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MEDICAL SCIENCES

The influence of systemic and local factors on the topological differences in deep vein thrombosis

Ștefan Cristian Vesa[1], Romeo Chira[2], Sonia Irina Vlaicu[2], Sergiu Pașca[3], Sorin Crișan[4], Adrian Trifa[5], Anca Dana Buzoianu[1]

1) Department of Pharmacology, Toxicology and Clinical Pharmacology, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Internal Medicine I, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Medfuture Research Center for Advanced Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

4) Department of Internal Medicine V, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

5) Department of Medical Genetics, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Ștefan Vesa
e-mail: stefan.vesa@umfcluj.ro

Background and objectives. Deep vein thrombosis (DVT) is a common cause of intra-hospital morbidity and mortality, and its most severe complication is pulmonary thromboembolism. The risk factors that influence the apparition of DVT are generally derived from Virchow's triad. Since the most severe complications of DVT occur in proximal rather than distal deep vein thrombosis, the aim of this study was to identify the factors influencing the apparition of proximal DVT.

Materials and methods. This was a transversal, cohort study. The study included 167 consecutive patients with lower limb DVT over a two-year period. The following data were recorded or determined: general data, conditions that are known to influence DVT, medical history and coagulation or thrombophilia-related genetic variations.

Results. In the univariate analysis, male gender, neoplasia, previous DVT and mutated factor V Leiden were all associated with proximal DVT, while bed rest was associated with distal DVT. In the multivariate analysis, male gender, previous DVT and factor V Leiden mutation were independently correlated with proximal DVT, while bed rest was independently associated with distal deep vein thrombosis.

Conclusion. Our observations point out that the factors indicating a systemic involvement of coagulation were correlated with proximal DVT, while local factors were associated with distal DVT.

CYP4F2 and VKORC1 polymorphisms amplify the risk of carotid plaque formation

Ștefan Cristian Vesa[1], Sonia Irina Vlaicu[2], Vitalie Văcăraș[3], Sorin Crișan[4], Octavia Sabin[1], Sergiu Pașca[5], Adrian Pavel Trifa[6], Tamas Rusz-Fogarasi[7], Mădălina Sava[8], Anca Dana Buzoianu[1]

1) Department of Pharmacology, Toxicology and Clinical Pharmacology, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Atherosclerosis represents the process by which fibrous plaques are formed in the arterial wall, increasing its rigidity with a subsequent decrease in blood flow which can lead to several cardiovascular events. Seeing as vitamin K antagonists are involved in the pathogenesis of atherosclerosis, we decided to investigate whether polymorphisms in genes that influence vitamin K metabolism might have an impact in modulating the risk of plaque formation.

2) Department of Internal Medicine I, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Neurology, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

4) Department of Internal Medicine V, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

5) Medfuture Research Center for Advanced Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

6) Department of Medical Genetics, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

7) Department of Surgery Clinic I, Emergency County Hospital, Cluj-Napoca, Romania

8) Department of Dermatology, Emergency County Hospital, Oradea, Romania

Corresponding Author:
Ştefan Vesa
e-mail: stefan.vesa@umfcluj.ro

Patients and methods. In the current study we included adult patients admitted in the Clinical Municipal Hospital of Cluj-Napoca without any carotid or femoral plaques clinically visible at the initial investigation, and a five year follow-up was subsequently performed. We recorded the following patient characteristics: age at inclusion, gender, area of living, smoking, presence of carotid and/or femoral plaques at five years, ischemic heart disease, arterial hypertension, atrial fibrillation, heart failure, diabetes mellitus, obesity, dyslipidemia, drug (oral anticoagulants, antihypertensives, hypolipidemic, anti-diabetic) use and status for the following gene polymorphisms: VKORC1 1639 G>A, CYP4F2 1347 G>T and GGCX 12970 C>G.

Results. We observed that the major predictor of both carotid and femoral plaque formation is represented by ischemic cardiac disease. VKORC1 and CYP4F2 polymorphisms did not predict plaque formation, except for VKORC1 homozygous mutants. Nonetheless, both VKORC1 and CYP4F2 interacted with ischemic cardiac disease, increasing the risk of developing a carotid plaque, while only CYP4F2, but not VKORC1, interacted with ischemic cardiac disease to increase the risk of femoral plaque formation.

Conclusions. We documented that CYP4F2 and VKORC1 polymorphisms boost the proinflammatory plaque environment (observed indirectly through the presence of ischemic heart disease), increasing the risk of plaque development.

Oral anticoagulants preference in hospitalized patients with acute deep vein thrombosis or non-valvular atrial fibrillation

Ştefan Cristian Vesa[1], Sonia Irina Vlaicu[2], Sorin Crișan[3], Octavia Sabin[1], George Saraci[4], Vitalie Văcăraș[5], Daciana Elena Popa[6], Paula Pârcălab[7], Valer Ioan Donca[8], Antonia Eugenia Macarie[8], Mădălina Sava[9], Anca Dana Buzoianu[1]

1) Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Internal Medicine I, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. The aim of the study was to assess the preferences of oral anticoagulants (OA) in patients diagnosed with deep vein thrombosis (DVT) of lower limbs or non-valvular atrial fibrillation (AF) requiring anticoagulation for medium/long term.

Material and methods. The study included consecutive patients admitted with a diagnosis of either acute DVT of the lower limbs (without signs of pulmonary embolism) or non-valvular AF who required oral anticoagulation, in a time frame of 18 months from January 2017 until June 2018. The following data were recorded: demographic variables, comorbidities (ischemic heart disease, arterial hypertension, heart failure, stroke, peripheral artery disease, diabetes mellitus, obesity), type and

3) Department of Internal Medicine V, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca

4) Department of Internal Medicine III, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

5) Department of Neurology and Pediatric Neurology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

6) Department of Cardiology, „Niculae Stăncioiu" Heart Institute, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

7) "Prof. Dr. Ion Chiricuţă" Oncology Institute, Cluj-Napoca, Romania

8) Department of Geriatrics-Gerontology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

9) Department of Dermatology, Emergency County Hospital, Oradea, Romania

Corresponding Author:
Octavia Sabin
e-mail: octavia.sabin@umfcluj.ro

dose of OA (acenocoumarol, dabigatran, apixaban, rivaroxaban), complications due to the use of OA.

Results. AF patients were older and had considerably more cardiovascular comorbidities that DVT patients. Vitamin K antagonists (VKA) were more likely to be administered in patients with AF, as they had an indication for indefinite anticoagulation. VKA were more frequently prescribed in patients with ischemic heart disease, heart failure, and diabetes compared with DVT patients. Also, complications related to OA use were more frequent in VKA group. Almost half of the patients with acute DVT (48.5%) were treated with direct OA (DOAC) rather than VKA, and only a quarter of AF patients (24.8%) were treated with DOACs.

