficacy of this agents but it appears that HAP has greater results than the other ones.

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## NON-CARIOUS CERVICAL LESIONS: THE CORRELATION BETWEEN THE CLINICAL ASPECTS AND ETIOLOGY

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**Introduction.** Non-carious cervical lesions (NCCL) involve loss of hard tissue in the cervical third of the crown unrelated to dental caries. Such lesions are common and have frequently been investigated. The factors incriminated in the formation and progression of NCCLs are:

- Biocorrosion (chemical, biochemical) caused by intrinsic and extrinsic acids;
- Friction (wear) caused by traumatic brushing;
- Traumatic occlusion (abfraction).

The aim of this study was to assess the potential relationship between the type and shape of the lesions and the etiology of the disease.

**Materials and methods.** The study was carried out on 40 patients aged between 20 and 60 years. The following investigations were performed:

1. Recording the type of occlusion;
2. Recording the nature of occlusal contact in maximum intercuspation and eccentric movements;
3. Establishing the tooth-brushing technique, brush force, brushing frequency, bristle stiffness and toothpaste abrasiveness;
4. Questioning the type of acidic foods and drinks.

**Results.** According to our examinations the patients present the following lesions in the cervical third of the crown, of different shapes:

- a. Wedge shaped lesions with sharp margins have been assessed in 32 patients; premature contacts and interference in eccentric movements were detected;
- b. Well defined typical lesions with sharp or grooved margins, with scratched surface and sharp line angles in 5 patients conducting horizontal brushing;
- c. Formations described as smooth disc-shaped rounded lesions, concave with no sharp edges, grooves or ridges in 3 patients. The teeth didn't show premature contacts or interference. Patients reported a high consumption of acidic drinks and foods.

**Conclusions.** This study demonstrated a highly significant positive relationship between the presence of premature contacts, eating habits, brushing technique and the lesions. The prevalence of the lesion increased with age, by repetitive predominant etiologic factor.

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## RISK AND PROTECTIVE FACTORS IN PERIODONTAL DISEASE AND CARDIOVASCULAR PATHOLOGY

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**Introduction.** Periodontal disease with systemic implications and metabolic disorders are highly prevalent among the population and their relationship could have an impact on the human life.

The current study aims at assessing the correlation between periodontitis and systemic diseases using a validated questionnaire.

**Materials and methods.** We designed a questionnaire with 42 items. A pilot study was carried out on 10 participants to evaluate the clarity, relevance and coherence of the questionnaire. All of them described it as open and easy to understand. Finally, the questionnaire was validated by the test-retest method, by applying at 30 volunteers, twice at an interval of 2 weeks.

The questionnaire was administrated then as a structured interview to 108 adults over 45 years from the counties of Cluj and Bihor, included by consecutive selection.

Data obtained was introduced in Microsoft Excel and statistical analysis was performed using SPSS software.

**Results.** The average age ( $\pm$ SD) of the participants was  $55.74 \pm 10.51$  years. A known diagnosis of periodontal disease was declared by 58.3% of participants, while 93.5% of the patients with periodontitis reported family history of periodontal disorders.

A statistically significant difference was observed between patients with a diagnosis of periodontal disease (PD+) and those without periodontal disease diagnostic (PD-) regarding the presence of edentulism ( $p=0.015$ ) and the duration of smoking in the patient history. ( $p=0.047$ ). Cardiovascular disease was declared by 38.1% of participants and diabetes was declared by 36.5% of subjects in the PD+ group. We observed a low prevalence of periodontal and cardiovascular disease in participants reporting a healthy lifestyle and sustained physical activity.

**Conclusion.** The high prevalence of chronic periodontitis and systemic disease — cardiovascular diseases or diabetes — as major risk factors for public health increase the interest in new studies evaluating their association.

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## PERIPHERAL NERVE REGENERATION IN THE SURGERY OF FACIAL DEFORMITIES

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**Introduction.** Orthognathic surgery implies osteotomies of the deformed maxillary bones to correct their position and/or dimension. The osteotomy lines of the mandible (bilateral mandibular sagittal split osteotomies - BSSO) split the bone through the mandibular canal, containing the inferior alveolar nerve, artery and vein. In the postoperative period, anesthesia of various degrees of the lower lip and chin can be noticed and evaluated. Repetitive peripheral magnetic stimulation (rPMS) is a noninvasive magnetic stimulation method, which operates on Faraday's principle of electromagnetic induction. Peripheral magnetic stimulation produces muscle contractions and sensory afferents via the depolarization of conductive structures within the peripheral nervous system, by creating voltage differences and ion flows, thus activating conductive structures beneath the stimulated region. The mechanisms potentially involved in PMS include local changes of muscle and nerve function.

**Material and methods.** The present study intended to evaluate the regenerative capacity of rPMS involving inferior alveolar nerve (IAN) in patients having undergone orthognathic surgery.

Pharmacological and non-pharmacological enhancement of ISN regeneration after MMA intervention: a tree-arms pilot study

**Objectives:**

- to test the efficacy of rTMS treatment vs control;
- to compare the efficacy between the 3 groups with rTMS treatment;
- to test the efficacy of CRB treatment in peripheral nerve injury (IAN).

A device MagPro X100 (MagVenture, Denmark) will be used for repetitive stimulation with a figure-8 coil (C-B60). The coil will be placed tangential to the skin, in the region of the mentalis muscle. The stimulation intensity will be gradually raised until the obtaining of a sensation, avoiding triggering pain.

**Results and Conclusion.** After 10 sessions, progressive reduction of the anesthetized region and intensity of the anesthesia is observed.

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## EXPERIMENTAL STUDY ON HEMATOLOGICAL BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN PERIAPICAL INFLAMMATORY LESIONS

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**Introduction.** Periapical lesions (PL) are the result of the bacterial infection of the root canals of teeth, inducing local inflammation, alveolar bone destruction and changes in the defense capacity of the body. The aim of the study was to evaluate the evolution of hematological parameters and oxidative stress markers (malondialdehyde MDA and glutathione GSH) in an animal model with experimentally induced PL and to investigate the relationships of these parameters with histopathologically detected periapical inflammatory changes.

**Material and methods.** The experimental study was performed on 3 groups of animals; adult Wistar rats divided into: Group I, control group; Group II, animals with PL induced experimentally in mandibular molars; and Group III with type 2 diabetes mellitus and experimentally induced PL. Venous blood samples were collected by retro-orbital sinus puncture, at 14 days, 30 days and 60 days. After the animals were sacrificed, the mandibles were processed and examined histopathologically.

**Results.** Group II had increases of total leukocytes ( $12.81 \times 10^9/l$ ) and neutrophils ( $4.09 \times 10^9/l$ ) compared to group I ( $9.39 \times 10^9/l$ ,  $p=0.014$  and  $2.47 \times 10^9/l$ ,  $p=0.036$ , respectively). High serum MDA concentration values ( $3.12 \text{ nmol/ml}$ ) were found in group II compared to group I ( $1.51 \text{ nmol/ml}$ ,  $p=0.021$ ). In group III, lymphocyte values increased from 14 days to 60 days ( $5.1 \times 10^9/l$  vs.  $7.76 \times 10^9/l$ ,  $p=0.034$ ). The mean MDA ( $2.99 \text{ nmol/ml}$ ) and GSH values ( $6.85 \text{ nmol/ml}$ ) in group III were higher than those in group I ( $1.52 \text{ nmol/ml}$ ,  $p=0.0007$ ,  $p=0.015$ , respectively). In group III, local inflammatory infiltrate was more intense and correlated with total leukocytes and neutrophils.

**Conclusion.** Changes in hematological parameters and oxidative stress markers were identified both in the active and the chronic stage of periapical inflammation. Local inflammatory infiltrate was associated with the values of total leukocytes and neutrophils in peripheral blood.

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## THE MANAGEMENT OF ACCIDENTS AND COMPLICATIONS OF DENTAL EXTRACTIONS

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**Introduction.** Nowadays, the techniques for the procedure of dental extractions are well studied and presented in literature. Unfortunately, the perfect dental extraction procedure can be performed only in rare situations, and it depends on multiple factors such as: the anatomical situation, morphology of the tooth, the local pathological process, bleeding, the movements of the tongue and on the dentist's safety and dexterity.

The aim of the present research is to observe the factors which determine the accidents and complications of dental extractions and their frequency of occurrence.

**Materials and methods.** The study included 5 clinical cases that were treated in the Department of Oral Rehabilitation, Oral Health and Dental Office Management, Faculty of Dentistry, Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca.

The method of the study is "clinical case report".

In order to evaluate the risk of accidents and complications of dental extractions, a set of clinical and complementary examinations -OPT, or retro-alveolar radiographs- were performed.

**Results.** In our study the most frequent accident was the fracture of the teeth that need to be extracted –in 2 cases. Postextraction bleeding and dry socket was noticed in other 2 cases. Dental extractions were followed by intense pain, but in most cases, the evolution led to a normal healing.

**Conclusions.** Dental extractions can be difficult for both the patient and the dentist. Also, the oral surgeon has a multitude of management methods for the prevention of accidents and complications of a dental extraction; however, first and foremost they should respect the bioethics' principle of 'PRIMUM NON NOCERE', according to which, it is easier to prevent than to treat.

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## THE USE OF SLM IN DENTAL IMPLANTS – A LITERATURE REVIEW

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**Introduction.** Dental implants are gaining popularity amongst the patients and doctors and frequently are being considered as a first treatment option. Although current survival results can be considered satisfying, improvements can be made. These changes may come from the actual structure of the implant, not only from changes in its design or coating. This is why, we are going to observe in the currently available literature if mentions are made regarding the efficiency of dental implants produced by SLM (Selective Laser Melting) with controlled porosity (reproducible pore size), in order to begin our studies in this field.