The visual stethoscope: hand-held ultrasound devices for diagnosing lung disease

Mariam Haji-Hassan[1], Lavinia Manuela Lenghel[2], Sorana D. Bolboacă[1]

1) Department of Medical Informatics and Biostatistics, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca

2) Department of Radiology, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Lung ultrasound is a relatively recent medical technique and sometimes considered a surprising turn for medical imaging technology. Since the air in the lung scatters and impedes sound waves transmission, the method was thought to be of little use for studying the lungs. However, the surface of the lung is a strong reflector of ultrasound waves and thus creates several reverberation artifacts. These artifacts contain valuable information and correlate with the current lung pathophysiology. We conducted a systematic review to assess the extent to which hand-held ultrasound devices can be used to diagnose lung disease.

Corresponding Author:
Mariam Haji-Hassan
e-mail:
mariam.hadassah33@gmail.com

Method. We searched three databases, namely PubMed, ScienceDirect, PMC Europe, on June the 7th 2020. We excluded studies where the ultrasound operators were not doctors and those who reported the use of hand-carried cart-based systems.

Results. We included 16 articles in the final evaluation. In these studies, portable ultrasound was employed to identify pleural effusions, parenchymal consolidation areas, and interstitial lung disease. The most frequently used hand-held device was Vscan (General Electric). While one of the studies was descriptive, the others compared the sensitivity and specificity of hand-held ultrasound devices with clinical examination and/or other imaging modes. Ultrasound showed a good association with different imaging techniques, up to 92% sensitivity and 80% specificity for diagnosing interstitial lung disease when ultrasound was compared with computed tomography.

Conclusion. While all lesions mentioned above can appear in COVID-19 infection, they are neither specific nor pathognomonic. However, point-of-care lung ultrasound with pocket-size devices has proven several advantages: good concordance with X-ray and CT scans, without repeated exposures to radiation, increased accessibility and decreased cost, the performance of the examination at the bed-side, with no contamination caused by transportation, ease of disinfection procedures (as these devices can be entirely enclosed in a protective sheath). Therefore, point-of-care lung ultrasound is likely to develop into an established diagnostic technique in the context of an increased need for lung examinations.

Isolated very low voltage in patients with reflex syncope

Călina-Patricia Țentea[1], Roxana Chiorescu[1], Sorin Crișan[2], Sorin Pop[1], Dan Blendea[1]

1) Department of Internal Medicine I, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Internal Medicine V, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Călina-Patricia Țentea
e-mail: tenteapatricia@yahoo.it

Introduction. This research was prompted by our observation that a significant number of patients with reflex syncope (RS) have QRS complexes of very low voltage (≤0.3 mV) in one, and, occasionally more than one frontal lead on the 12-lead electrocardiogram. We hypothesized that the changes in QRS voltage might be related to the specific geometry and/or ventricular activation pattern in RS. Given these considerations the aim of this study was to assess comparatively the presence of very low voltage (VLV) in the frontal plane leads in patients with syncope and with no history of syncope.

Material and methods. We included 169 patients (age 52 ± 20 years, 90 women) 114 with RS and 55 controls with no syncope and without structural heart disease. The QRS voltage was measured in mV on 12-lead ECGs. The lowest QRS voltage (QRSmin) in the frontal plane leads was determined.

Results. Very low voltage in the frontal leads was observed in 71 (42%) of the patients with 12 (7%) patients having VLV in two or more frontal plane leads.

The ECG lead that had the QRS with lowest amplitude in the frontal leads (aVL in 56% of the patients), was close to perpendicular (average 84 degrees) to the QRS axis.

Very low voltage in the frontal leads was significantly more prevalent in the RS group than in the control group (64 (56%) vs 14 (25%), respectively; $p<0.001$). VLV in two or more frontal leads was present in 12 (11%) of patients in the RS group and in no patient from the control group ($p=0.001$).

Patients in the RS group had significantly lower QRSmin when compared to patients in the control group (0.34±0.17 mV vs. 0.42±0.16 mV; $p=0.014$).

ROC analysis showed that QRSmin of ≥0.3 mV discriminated between syncope and no syncope patients in this population with a sensitivity of 79% and specificity 49% (AUC=0.652).

Only 27 (23%) of the RS patients had a QRSmin of ≥0.5 mV.

QRSmin in the syncope group correlated significantly with the left ventricular end-diastolic diameters (r = 0.2, p= 0.050) measured by echocardiography.

Conclusion. The presence of isolated very low QRS voltage in the frontal leads may help identify predisposition for reflex syncope.

Predictors of adverse events after peripheral angioplasty – gender related characteristics

H. Comșa[1,2], G. Cismaru[1,2], A. David[2], G. Gușetu[1,2], B. Caloian[1,2], F. Frîngu[1,2], R. Tomoaia[2], D. Zdrenghea[2], D. Pop[1,2]

1) Department of Cardiology Rehabilitation, Faculty of Medicine, Iuliu Hațieganu Univesity of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Clinical Rehabilitation Hospital, Cluj-Napoca, Romania

Corresponding Author:
Dan Horațiu Comșa
e-mail: dh.comsa@gmail.com

Introduction. Indications for percutaneous treatment are the same regardless of gender and it should not influence peripheral angioplasty outcomes. The aim of the study was to compare the outcome of subjects who benefited from endovascular therapy and to identify differences in term of medium-term results according to gender, and also identify negative prognostic factors for each specific group.

Material and methods. A total of 104 consecutive subjects (50 women and 54 men) who received endovascular treatment for symptomatic PAD in the between January 2017 and October 2019 were included. Values of biological parameters, PAD anatomical data, medical treatment and type of percutaneous intervention were documented. Results were assessed at 1 month, 6 months, 1 year, and 2 years after index revascularization, documenting MALE (major adverse limb events), defined as a composite endpoint of severe limb ischemia requiring revascularization or major amputation.

Results. Mean follow-up was 21 months, with no significant gender differences Ankle-brachial index (ABI) was similar in the two groups, with an average value of 0.5 ($p > 0.05$). The occurrence of MALE endpoints was similar in the two groups, at around 20% ($p = 0.914$). The only variable found to have a significant influence on the occurrence of MALE was the ABI. A value below 0.5 was found to be an independent predictor for MALE ($p = 0.001$), with no other parameter able to predict an adverse post-PTA outcome. Literature data on the medium- and long-term outcomes of percutaneous and surgical revascularization in women are contradictory. Previous studies comparing the results of peripheral percutaneous interventions for the two genders did not highlight female sex as an independent predictor for an unfavorable post-PTA outcome. We found no significant differences in the occurrence of MALE over a mean follow-up period of 2 years. The rates of major amputation and the need for reintervention were similar between the two groups. The only independent predictor for MALE, regardless of gender, was ABI. A threshold value below 0.5 was a strong negative predictor of limb-related adverse events.

Conclusion. No significant differences were observed in the rates of MALE post-PTA between the two genders at 2 years. An ABI of less than 0.5 is an independent predictor of MALE, regardless of sex.