**Materials and method.** A systematic review of PubMed indexed literature was conducted in November 2015, to identify studies focusing on dental and orthopedic implants produced by SLM using specific inclusion criteria. Data regarding the purposes of such implants, parameters of interest and other relevant information was taken into focus.

**Results.** 23 studies were taken into consideration out of 60 results. Relevant purposes for implants produced using SLM were considered: dental implants, individual biodegradable bone substitute, mandibular condyle implant, bone augmentation implants, cranioplasty implants, zygomatic implants and other craniomaxillofacial bone defects. Elastic modulus, fatigue, bending strength, static and dynamic compressive properties, vascular endothelial growth factor (VEGF) in the surrounding bone were considered some of the parameters of interest.

**Conclusion.** After an extensive literature search, we came to the conclusion that the use of dental implants with controlled porosity produced by SLM can be an improvement on present dental implants and can lead to further research. Our next study will aim to modify the implants' elastic modulus in order to bring it closer to the surrounding bone to increase the survival and behavior of the implant, while not compromising the static and dynamic compressive properties of the implant.

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## ORAL REHABILITATION OF THE ANTERIOR AREA USING ADHESIVE SYSTEM RESTORATIONS

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**Introduction.** Restoration of the anterior area is a paramount importance due to increasing esthetical needs of our patients. Using adhesive systems restorations we can replace the lost teeth with minimum or even without preparation of the adjacent teeth, with perfect esthetic outcomes.

The aim of this presentation is to illustrate the results obtained using adhesive system restorations.

**Material and methods.** For patients with periodontal disease, MOD cavities were prepared in the middle third of the oral and occlusal side of the adjacent teeth of the edentation. Based on the impression, an adhesive bridge was made in the lab from CR-CO alloy and Premise Indirect composite.

For patients without periodontal disease, no preparation was performed for the adjacent teeth of the edentation. The strength of that bridge was made using Construct from Kerr.

**Results.** Both in patients diagnosed with periodontal disease and without periodontal disease good esthetic and functional outcomes were obtained using adhesive system restorations. In the same time we succeeded in immobilization of teeth.

**Conclusions.** Adhesive systems restorations is an alternative for the restoration of anterior edentation.

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## SPECTROPHOTOMETRIC COLOR EVALUATION OF PERMANENT INCISORS, CANINES AND MOLARS. A CROSS-SECTIONAL CLINICAL STUDY

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**Introduction.** An accurate color reproduction represents the final validation level of an esthetic anterior or posterior restoration. The aim of this study was to evaluate the color of permanent maxillary incisors, canines and molars, using a clinical spectrophotometer.

**Material and methods.** The Vita Easyshade Advance 4.0® intraoral spectrophotometer was used by one clinician to determine the color of 369 permanent maxillary incisors, canines and molars. The best matches to Vitapan Classical® and 3D-Master® shade guides were recorded. A one-way analysis of variance and Kruskal-Wallis test were used to compare L\*, a\*, b\*, c\* and h\* color coordinates among the 3 types of teeth. Differences between the mean values of all color coordinates were evaluated by use of Bonferroni corrections. Color difference (DE\*) between incisors, canines and molars was calculated from DL\*, Da\* and Db\* data and the results were compared to DE\*=3.3 acceptability threshold.

**Results.** Except for Da\* and Dh\* between canines and molars, statistically significant differences among the mean differences of all color coordinates were found when the 3 types of teeth were compared by pairs. The most frequently measured shades were A1 (48.4%), respectively 1M1 (31.5%) for incisors, B3 (36.6%), respectively 2M3 (39.8%) for canines and B3 (44.7%), respectively 2M3 (52%) for molars. Incisors had the highest lightness values, followed by canines and molars. Molars were the most chromatic with the highest a\* and b\* values.

**Conclusion.** Despite the limitations of this study, color differences among incisors, canines and molars were found to be statistically significant, above the clinical acceptability threshold established. In conclusion, successful esthetic restorations of permanent teeth of the same patient need an individual color assessment and reproduction of every type of tooth.

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## THE TREATMENT OF TEMPORO-MANDIBULAR DYSFUNCTION. REVERSIBLE PHASE

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Often we see patients who experience muscle or joint pain in the maxillo-facial area. Sometimes we are facing patients who complain of clicking or 'popping' of temporomandibular joints (TMJ). In all these situations, occlusion can be the main factor or a contributing factor that maintains the pathology.

The complexity of structure and functions of the TMJ make the diagnosis of its diseases/disorders difficult. Treatment decisions are based on the clinical exams and imaging of the temporo- mandibular joint. Magnetic Resonance Imaging is the most specific and sensitive for interpretation of soft tissue and inflammatory conditions in the joint, whereas CT examination produce excellent image for osseous morphology and pathology. Recently, ultrasonography is used in diagnosis of TMJ disorders. The advantages of ultrasound imaging for TMJ are: direct observation of the joint disc movement during the opening and closing of the mouth, non invasive, low cost.

Treatment of these patients consists of two phases: a reversible phase followed by the definitive treatment (final occlusal equilibration). The reversible phase is performed using the bite splints. There are different shapes of bite splints, made out of different materials, and used for different goals. Full or partial coverage, hard or soft bite splints can be used.

Choosing the right type of the bite splint is an important factor for the outcome of the treatment. This can be done only based on a accurate diagnosis.

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## INDIRECT RESTORATIONS IN THE ANTERIOR AREA

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**Introduction.** Dento-facial harmony plays an important role in social interactions of every individual. The appearance of dental elements is also an important feature in determining the attractiveness of a facies.

**Material and methods.** We analyzed indirect restorations made in the anterior area, surprising how the patient perceives the restoration.

**Results and conclusions.** The ideal prosthetic restoration of anterior area is overlapping vision of the team dentist- patient- dental technician with the patient's one. Aesthetics is perceived differently, being determined by cultural and temporal influences, and especially the personality of each individual.

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## IN VIVO ULTRASONOGRAPHIC EVALUATION OF PERIODONTAL CHANGES DURING ORTHODONTIC TOOTH MOVEMENT

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**Objective.** Orthodontic tooth movement (OTM) is a process whereby the application of a force induces bone resorption on the pressure side and bone apposition on the tension side. However, only limited data are available on the in vivo behavior of the periodontal tissues. The aim of this pilot study was to measure the changes of periodontal tissues induced by the orthodontic canine retraction, using 40 MHz ultrasonography.

**Materials and methods.** The ultrasonic evaluation of the periodontal tissues were conducted on a patient with indication for orthodontic treatment. His bilateral upper first premolars were extracted due to severe crowding, and the canines were distalized using elastomeric chain, with a net force of 100 g. Ultrasonographic scans were taken before, during and after retraction, in three distinct areas of the canines' buccal surface: mesial, middle and distal. The reference point was the bracket, which appeared hyperechoic on the us scan.

**Results.** Four different dimensions were obtained: d1 (depth of the sulcus), d2 (thickness of the gingiva), d3 (length of the supracrestal fibers), d4 (width of periodontal space). An increase of d1 was observed in all three areas of the periodontium, during the orthodontic treatment. The width of the periodontal space (d4) became larger mesially and distally, whereas on the middle part of the buccal surface the dimension decreased.

**Conclusions.** Changes in periodontal ligament space and free gingiva during orthodontic tooth movement were observed using high resolution ultrasonography. Future studies will be carried out on a larger sample of patients, to demonstrate the dimensional modifications during OTM.

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## EFFECTIVE DOSE OF DENTAL X-RAYS EXAMINATIONS IN A PEDIATRIC POPULATION: A RETROSPECTIVE STUDY

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**Introduction.** The aim of this study is to estimate the effective doses of dental radiological investigations in children and young population, as a measure of stochastic risks from exposures to low doses of ionizing radiation.

**Material and method.** A cohort group of patients aged between 0-22 referred to a radiological exam during 1st January 2014 and 30th June 2015 in four different oral radiological centers in Cluj-Napoca. In all centers were available the following radiological exams: CBCT, skull radiography, panoramic and intraoral radiography. The effective dose for CBCT was estimated by using the DAP value and the average conversion coefficients for CBCT. The effective dose for panoramic and cephalometric radiographies was calculated by using conversion factor of Helmrot and Alm Carlsson and Batista equation. The effective dose to children was retrospectively estimated, based on the type of examination, age and gender of the patients and settings for exposure.

**Results.** A total number of 5349 of radiological examinations in children had been done and the most frequent examination was the panoramic radiograph with 65%. Even the number of CBCTs was lower compared to 2D X-rays, we noticed a higher value on mean DAP for all CBCT machines comparing to mean DAP for 2D exams. The lowest DAP value was identify for cephalometric radiography. The effective dose for CBCT was higher compare to effective dose of 2D radiological examination. The effective dose increased to children over 10 years old. Dose from CBCT from different tissue showed that highest dose was for salivary gland 47% followed by the thyroid gland with 16% and the brain tissue with 15%.

**Conclusions.** Children are more susceptible to radiation than adults. This study reveals the number of dental radiographs performed to children and the comparative doses of CBCT and 2D radiographs.

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## MICRO-CT ANALYSIS OF Ti6Al7Nb CUSTOM MADE IMPLANTS

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**Introduction.** The objectives of the study were to test the osseointegration of Titanium–Aluminum–Niobium (Ti6Al7Nb) alloy used for maxillo-facial reconstruction, and the osseointegration of this type of alloy with different coatings (hidroxyapatite and SiO<sub>2</sub>-TiO<sub>2</sub>). Micro-CT analysis and histological analysis were compared, in order to assess the osseointegration of these alloys.