Metabolomics - a new tool in diagnosis and prognosis of early hepatocellular carcinoma

Iuliana Nenu[1,2], Iulia Minciună[1,2], Sorana Pașcalău[1], Carmen Socaciu[3], Adriana Filip[4], Bogdan Procopeț[1,2], Horia Ștefănescu[1]

1) Regional Institute of Gastroenterology and Hepatology „Prof. Dr. Octavian Fodor", Cluj-Napoca, Romania

2) Department of Internal Medicine III, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Center for Applied Biotechnology BIODIATECH, SC Proplanta, Cluj-Napoca, Romania

4) Department of Physiology, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Iuliana Nenu
e-mail: iuliana.nenu@gmail.com

Introduction. In the last years, metabolomics, the youngest "omic" science, has played an important role in the struggle to discover new biomarkers and biochemical networks and to improve diagnosis, prognosis and treatment of hepatocellular carcinoma (HCC). Sphingolipids, a family of membrane lipids, involved in numerous cell functions, including cell death pathways have an altered expression in many types of cancer. The aim of the study was to identify a metabolomic profile for HCC diagnosis and prognosis to compare its diagnostic accuracy with alpha fetoprotein (AFP).

Methods. We conducted a case-control study, in which 69 patients were included. The patients were admitted between 2016-2020 at the "Prof. Octavian Fodor" Regional Institute of Gastroenterology and Hepatology Cluj-Napoca, Romania as following: 37 patients with early HCC (BCLC stages 0 and A) and 32 with compensated cirrhosis and a period of three years of follow-up was performed. Common workup for the assessment of liver disease and AFP was carried out in each patient. High pressure liquid chromatography (HPLC) coupled with quadruple time of flight electrospray in a positive ionization mode mass spectrometry (QTOF-ESI+-MS) was performed from serum samples of each patient. MetaboAnalysis, performing univariate and multivariate statistical analysis was used to identify candidate biomarkers. Their performance for detection of early HCC was evaluated using semi-quantitative assessment and through a leave-one-out cross-validation based on area under the receiver operating characteristics (ROC) curve.

Results. 15 metabolites were identified. Sphingolipids are the most upregulated in HCC patients, particularly C16 sphinganine (C16-SPH) ($p < 0.001$ vs. compensated cirrhosis). The expression of C16-SPH was 4.869 times higher in HCC than in cirrhotic controls ($p < 0.005$). The area under the curve (AUC) of C16-SPH for the diagnosis of HCC was significantly higher compared to AFP [0.969 (95%CI, 0.923-1) vs. 0.544 (95%CI, 0.415-0.673), p (deLong test) <0.001.

Conclusion. Sphingolipids expression is upregulated in HCC patients compared with cirrhosis control and might represent novel diagnostic markers for the identification of early hepatocellular carcinoma in patients with chronic liver diseases.

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The relationship between leptin, ghrelin, leptin/ghrelin ratio – obesity and metabolic syndrome

Adela-Viviana Sitar-Tăut[1], Angela Cozma[1], Adriana Fodor[2], Sorina-Cezara Coste[1], Olga Hilda Orășan[1], Vasile Negrean[1]

1) Department of Internal Medicine IV, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca

2) Department of Diabetes and Nutrition, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Adela-Viviana Sitar-Tăut
e-mail: adelasitar@yahoo.com

Introduction. Metabolic syndrome represents a cluster of risk factors associated with increased cardiovascular risk. Adipose tissue is considered an endocrine organ that secretes adipocytokines. Leptin and ghrelin are two hormones with the opposite effect. The purpose of the study was to assess the relationship between adipocytokines and theirs' ratio–obesity–metabolic syndrome.

Material and methods. 60 patients (44 women), mean age 61.88±10.08 were evaluated. CV risk factors were assessed. The values of leptin, ghrelin, insulin were measured. The insulin resistance score was calculated using HOMA index. Patients were categorized according to BMI in normal weight, overweight, and obese. The classification of MetS was based on IDF guidelines.

Results. 20% patients were normal weight, 51.7% overweight and 28.3% obese, 71.7% with metabolic syndrome. The overall mean±SD (median) values were for leptin 26201±23946 (20195) pg/ml, for ghrelin 39.55±18.90 (34.25) pg/ml, for HOMA-index 2.07±1.19 (1.72), and for leptin/ghrelin ratio 771.68±791.43 (508.61). Globally, significant correlations were found between leptin and BMI (rho=0.402, p=0.001), leptin/ghrelin and BMI (r=0.304, p=0.018). Obese patients presented greater leptin values (in obese 34360 pg/ml vs overweight 18000 pg/ml vs normal weight 14350 pg/ml, p=0.0049), leptin/ghrelin ratio (1055±641 vs 771.36±921 vs 370.7±257, p=0.0228), HOMA index (p=0.0030). Mean leptin/BMI ratio was significantly higher in obese ones. Mean ghrelin/BMI ratio significantly lower in obese ones. No significant differences were found regarding leptin and ghrelin values between patients with MS vs without MS. MS patients presented greater values of leptin/ghrelin values (869.19±845 vs 525.03±584, p=0.07) and HOMA index (1.83 vs 1.57, p=0.004). The determined area under the ROC curve for MS identification was for leptin/ghrelin ratio globally 0.687 (0.690 in women cut-off value 600.54 pg/ml, 0.923 in men – cut-off value 101.98 pg/ml). For the HOMA index AUROC globally was 0.740 (0.752 in women, 0.654 in men).

Conclusion. Obese patients have significantly increased values of leptin/BMI and decreased ghrelin/BMI values. The leptin/ghrelin ratio seems to have a good capacity to identify metabolic syndrome patients, especially in men.

Pruritus and insomnia predict the risk of death in patients on hemodialysis

George Ciulei[1], Flaviu Mureșan[2], Adela Sitar Tăut[1], Vasile Negrean[1], Angela Cozma[1], Olga Hilda Orășan[1]

1) Department of Internal Medicine IV, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Pruritus and insomnia are associated with a poor quality of life in hemodialysis patients (HD), but their coexistence and impact have rarely been studied in this group. We investigated the survival of HD patients presenting either none, one, or both disorders and compared certain features between these groups.

2) Department of Surgery IV, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author: George Ciulei
e-mail: geo.ciulei@yahoo.com

Material and methods. 170 patients treated by HD or online hemodiafiltration were included and were assigned in 4 study groups depending on the presence of either, neither, or both pruritus and insomnia. The survival difference between groups after 20 months was analyzed, and we searched for significant differences in terms of clinical and laboratory features.

Results. Patients with both pruritus and insomnia had lower survival at 20 months. Patients with pruritus alone had a lower Kt/V than those with no complaints or insomnia alone. Those with no complaints had lower C-reactive protein and higher albumin levels than patients with insomnia alone or both conditions.

Conclusion. Pruritus and insomnia should be actively investigated and correlated with clinical and laboratory features as they have a significant impact on survival in HD patients.

Association between RAAS polymorphism with carotid atheromatosis in hypertensive patients

Oana Mocan[1], Dan Rădulescu[1], Elena Buzdugan[1], Angela Cozma[2], Daniel Corneliu Leucuţa[3], Cristina Cătană[4], Lucia Maria Procopciuc[4]

1) Department of Internal Medicine V, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Internal Medicine IV, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Medical Informatics and Biostatistics, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

4) Department of Medical Biochemistry, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author: Oana Mocan
e-mail: oanamocan@yahoo.com

Introduction. Renin–angiotensin–aldosterone system influences atherosclerosis and may be associated with carotid atheromatosis (CA). Thus we aimed to assess the relation between genetic variants of RAAS, M235T-angiotensinogen (AGT) and insertion/deletion of angiotensin conversion enzyme (I/D-ACE), with CA in patients with essential hypertension (EHT), as predictors of ischemic cerebrovascular events.