**Material and methods.** The Ti6Al7Nb implants were manufactured using a selective laser melting technology and had a cylindric screw-type shape with a total porosity of 25%. Thus, 18 rabbits were used in this study which received intrafemoral implants with and without coating. The samples were harvested at 1, 3 and 6 months after implantation and histopathological examination of the bone surrounding the implants was performed after the Micro-CT scan.

**Results.** Both examinations compared the amount of mineralized bone and osteoid formed at the implant site, revealing more bone formation for the implants with coating at 3 months and similar mineralized bone for all of the implants at 1 and 6 months.

**Conclusion.** The study revealed better and faster osseointegration for the implants with coating. Demineralization at the implant site was noticed for Ti6Al7Nb implants at 3 months, both with micro-CT and histological exam, proving that micro-CT can be a fast and reliable test of osseointegration.

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## INVESTIGATION OF DENTISTS' AND PATIENTS' PREFERENCES REGARDING TREATMENT IN CASE OF APICAL PERIODONTITIS

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**Introduction.** Therapeutic decision in dentistry is a complex cognitive process for the dentists because it involves taking into consideration several factors, including patient's preferences. The hypothesis of this study was that apicoectomy is indicated more often than necessary. In order to confirm or invalidate this assertion two questionnaires were created (one for the dentists and the other one for the patients).

**Material and methods.** The questionnaire addressed to the dentists included questions about treatment options in case of two scenarios concerning an anterior and a posterior tooth with apical periodontitis, with or without previous endodontic treatment and also general questions, such as medical specialties, age and years of experience. The questionnaire addressed to the patients included questions about treatment options for various scenarios presented, concerning anterior or posterior teeth with apical periodontitis. The questionnaire included a question about the role that each patient wants to play in establishing the treatment plan, but also general questions on age, gender, monthly income, etc. Both questionnaires included questions about dentist's and patient's opinion on factors that could influence the patient in choosing the final treatment.

**Results.** After descriptive statistical analysis was performed, preferences rates were subjected to Chi-square test (including McNemar test for significance). In order to evaluate dentists' versus patients' perspective on the factors related to decision-making Kappa test was applied.

**Conclusion.** The results indicate that both physicians and patients prefer nonsurgical endodontic treatment in case of a tooth with apical periodontitis, the second option being surgical endodontic therapy - apicoectomy. Regarding the role that the patients prefer to have in the decision-making stage, we observed a predominantly passive role for the participants in this study.

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## CYTOTOXICITY EVALUATION OF A NEW EXPERIMENTAL GIOMER

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**Introduction.** Giomers represent a hybrid adhesive bio material based on the pre-reacted glass technology. Their behavior regarding the fluoride ion charging, release and recharging included them in the smart material category. Because their advantages combine the advantages of a composite material and a glass-ionomer cement they are used in many dental fields.

**Objective.** Evaluate the local effect of giomer biomaterial samples after implantation subcutaneous and intramuscular and compare with the results obtained at the control sample according to ISO 10993.

**Material and methods.** The sample were prepared from the experimental giomer with a diameter of 2mm in order to be implanted subcutaneous and intramuscular in 20 lab rats specimens under anesthesia. Resorbable suture were used to close the intramuscular site and non-resorbable sutures for the skin. After 2 weeks the rats were euthanized with an overdose of anaesthetic (ISO 10993) and the sample were analyzed macroscopically and microscopically; the response was evaluated and compared

**Results.** The new experimental giomer according to ISO 10933 standard was moderately irritant (with a score between 9.0 up to 15.0) subcutaneously, and slight irritant (with a score between 3.0 up to 8.9) intramuscularly, comparable with the control samples.

**Conclusion.** Giving the tissue response to the giomer sample, the experimental giomer can provide good response in clinical situations.

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## BOND STRENGTH OF TWO ORTHODONTIC ADHESIVES AFTER DENTAL BLEACHING

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### Objectives:

1. There is a significant difference between two orthodontic adhesives, with higher values of Shear Bond Strength (SBS) for Transbond XT (3M) compared to Light Bond (Reliance Ortho).
2. There is no significant difference between SBS values for unbleached and bleached teeth seven days after the procedure with similar Adhesive Remnant Index (ARI) between the groups.

**Methods.** Forty human premolars were divided into four groups: two control groups were bonded using 2 different adhesives and two test groups have been bleached with 35% hydrogen peroxide and preserved for one week prior to bonding with the two adhesives. Brackets were debonded using an Instron machine, ARI scores were evaluated and certain surfaces were examined under Scanning Electronic Microscopy (SEM).

**Results.** One-Way ANOVA showed no statistically significant differences between values obtained for the two adhesive systems neither in the control, nor in the test groups. No statistically significant differences were obtained between values for bleached teeth seven days after bleaching compared to unbleached teeth. Chi-square test has shown a statistically significant difference between ARI scores in the control and test groups, with ARI scores significantly lower for bleached teeth.

**Conclusions.** SBS values obtained for the two adhesives are similar. There is no difference between SBS of unbleached and bleached teeth seven days after the procedure using 35% hydrogen peroxide. ARI scores are significantly different for bleached teeth showing lower values, meaning higher prevalence of adhesive fracture at the enamel-adhesive interface.

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## DIFFERENT TYPES OF INTRAORAL ANCHORAGE APPLIANCES USED IN ORTHODONTIC TREATMENTS AND THEIR EFFICIENCY IN CASES WITH FIRST PERMANENT MOLAR EXTRACTIONS

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**Introduction.** Over the last years dentists have reported a large number of missing first molars, due to extractions, even at young ages.

The purpose of this study was to compare different intraoral anchorage appliances used in orthodontic treatments in such cases and their efficiency regarding anchorage loss.

**Material and methods.** The study included 33 patients with class II/2 and 34 patients with class II/2, all at the age of 12 years, and with at least one missing first permanent molar.

For intraoral anchorage we used the transpalatal arch, Nance appliance, lingual arch and mini-implants. We observed the anchorage loss, measured in mm.

**Results.** The study showed a loss of anchorage ranging between 1 and 3 mm by using the transpalatal arch, Nance appliance and lingual arch.

Three of the ten mini-implants were lost, but ultimately, the remaining ones showed no loss of anchorage.

**Conclusion.** In cases with missing first permanent molars, mini-implants represent the best appliance to prevent any loss of anchorage.

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## INFLUENCE OF POLYMERIC MATRIX ON THE BIOCOMPATIBILITY OF FIBER-REINFORCED COMPOSITES

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**Introduction.** The reconstruction of the bone defects has always been an issue of primary importance. In spite of all the improvements brought to the techniques of bone reconstruction, present day biomaterials still have some shortcomings that limit their application and sometimes cause clinical problems. Our study aims to elaborate and test in vitro and in vivo new advanced biomaterials based on fiber-reinforced composite (FRC) that will serve cranial bone reconstruction, in case of large bicortical calvarias defects.

**Material and methods.** Four different resins were prepared starting from Bis-GMA, TEGDMA, UEDMA and HEMA (in different proportions). Fiberglass E300 g/m<sup>2</sup> was selected to reinforce the polymeric matrix. To ensure the chemical connection between the organic and inorganic phases, 3-metacrililoiloxipropil-1-trimetoxisilan was used. The FRC were obtained through chemical polymerization followed by post-cure thermic treatment. The conversion of resins was analyzed by FTIR (Fourier Transform Infrared Spectroscopy). Soft tissues reaction to implantation of FRC was assessed by histopathological examination, according to ISO 10993-6.

**Results.** Better conversion rates (above 85%) were observed when UDMA monomers were used. Polymeric matrix containing UDMA base monomer determined a lower inflammatory reaction when implanted in soft tissues (inflammation score  $1.2 \pm 0.2$  for subcutaneous implantation test and  $0.8 \pm 0.3$  for muscle reaction).

**Conclusion.** Adequate choice of polymeric matrix betters conversion rate and optimizes the biological behavior. Among the FRC studied, polymeric matrix based on UDMA (60%), TEGDMA (30%) and bis-GMA (10%) is the best formulation in terms of biocompatibility.

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## **DENTAL EROSION ASSOCIATED WITH GASTROESOPHAGEAL REFLUX DISEASE: TREATMENT OPTIONS AND CASE REPORT**

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Dental erosion is an irreversible tooth pathology that implies chemical dissolution of enamel and dentin without bacterial involvement. There are intrinsic and extrinsic factors involved in the etiology of dental erosion. Among intrinsic factors gastro-esophageal reflux disease is frequently encountered. Currently the Basic Erosion Wear Examination (BEWE) index allows a quick, valid diagnosis for this tooth pathology, as well as a guide for proper treatment plan and execution. In early stages treatment of dental erosion is simpler, while in advanced stages complex treatment schemes are required, including dental and periodontal structures and prosthetic rehabilitation. In this case report, a complex approach to restore the severe dental erosion due to gastro esophageal reflux disease by proper techniques and materials is presented.

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## THE NECESSITY OF CBCT IN IMPLANT SUPPORTED REHABILITATION

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**Introduction.** Cone Beam CT has proven its efficiency in the medical field by providing a substantial volume of information, using a reduced radiation dose, low cost and high accessibility, compared with multi-slice computerized tomography.

The use of CBCT in dental implant treatment planning regards linear measurements, three-dimensional evaluation of alveolar ridge topography, proximity to vital anatomical structures, the fabrication of surgical guides and also bone density measurements.

The aim of this report is to present the case of a patient referred for dental implant treatment planning, which proves CBCTs necessity in the evaluation of the information required about the implant receptor.

**Material and methods.** A 42 years old female patient was referred for dental implant treatment. Clinical examination and a panoramic radiography were performed to establish the treatment plan. A CBCT scan was indicated to assess the quality and the volume of the available bone for implant supported rehabilitation.