Material and methods. We designed a prospective cross-sectional study on a group of 162 hypertensive subjects, in three tertiary regional medical centers. We determined the genotypes for M235T-AGT and I/D-ACE using PCR-RFLP methods. The relationship between the studied RAAS gene polymorphisms and CA was assessed by multiple logistic regressions.

Results. The studied group of hypertensive subjects with CA, compared to the group without CA, had the following distribution of M235T-AGT genotypes: MT235 (56.2% vs. 43.8%), TT235 (23.35% vs. 20.2%), MM235 (20.5% vs. 36%). Regarding I/D-ACE gene variant we found the following distribution of the genotype: I/D (20.5% vs. 29.2%), D/D (37% vs. 34.8%), I/I (42.5% vs. 36%) in the CA group of hypertensive subjects, compared to the group without CA.

The chance for a patient to have CA was 2.3 times (95%CI=1.05-5.26) higher for the MT/TT235-AGT genotypes (p=0.041) vs. MM235-AGT, after adjustement for age, gender, hepatic steatosis, and low-density lipoprotein cholesterol. In the codominant model, the chance for a patient to have CA was 2.24 times (95%CI=1.07-4.86) higher in the MT235-AGT genotype (p=0.036) vs MM235-AGT. We didn't find a statistically significant association between any model of I/D-ECA genotype and CA.

Conclusion. We found statistically significant higher odds of having CA for the MT/TT235-AGT genotype than for the MM235-AGT genotype, in hypertensive patients, even after adjustment for confounders. We could not find a relation between I/D-ECA and CA.

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Accelerated atherosclerosis in patients with venous thromboembolism and cancer - myth or reality?

Elena Cristina Buzdugan[1], Iulia Denisa Bulbucan[2], Kinga Zsakay[2], Ionel Lucian Stan[2], Alexandra Popov[2], Sorin Crișan[1]

1) Department of Internal Medicine V, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Municipal Hospital Cluj-Napoca, Romania

Corresponding Author: Sorin Crișan
e-mail: Sorin.Crisan@umfcluj.ro; crisan.sorin@gmail.com

Introduction. In patients with venous thromboembolism (VTE) about 20% have concomitant cancer, with considerable impact on survival rates. Their death is caused, apart from the progression and complications of neoplasia, by acute arterial events. Atherosclerotic (ATS) disease within VTE population has often been documented, favored by shared risk factors, and is considered an accelerated process if cancer coexist. Our aim was to test the hypothesis of a particular ATS disease in patients with VTE and concomitant cancer.

Material and methods. In a 18 month period, all VTE patients admitted in the medical departments of our hospital were selected. They were divided, taking into account the malignancy, into group A (cancer+) or group B (cancer-). All patients were analyzed accordingly to traditional risk factors (except for smoking, this information being inconstantly recorded), C-reactive protein (CRP) and pulse pressure (PP). We considered ATS disease present if proofs for coronary heart disease (CHD) were found in medical history and/or carotid artery disease was proven using ultrasound.

Results. Out of a total of 156 VTE patients, 29 (18.58%) have been selected in group A and 127 (81.42%) formed the control group B. The median age for the study groups was 68.21 y/o. We found no significant differences between groups, according to age (67.27 vs 68.42 y/o, p=0.90) or male sex (48.27 vs. 44.09%, p=0.68). No other traditional risk factors differ significantly (obesity 34.48 vs 40.15%, p=0.57; hypertension 68.96 vs 70.07%, p=0.90; diabetes 17.24 vs 17.32%, p=0.99; dyslipidemia 51.72 vs 60.62%, p=0.38). CRP levels (18.91 vs 21.03 mg/dl, p=0.83) and PP values (56.41 vs 58.56 mmHg, p=0.83) were similar. Proportion of CHD (41.3 vs 32.28%, p=0.35) and carotid involvement (34.48 vs 25.19%, p=0.82) resembled. When we considered age subgroups (<65 y/o and ≥65 y/o), differences did not reach significant values.

Conclusion. We found similar arterial risk factors and ATS disease prevalence, regardless of the presence of neoplasia. We do not confirm accelerated ATS disease in patients with VTE and concomitant cancer.

Eosinophilic esophagitis – case presentation

Valentin Militaru[1], Constance Chapuis-Biron[2], Josselin Questiaux[2], Lea Juif[2], Stephane Koch[2], Lucine Vuitton[2]

1) Department of Internal Medicine V, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Gastroenterology, University Hospital of Besançon, France

Introduction. Eosinophilic esophagitis (EoE) is a chronic disease of the esophagus characterized by eosinophilic inflammation, clinically manifested as esophageal dysfunction, showing an important increase in frequency lately. Our aim was to present the clinical, endoscopic, and histological findings in a case of EoE manifested by food impaction.

Material and methods. Clinical, endoscopic, and histological data concerning our patient were collected from the medical digital record in the University Hospital of Besançon (France).

Corresponding Author: Sorin Crișan
e-mail: Sorin.Crisan@umfcluj.ro;
crisan.sorin@gmail.com

Results. A 57-year-old male having no relevant antecedents or treatment, complaining of non-progressive dysphagia during the last 5 years, presented for complete aphagia after ingestion of chicken meat. At the emergency endoscopy an impacted fragment of meat was found and extracted with a polypectomy snare. The esophagus displayed esophagitis and successive stenoses. Histological examination of biopsies showed 40 eosinophils/40x microscopic field. After several weeks of proton pump inhibitor dysphagia persisted, making balloon dilation necessary. Consequently, upper gastrointestinal endoscopy was done, completing the diagnosis with Barrett's esophagus C1M2.

Conclusion. The most frequent symptoms of EoE are dysphagia and food impaction. The pseudo-tracheal aspect in endoscopy is typical. The association of EoE and gastroesophageal reflux disease has been described. Balloon dilation is effective for stenoses, in association with medication.

Genetic profile and Hippo signaling expression in serous ovarian adenocarcinoma

Laura-Ancuța Pop[1], Paul Kubelac[2,3], Paul Chiroi[1], Lajos Raduly[1], Cornelia Braicu[1], Patriciu Achimaș-Cadariu[3,4], Ioana Berindan-Neagoe[1,5]

1) Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Oncology, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj Napoca, Romania

3) Department of Surgery, „Prof. Dr. Ion Chiricuță" Oncology Institute, Cluj-Napoca, Romania

4) Department of Oncological Surgery and Gynecologic Oncology, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

5) Department of Genetics, Functional Genomics and Experimental Pathology - The Oncology Institute "Prof. Dr. Ion Chiricuță", Cluj-Napoca, Romania

Corresponding Author:
Laura-Ancuța Pop
e-mail: laura.pop@umfcluj.ro

Background. Ovarian cancer is one of the most common cancers worldwide. Truthfully, the clinicopathological and molecular features of these neoplasms collection are so miscellaneous that over 70% of the cases do not get diagnosed until late III or IV stages, where the five-year survival rate has a dismal 25%.