**Results.** The Panoramic view showed a satisfying bone height, whereas the CBCT acquisitions exhibited an abnormal bone conformation and an insufficient bone width.

Based on the findings on the CBCT images, the implant rehabilitation of the patient can be performed only after bone reconstruction.

**Conclusion.** The use of CBCT is mandatory in implant supported rehabilitation, as it gives the clinician the necessary information about the three-dimensional anatomical presentation of the receptor bone site. Treatment planning is necessary for a good functional and aesthetic outcome of the rehabilitation.

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## DETERMINING THE POSITION OF THE ROTATION CENTER OF THE MAXILLARY CENTRAL INCISOR, DURING THE ORTHODONTIC TREATMENT, WITH THE HELP OF NUMERICAL SIMULATION PROGRAMS

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**Introduction.** The orthodontic treatment implies a force applied at the level of the clinical crown of the tooth. Due to this application point, the dental movement obtained is an inclination of the long axis of the tooth (for a bodily movement –translation- the forces should pass at the level of the resistance point of the tooth, situated at the level of the tooth root). The aim is to determine the location of the rotation center of the central maxillary incisor, with the purpose of using this data in future clinical and mathematical studies, clarifying the orthodontic movement processes.

**Material and methods.** Based on a study carried out on 30 panoramic X-rays on a 1:1 scale, we determined an average length of 24.8 mm for the central maxillary incisor. Applying a force of 100 g on the clinical crown (at 4 mm of the incisor edge), using the ORTODØ1(Ø2) program developed specifically for this study in MATLAB R2007B, we can determine the position of the rotation center of the central maxillary incisor. For an  $\alpha$  point (situated on the incisal edge of the crown) movement of +1mm, the  $\beta$  point (situated at the apex) has the possibility to move anywhere between -1 and +1mm.

**Results.** For  $y\alpha=1$  and  $y\beta=-1$  the rotation center is located at 19.65 mm from the incisal edge.

For  $y\alpha=1$  and  $y\beta=0$  the rotation center is located at 27.81 mm from the incisal edge.

For  $y\alpha=1$  and  $y\beta=+1$  the rotation center does not exist; the movement obtained is a pure translation; it is a hypothetical theory given the fact that for a pure translation movement the force application point should be at the level of the resistance point.

**Conclusions.** Through this numerical simulation program we can determine the location of the rotation point of the central maxillary incisor during the orthodontic treatment, and, with the use of clinical data we can further develop an improved version of the program, which will be able to determine the simulation of this tooth movement in the orthodontic treatment.

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## PHYSICAL METHODS OF EVALUATION FOR A NEW ENDODONTIC SEALANT

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**Introduction.** Several evaluation tests for physical, chemical and biological properties of a new endodontic sealer are required in order to make possible its clinical usage.

**Material and methods.** The current paper presents the evaluation of two physical properties (adhesion and radioopacity) of a new endodontic hydroxyapatite based filling material realized in collaboration with “Raluca Ripan” Institute for Research in Chemistry. For accurate results we have compared the new material with two commercial consecrated sealers- AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) and Real Seal SE (SybronEndo, Orange, CA, USA).

**Results.** The experimental sealer has similar adhesion comparing to the commercial consecrated sealers. Better adhesion to dental structures might be obtained by choosing the appropriate radicular filling technique. ISO standards regarding the radioopacity have been respected for all tested materials.

**Conclusion.** The new endodontic sealer has similar physical properties comparing to other consecrated endodontic filling materials. Further chemical and biological testes are required.

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## MANAGING PEUTZ JEGHERS SYNDROME: A CLINICAL CASE

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**Introduction.** Peutz Jeghers Syndrome is a rare, autosomal dominant intestinal polyposis, diagnosed by the presence of intestinal hamartoma, mucocutaneous melanotic lesions and a positive family history. Alongside the risk of intussusceptions, this syndrome increases the risk of gastrointestinal neoplasia in affected patients. This brings forth the stringent need for a rigorous clinical management of such cases.

**Case Description.** A 44 year old male patient, with Peutz Jeghers Syndrome diagnosed in 1989, presents with symptoms consistent with a subocclusive syndrome. The clinical examination has not yielded any significant information. Considering the risk of retaining the videocapsule, small bowel investigation was carried out via enteroCT, which showed the presence of an intraluminal polypoid growth in the proximal jejunum.

For the polypectomy procedure, the spiral enteroscopy technique was used. The hamartomatous polyp was removed, allowing favorable postoperative recovery. This technique did not show other lesions in the proximal small bowel. The histopathological analysis of the polyp confirmed its hamartomatous, benign character.

**Discussion.** Peutz-Jeghers polyposis involves an active monitoring regimen for the patient. Choosing modern techniques which involve minimal risk and discomfort is essential for ensuring compliance and a good patient-doctor collaboration. Spiral enteroscopy is a safe, rapid and effective technique in monitoring and treating small bowel lesions which occur in these patients.

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## PREVALENCE OF DISC DISPLACEMENT AMONG ORTHODONTIC PATIENTS

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**Introduction.** The purpose of this study was to evaluate the prevalence of Disc Displacement among orthodontic patient with different malocclusion and CO-CR discrepancy.

**Material and methods.** The study included 25 patient with CO-CR discrepancy of 0.5,1 and 2 mm in vertical plane and 0-0.5 mm in transversal plane. Disc displacement (DD) was evaluated clinical and with MRI images.

**Results.** Subject with CO-CR discrepancy of 2 mm in vertical plane and 0.5 mm have an anterior or medial DD and subject with less than 0.5 mm vertical discrepancy show no DD.

**Conclusion.** This study suggest that a patient with transverse discrepancy have a bigger chance to develop DD.

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## CURRENT PERSPECTIVES OF SILVER NANOPARTICLES IN DENTAL BIOMATERIALS

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**Introduction.** Nanotechnology is the most popular and intriguing area for research and development in basically all fields and disciplines. Evidence based studies proved the safety of nano-structured materials. The main focus is integration for development of biosynthetic and environment-friendly technologies and also synthesis of nanomaterials. Metal oxide nanoparticles have high antibacterial activity, while antimicrobial formulations comprised of nanoparticles could be effective bactericidal agents. Antimicrobial materials act against bacteria and avoid, delay or reduce biofilm formation on various materials. Silver nanoparticles (AgNPs) have been successfully applied in restorative dentistry, in endodontics, dental prostheses and implantology. AgNPs incorporation aims at avoiding or decreasing the microbial colonization over dental materials, increasing oral health status.

**Material and methods.** A systematic literature review was conducted by two independent reviewers using PubMed, Embase, Web of Science, Cochrane and Scopus databases in order to identify studies focusing on AgNPs and their application in dentistry. The keywords used were “nanotechnology”, “silver nanoparticles”, “antimicrobial activity” and “dental biomaterials”.

**Results.** The research included original articles, in vitro studies, in vivo studies and other review articles, written in English. From a total of 836 articles, 41 formed the basis of this research.

**Conclusion.** According to the findings of this study, silver nanoparticles are one of the most effective antibacterial agents due to their large surface area to volume ratios. The long-term antibacterial, physical and clinical effects of silver nanoparticles on dental and medical biomaterials should be investigated in future studies.

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## WAX CONSTRUCTIONS: INSTRUMENT OF COMMUNICATION AND TRANSFER OF CLINICAL INFORMATION TO OPTIMIZE AESTHETICS IN FIXED PROSTHODONTICS

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**Introduction.** One of the most challenging aspects of cosmetic dentistry is the ability to visualize the final result before the treatment is initiated. Communication among the restorative dental team must include the patient. The patient wants to know the details of the anticipated restorations; but because a patient is not aware of many dental innovations, care must be taken to provide them with a clear explanation of all anticipated treatment parameters. Through effective communication the patient gains confidence in the dental team and better understanding of the proposed restorative treatment. Patient satisfaction is significantly enhanced through effective communication.

**Materials and methods.** the objective of this study was to examine three methods for obtaining the information through wax-up constructions : diagnostic , full- contour and try- in . Each of those three types of wax-up are used as communication instruments. For wax-up construction the materials used are: grey modelling wax, acrylic resin for patterns and polyethylene foil.

**Results.** The wax-up constructions, was properly used and provided valuable information to the dentist, dental technician and patient in a three-dimensional manner. It helped dentist to determine the amount of reduction (minimally invasive) achieving the final result, keeping preparations as conservative as possible. The wax-up construction provided a blueprint of the final restorations. The wax-up also allowed the manufacture of putty keys for the temporary restorations and reduction guides for tooth preparation.

**Conclusion.** Wax-up is the method that optimizes the communication between dentist, patient and dental technician, so the final results of the treatment can be predicted.

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## FROM NON INVASIVE TO MINIMALLY INVASIVE DENTAL TREATMENTS BY USING FIBER-REINFORCED COMPOSITE RESTORATION: INLAY, ONLAY AND ADHESIVE BRIDGE

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**Introduction.** Fiber-reinforced composite materials are a solution in noninvasive and minimally invasive dental treatments.

**Materials and methods.** Fiber reinforcement is a fiber mesh that adds strength and toughness to composites in several directions. Fiber reinforcement is made from bidirectional glass fibers and a polymer/resin gel matrix.

**Results.** On-lays, inlays, table tops and adhesive bridge when are made from light-cured restorative composite materials look better with optic glaze, that provide long-lasting color and surface gloss in an extremely easy way. Components and physical properties of fiber-reinforced composites work system reveal a simple way to reach aesthetics.

**Conclusions.** Thru composite materials reinforced with fiber the specialists can develop a longevity restoration in different ways: direct, direct-indirect or indirect, and various kind: on-lays, inlays, overlays and adhesive bridge.

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