Methods. In this study, we evaluated the genetic profile ovarian serous carcinoma samples using next generation sequencing technology. In addition, the expression level of hippo signaling genes was evaluated.

Results. We identified four patients with pathogenic *BRCA1/BRCA2*, and 13 genes mutated in our cohort of patients. The most frequently mutated genes were: *TP53, ERBB4, STK11* and *PIK3CA*, which are known cancer driver genes. We also observed a statistically significant increased level for *TP53* and reduced expression level for *LATS1, LATS2, MST1, TAZ* and *TEF,* genes involved in the conserved pathway of Hippo signaling.

Conclusion. This study was able to demonstrate that ovarian cancer patients present also *BRCA1/2* pathogenic mutations and mutations in specific cancer driver genes. The expression levels of *LAST1* and *TAZ* was correlated with the stage of ovarian patients. These data could be used as starting point for new biomarkers of early diagnosis of ovarian cancer.

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Analysis of the expression and prognosis values of key genes related to Hippo signaling in lung adenocarcinoma

Lajos Raduly[1], Cornelia Braicu[1], Antonia Haranguș[1,2], Ancuța Jurj[1], Oana Zanoaga[1], Roxana Cojocneanu[1], Cecilia Pop-Bica[1], Liviuța Budișan[1], Marioara Simon[2], Ioana Berindan-Neagoe[1,3]

1) Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Bronchology, Leon Daniello Pneumology Clinical Hospital, Cluj-Napoca, Romania

3) Department of Genetics, Functional Genomics and Experimental Pathology, „Prof. Dr. Ion Chiricuță" Oncology Institute, Cluj-Napoca, Romania

Corresponding Author:
Lajos Raduly
e-mail: raduly.lajos78@gmail.com

Introduction. Non-small cell lung cancer involves more than 80% of pulmonary tumors, adenocarcinoma (LUAD) representing the predominant subtype. At the time of diagnosis, most NSCLC patients are already at the metastatic stage and with poor prognosis. The Hippo signaling cascade is involved in different cellular processes such as cell differentiation, proliferation and apoptosis.

Materials and methods. In our study, we intend to identify key Hippo signaling genes involved in the NSCLC tumorigenesis and disease progression by integrated bioinformatics analysis.

Results. Using UALCAN data based it was observed overexpression of BBC3, MST1, STK3, TAZ, TP53, TP73 and MALAT1 and downregulation of LATS2, TEAD1, TEF, YAP. Among these genes only TEF and MALTA1 were correlated with survival rate. An additional analysis was done to identify key miRNAs related to hippo signaling and identified five miRNAs overexpressed and three downregulated miRNAs, only let-7b was able to predict overall survival in LUAD patients, transcript that directly regulated TAZ and TP53 gene (frequently mutated, in LUAD).

Conclusion. Finally, we investigated the potential prognostic role and therapeutic targeting of the Hippo pathway, being mainly related to TEF, MALAT1 and let-7b.

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BASIC SCIENCES

Is there a link between vitamin D and rheumatoid arthritis?

Cristina-Sorina Cătană[1], Erin-Manuela Grigorean[2]

1) Department of Medical Biochemistry, Faculty of Medicine Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Erin Grigorean
e-mail: eringrigorean@yahoo.com

Introduction. Vitamin D has always appealed to the rheumatologic comunity. Besides its role in the phosphocalcic metabolism, various evidence has been found regarding its action on other systems of the human body. Since the discovery of the VDR receptors (Vitamin D Receptor) in almost all immune cells, it has been supposed that vitamin D would is also involved in immune homeostasis. Research has demonstrated that the molecule plays a role in autoimmune diseases such as multiple sclerosis and Hashimoto thyroiditis. The correlation between low serum levels of vitamin D and the severity of rheumatoid arthritis has been debated in recent publications. Despite this, studies have shown mixed results.

Objective. The objective of this study was to determine if there exists a correlation between vitamin D and rheumatoid arthritis.

Materials and methods. Using PubMed, we have examined four studies based on pacient groups and control groups analysis. Statistical methods have shown if vitamin D deficit is more frequent in rheumatoid arthritis pacients.

Results. A lack of vitamin D was found in both rheumatoid arthritis patients, as well as the general population. The analysed studies have emphasised the importance of future research in therapeutic eficiency in rheumatoid arthritis. The absence of an international agreement on vitamin D serum levels, as well as the period in which the studies have been conducted, remained the main obstacles in result interpretation.

Conclusion. It is essential to find an association between vitamin D deficiency and disease severity in rheumatoid arthitis.

Multimarker approach for acute myocardial infarction's diagnosis and treatment

Cristina-Sorina Cătană[1], Andreea Iosub[2]

1) Department of Molecular Biochemistry, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Andreea Iosub
e-mail: andreeaiosub17@yahoo.com

Introduction. Acute Myocardial Infarction (AMI) is one of the leading causes of mortality and morbidity worldwide. Ischemic heart disease is responsible for 20% of all deaths in Europe. Every day, in Romania, 40 people die from myocardial infarction, and 2 out of 3 Romanians have cardiovascular diseases. An early diagnosis it is necessary, and an optimal therapeutic management of these patients, because the highest mortality rate appears in the first hours after the onset of ischemia.

Objectives. The aim of this study was to present and analyze the various biochemical markers released in patients' serum during acute myocardial infarction, in order to establish the effectiveness of a multimarker approach for early diagnosis.

Material and methods. The levels of markers detected in the blood of 94 patients selected from those presented at the Heart Institute „Nicolae Stăncioiu" in the first half of 2020 were analyzed following the variability of levels of cardiac troponin with high sensitivity (hs-cTnT), creatine kinase-MB (CK-MB), lactate dehydrogenase

(LDH), aspartate aminotransferase (ASAT), alanine aminostransferase (ALAT) in patients diagnosed with ST-segment elevation myocardial infarction (STEMI), compared with patients with acute myocardial infarction without ST segment (NSTEMI). We also aimed to highlight a possible correlation between the level of hs-cTnT and the glomerular filtration rate (GFR), and respectively hs-cTnT and body mass index (BMI).

Results. We used specific statistical methods to analyze the values of the biochemical markers considered, hs-cTnT, CK-MB LDH, ASAT, ALAT and highlighted their differences in variability within the two compared groups. We also established correlations between the values of hs-cTnT and RFG, respectively hs-cTnT and BMI.

Conclusion. We have shown that a multimarker approach is useful in the diagnosis of AMI.

The role of vitamin D in Hashimoto thyroiditis: a comprehensive meta-analysis

Cristina-Sorina Cătană, Diana-Elena Deaconescu

Department of Medical Biochemistry, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Diana Elena Deaconescu
e-mail: diana.dde25@yahoo.com

Introduction. Recent research has shown that vitamin D has not only an important role in bone and calcium homeostasis, but also in modulating innate and adaptative immune response. Its receptors are expressed on immune cells – B cells, T cells and antigen presenting cells. In the present meta-analysis, our main objective was to evaluate the relation between vitamin D deficiency and a specific autoimmune disease- Hashimoto's thyroiditis (HT). More exactly we analyzed the discriminative value of vitamin D between HT and control subjects.

Material and methods. We searched in PubMed and Medline for articles that we reviewed in the present meta-analyses. There were included 4 articles published in the last 4 years that provided data for quantitative meta-analysis. We combined and standardized mean differences in those case-control studies.

Results. The present study shows that vitamin D is lower in HT patients comparative with control group. Also, the level of vitamin D is negatively correlated with TSH.

Conclusions. The correlation between vitamin D deficiency and autoimmune thyroiditis is a big step in further prevention and better treatment in HT patients.

Students' lifestyle and behavior during Covid-19 pandemic lockdown: use of recreational products

Irina Brumboiu[1], Irina Iaru[2]

1) Department of Epidemiology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. The quarantine period suddenly changed the daily activity of the students. This involved both the educational process and the daily living conditions. To adapt to the new situation, the use of recreational products may be different from previous periods. The objective of the study was to evaluate how different recreational products were used by students in the context of the current conditions of the university education process.

2) Department of Pharmacology, Physiology, Pathophysiology, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Irina Brumboiu
e-mail: ibrumboiu@umfcluj.ro

Materials and method. The study is part of a larger project that assesses students' lifestyles and behaviors, initiated in 2015. The descriptive analysis included a comparison between the results obtained during the quarantine and those observed in previous periods.

Results. Among the respondents (n = 901), 14.9% reported smoking (almost) daily before the state of emergency, while 10.8% during the state of emergency. The number of cigarettes/day was <10 for 10.8% of students, between 10 and 20 cigarettes/day for 9.4% before the state of emergency, and during this period <10 cigarettes/day for 7.9% and between 10 and 20 cigarettes/day for 5.8% of students. More students (26.5%) reported alcohol consumption before quarantine compared to the quarantine period (7.3%). In case of cannabis use, 5.1% of students reported previous use compared to 1.2% during the restrictions.

Conclusions. The conditions of living and of educational process of the students, suddenly changed in the context of the Covid-19 pandemic control measures, were associated with a different way of using recreational products. Changed behavioral practices can reduce the risk of addiction and consequently exposure to risk factors known for pathologies that are also among the most common in our country: cardiovascular and neoplastic disease.

Antioxidant activity of *Nigella sativa* L. extracts in a cellular model of doxorubicin-induced cardiotoxicity

Raluca Maria Pop[1], Ioana Corina Bocşan[1], Veronica Sanda Chedea[2], Sonia Ancuţa Socaci[3], Anca Dana Buzoianu[1]

1) Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Research Station for Viticulture and Enology, Blaj, Romania

3) Department of Food Science, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Corresponding Author:
Raluca Maria Pop
e-mail: raluca_parlog@yahoo.com

Introduction. Black cumin - *Nigella sativa* (N. sativa) is known for its benefic effect in reducing multiple cardiovascular risk factors. Antracycline chemotherapy is one of cardiovascular high-risk factor. Within this study, the effect of N. sativa water and methanolic extracts was investigated using a cellular model of doxorubicin (doxo)-induced cardiotoxicity.

Materials and methods. Prior analysis, the extracts were characterized using complementary techniques like Fourier-transform infrared (FT-IR) spectroscopy, Ultraviolet-visible (UV-VIS) spectroscopy, Gas Chromatography coupled with Mass Spectrometry (GC-MS) and Liquid Chromatography coupled with Mass Spectrometry (LC-MS). The antioxidant activity was evaluated using H9c2 cells. Cytosolic and mitochondrial reactive oxygen species (ROS) release was evaluated using 2′,7′-dichlorofluorescin-diacetate (DHCF-DA) and mitochondria-targeted superoxide indicator (MitoSOX red), respectively. Mitochondrial membrane depolarization was investigated by flow cytometry.

Results. The compounds identified in *N. sativa* seeds by LC-MS analysis showed the presence of 12 phenolic compounds, with flavonols as predominant compounds. The FT-IR analysis identified the carbohydrates, amino acids and lipids. The GC-MS analysis further identified the volatile compounds. *N. sativa* seeds methanolic extract had the highest antioxidant activity with positive effect in reducing the intracellular and mitochondrial ROS release. Both water and methanolic extract preserved H9c2 cells viability. The mitochondrial membrane depolarization was not affected.

Conclusions. *N. sativa* is an important sources of phytochemicals that possess increased antioxidant activities. To maximize the pharmacological effects, different extraction solvents are required to obtain the bioactive compounds.

A pilot study to evaluate the correctness of the medication history using Structured Medication Questionnaire

Octavia Sabin[1], Amalia Ioana Radu[2], Anca-Daniela Farcaș[3]

1) Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Graduate, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Internal Medicine I, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Octavia Sabin
e-mail: octavia.sabin@umfcluj.ro

Aim. This study is to evaluate the opportunity of using a Structured Medication Questionnaire (SMQ) for drug history to better identify current medication and to potentially reduce medication errors. The hypothesis is that through SMQ the accuracy of medical history can be improved and the drug errors in hospitalized patients can be reduced.

Methods. The study was a pilot, interventional, pre-post, single-arm study design. The study enrolled all consecutive patients admitted to a cardiology ward in Cluj-Napoca in 2 weeks interval in February-March 2020.

Results. Fifty-six patients included in this study had a total of 429 drugs identified by SMQ compared to 338 drugs in the observation sheets (21%). Forty-six patients (76.78%) had at least one error in prescription drugs; if dietary supplements are added, 49 patients (87.5%) had at least one error. The classes of drugs where the most common errors were omitted were non-steroidal anti-inflammatory drugs (ATC code M01A), psycholeptics (ATC code N05), and ophthalmic products with 7 errors each (7.6% of the total errors by omission).

Conclusion. A structured questionnaire implemented in the patient interview may improve the accuracy of the medication history and may reduce the frequency of omission errors.

Muscle soreness and muscle fatigue modulation, by Coenzyme Q10, in physical stress

Ramona-Niculina Jurcău[1], Ioana-Marieta Jurcău[2], Nicolae-Alexandru Colceriu[3], Cornelia Popovici[4], Mihai Kiss[4]

1) Department of Pathophysiology, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Emergency Clinical Hospital for Children, Cluj-Napoca, Romania

3) Faculty of Horticulture, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

4) Department of Sports, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Ramona-Niculina Jurcău
e-mail: ramona_mj@yahoo.com

Introduction. Intense exercise has multiple consequences, among which are muscle soreness and muscle fatigue. Acute and chronic supplementation of CoQ10 may affect acute and/or chronic responses to various types of exercise. The objective is to highlight the muscle soreness (MSR) and muscle fatigue (MSF) modulation by Coenzyme Q10, on physical stress.

Material and methods. Voluntary men were divided into placebo group (P) and CoQ10 group (CoQ). Stress was represented by a physical effort on a treadmill. Analyzed parameters: MSR and MSF.

Results. MSR anf MSF were significantly reduced immediately after the stress in CoQ compared to P. For both parameters there were differences on dynamic developments between groups. CoQ10 influence was more intense on MSS than on MFS.

Conclusions. 1) CoQ10 acted more efficiently on MSS than on MFS. 2) MSS and MFS were reduced after the stress under CoQ10 influence. 3) Immediately post-stress, CoQ10 influence was higher. 4) CoQ10 could be useful to modulate muscle soreness and muscle fatigue sensation in acute intense psychical stress.

Influence of *Symphytum officinale* and moderate physical exertion, on low back pain

Ramona-Niculina Jurcău[1], Ioana-Marieta Jurcău[2], Nicolae-Alexandru Colceriu[3], Mihai Kiss[4], Cornelia Popovici[4]

1) Department of Pathophysiology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Emergency Clinical Hospital for Children, Cluj-Napoca, Romania

3) Faculty of Horticulture, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

4) Department of Sport, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Ramona-Niculina Jurcău
e-mail: ramona_mj@yahoo.com

Introduction. Pain is an unpleasant experience. The low back pain is a common condition and a substantial economic burden in industrialized societies. *Symphytum officinale* (SO) has been used as a medicinal plant. SO it has proved to be effective in various types of pain. Thus, it has effectiveness in osteoarthritis, sprains, muscle pain. The objective is to highlight the influence of *Symphytum officinale* and moderate physical exertion on low back pain.

Material and methods. Voluntary subjects were selected into two groups: control (C) and experimental (E), which has been administered SO. Both groups were subjected to the same type of physical effort on the stepper. SO ointment was administred to E, before beginning the exercise stepper, daily, in two stages of three weeks each. Analyzed indicators were: local pain sensation (P) and anxiety (A).

Results. Local SO ointment application to the E, had the most significant results after the second SO treatment period. Following SO ointment administration, P decreased in previous exercise period, and P and A were significantly reduced during exercise, compared to the untreated group, at all testing times.

Conclusions. 1) SO therapy significantly reduced pain sensation. 2) Both pain sensation and anxiety were significantly reduced in subjects treated with SO, compared to control. 3) SO provided pain and emotional protection for treated subjects. 4) SO ointment, due its therapeutic qualities of good tolerance, absence of toxicity and economic accessibility, could be use for antalgic protection, in subacute low back pain, in rest and moderately exercise conditions.

Antioxidant and anti-fatigue effects of a green tea extract in physical effort

Ramona-Niculina Jurcău[1], Ioana-Marieta Jurcău[2], Nicolae-Alexandru Colceriu[3], Cornelia Popovici[4], Mihai Kiss[4]

1) Department of Pathophysiology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Emergency Clinical Hospital for Children, Cluj-Napoca, Romania

3) Faculty of Horticulture, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Introduction. Tea is grown in 30 countries but is consumed worldwide, although at greatly varying levels. The ingestion of tea extract induces antioxidant activities. Green tea may lower levels of malondialdehyde. The objective is to highlight the antioxidant and anti-fatigue effects of an green tea extract (GTE), in physical effort.

Material and methods. Voluntary subjects were selected into two groups: control (C) and experimental (E), which has been administered GTE. The stress was conducted at the end of the one week training period on treadmill. Both groups have been subjected to the same type of physical effort on the treadmill. GTE was given the E, daily, for 3 weeks, in 3 doses a day. Analyzed indicators were: malondialdehyde (MDA) and muscle fatigue sensation (MFS).

4) Department of Sports, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Ramona-Niculina Jurcău
e-mail: ramona_mj@yahoo.com

Results. MDA has significantly increased for C, but it insignificantly increased for E. At all assessments times, the MDA values for C were higher than for E. MFS was perceived by E significantly more intense than E, both during the moderate physical training and after stress caused by acute physical effort.

Conclusions. 1) There were differences between C and E, both for MDA and MSF. 2) For E, MDA and MFS were significantly reduced, in moderate physical training and stress. 3) GTE effect was significantly higher on the stress than over the period of physical training. 4) GTE could be used for the modulation of oxidative stress and muscle fatigue sensation, both for moderate physical training, and stress caused by intense effort, in sedentary people.

Cardioprotective effects of curcumin nanoparticles on drug-induced acute myocardial infarction in diabetic rats

Paul-Mihai Boarescu[1], Ioana Boarescu[2,3], Ioana Corina Bocșan[4], Dan Gheban[5], Adriana Elena Bulboacă[1], Cristina Nicula[6], Raluca Maria Pop[4], Ruxandra-Mioara Râjnoveanu[7], Sorana D. Bolboacă[1]

1) Department of Pathophysiology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Medical Informatics and Biostatistics, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Zalău County Emergency Hospital, Zalău, Romania

4) Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

5) Department of Pathological Anatomy, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

6) Department of Ophthalmology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

7) Department of Pneumology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Paul-Mihai Boarescu
e-mail: boarescu.paul@umfcluj.ro

Aim. This study aimed to investigate the cardioprotective effects of pretreatment with conventional curcumin (CC) and curcumin nanoparticles (CNP) in acute myocardial infarction (AMI) on rats with streptozotocin (STZ)-induced diabetes mellitus (DM).

Material and method. Eight groups of 7 Wister Bratislava male rats were evaluated. Group 1 was the normal control group; group 2 was the group with AMI, group 3 with DM, and group 4 with DM and AMI. Groups 1-4 were treated with saline. Group 5 and group 7 received saline for 7 days before DM induction and continued with CC for group 5 and CNP for group 7, for 15 days before AMI induction. Group 6 received CC, while Group 8 received CNP for 7 days before DM induction and continued the treatment for 15 days before AMI induction.

Results. CC and NPC increased the RR interval and decreased heart rate in all groups, with better results for NPC compared to CC ($p = 0.0021$). NPCs were more effective than CC in preventing QRS complex enlargement and elongation of QT and QTc intervals ($p \leq 0.0057$). The administration of NPC before DM induction proved the most effective in the prevention of the reduction in R-wave amplitude ($p \leq 0.0048$). Curcumin nanoparticles effectively reduced ST-segment elevation ($p \leq 0.0021$). The best effect in reducing ST segment changes was obtained for NPCs administered before DM induction ($p \leq 0.0066$). The groups that received CC and NPC showed lower serum levels of creatine kinase, creatine kinase-MB and lactate dehydrogenase compared to the group with DM and AMI treated with saline ($p \leq 0.0049$). The best results in preventing the elevation of creatine kinase, creatine kinase-MB, and lactate dehydrogenase were obtained for the groups that received NPC ($p \leq 0.022$).

Conclusions. Curcumin nanoparticles administered prior to DM induction are more effective than conventional curcumin in maintaining cardiomyocyte function in acute myocardial infarction, more effectively preventing QRS complex enlargement, QT and QTc intervals elongation, and also in reducing ST-segment elevation. Curcumin nanoparticles also have better effects in maintaining a normal structure of the cardiomyocytes as they prevented serum elevation of intracellular enzymes such as creatine kinase, creatine kinase-MB, and lactate dehydrogenase.

Understanding the pathogenetic mechanism in disorders of sex development

D. Miclea[1,3], S. Bucerzan[1,2,3], C. Asavoaie[3], A. Zimmermann[4], R. A. Popp[1], V. Creț[3], M. Crișan[3], M. Stratan[1,3], F. Nazarie[1,3], D. Ștefan[1,3], P. Grigorescu-Sido[3], C. Alkhzouz[1,2,3]

1) Department of Medical Genetics, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of First Pediatric Clinic, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Medical Genetics Department, Clinical Emergency Hospital for Children, Cluj-Napoca, Romania

4) I[st] Clinic Internal Medicine, Department of Endocrinology and Metabolic Diseases University Johannes Guttenberg Mainz, Germany

Corresponding Author:
Diana Miclea
e-mail: diana.miclea@umfcluj.ro

Introduction. Disorders of sex development (DSD) are defined by an abnormal development of gonads, internal or external genital structures. Their understanding is very important for an adequate management and better long term prognosis in terms of physical, psychical, social and fertility. The aim of the study was to characterize a group of patients with DSD.

Material and method. We studied 267 patients investigated at Clinical Emergency Hospital for Children. All these patients were analyzed using a clinical protocol of evaluation. Gonads and internal genital organs morphology was investigated by ultrasounds or MRI. Hormonal investigations included 17OHprogesteron, DHEAs, delta4 androstendion, testosteron, DHT, AMH. Genetic testing consisted in karyotype and SRY gene evaluation, CYP21A2 gene evaluation (Stripassay analysis,ViennaLab), whole genome analysis of structural variation (>1kb) (SNParray, Illumina) and sequencing of 4800 gene associated with human pathology (NGS, Illumina).

Results. 13/267 patients (4.8%) presented sex chromosomes abnormalities. 2/64 patients (3.1%) tested for SRY gene presented SRY gene, discordant to karyotype sex (46,XX with SRY translocation). 28/131 patients (21.3%) tested for CYP21A2 mutations were confirmed with 21 hydroxylase deficiency. 9/37 patients (24.3%) analyzed by SNP array presented pathogenic copy number variants (CNVs) and 6/37 patients (16.2%) presented variants of uncertain significance (VUS). 13/22 patients (59%) analyzed by NGS presented pathogenic or potential pathogenic variants and 7/22 (31.8%) presented VUS.

Conclusions. Genetic evaluation permitted an elucidation of etiologic evaluation in a high percentage of the patients tested. However, there are still great challenges due to the necessity to perform the same protocol of genetic testing in each case, at an early age, to do functional analysis to better elucidate the importance of VUS and to adapt the therapeutic interventions according to different mechanisms.

Do journals encourage the publication of negative results in medicine? The study protocol

Alina-Georgiana Cozma[1], Maria Aluaș[2], Sorana D. Bolboacă[1]

1) Department of Medical Informatics and Biostatistics, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Oral Health, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Positive and negative results are essential for scientific progress, but there has been a hesitation in publishing negative results over time. Generally, negative results are more challenging to publish, while positive results are prone to generate more recognition, including funding. Negative research results usually refer to studies that fail to prove the study hypothesis. At least three main reasons could be listed: the original premise was defective, technical errors, or the researchers could not validate the previously reported results. Out of the three mentioned above, only the third category qualifies for publication. Our study aims to assess if journals indexed in Web of Science (WoS) encourage or not the publication of negative results.

Corresponding Author:
Alina-Georgiana Cozma
e-mail: Dr.alinacozma@yahoo.com

Method and results. The journals indexed by Web of Science (WoS), SCIE edition, represented the input data in our evaluation. The list of journals classified in thirty-eight medical-related categories was retrieved. The unrefined retrieved list (possible duplication) included 3400 journals. The following steps will be applied: [a] assign an identification number on each category; [a] classify the journals using quartiles according to impact factor in each category; [a] add together all journals and exclude duplicates; [a] decide the number of journals that must be screened, using an empirical rule for sample size calculation (e.g., for a population with a volume between 1001 and 5000 we expect to be sufficient to evaluate 5% of journals), [a] apply a stratified random method to identify the journal to be screened (the strata are the category and in each category, the quartile is a sub-stratum); [a] screen the included journals to evaluate if specific indications are provided relative to the publication of negative results.

Endometrial cancer as a possible etiology for stroke in a young patient

Ioana Boarescu[1,2], Paul-Mihai Boarescu[3], Adriana Elena Bulboacă[3], Sorana D. Bolboacă[1]

1) Department of Medical Informatics and Biostatistics, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Zalău County Emergency Hospital, Romania

3) Department of Pathophysiology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Ioana Boarescu
e-mail: ioana.boarescu@gmail.com

Introduction. Contrary to trends in most other diseases, the average age of ischemic stroke onset decreases. Several different diseases can result in ischemic stroke in a young patient. Numerous types of cancer have been shown to be associated with either ischemic or hemorrhagic stroke. Cancer may mediate stroke pathophysiology either directly or via coagulation disorders that establish a state of hypercoagulation, as well as via infections.

Case presentation. A 45 years old female patient was referred to the Neurology Department of the Zalău County Emergency Hospital with left hemiparesis that started one day before admission. The patient is known to have stage 2 hypertension, class 2 obesity, and left leg venous thrombosis, for which she received treatment at home in the past weeks. The patient's medical history revealed that she had had four pregnancies, but she lost 3 of them. During the last year, she presented irregular menstrual cycles. Neurological evaluation and a cerebral computed tomography scan revealed an ischemic stroke in the middle cerebral artery territory. Laboratory investigations revealed iron deficiency anemia and dyslipidemia. Considering the medical history, the clinical presentation, and the cardiovascular risk factors the following causes of ischemic stroke were investigated: paradoxical embolism, thrombophilia, antiphospholipid syndrome, and atherosclerosis. During hospitalization, she presented menometrorrhagia with aggravation of anemia. The performed pelvic ultrasound revealed endometrial hyperplasia. Given the presence of irregular menstrual cycles before hospitalization, a uterine curettage was done, and it established the diagnosis of endometrial cancer stage 2.

Conclusion. We report an unusual case of a patient who presented with stroke but was later diagnosed as a case of endometrial cancer. Cancer is not always seen as a cause of stroke in a young patient, but if a source of it is not found, screening for tumors should be done.

Quercetin attenuates naso-sinusal inflammation and inflammatory response in lungs and brain on an experimental model of acute rhinosinusitis in rats

C. N. Ţiboc (Schnell)[1], G. A. Filip[2], S. C. Man[1], N. Decea[2], R. Moldovan[2], R. Opriș[2], V. Sas[1], F. Tabaran[3]

1) Department of Pediatrics III, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Physiology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Pathology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Corresponding Author:
Cristina Schnell
e-mail: cristina_tiboc@yahoo.com

Introduction. The present study aimed to investigate the effect of intranasal Quercetin against inflammation and oxidative stress in nasal and sinus mucosa, but also in blood, lungs and brain, in a rat model of acute nasal and sinus inflammation induced by intranasal administration of LPS (from Escherichia coli).

Material and methods. Wistar rats were divided into five groups of 10 animals each. The control group received intranasal saline solution once/day, for seven consecutive days. Rats in groups 2 and 3, received low-dose (5 µg) and high-dose (10 µg) of LPS, once/day, for seven consecutive days. Rats in groups 4 and 5, received low-dose (5 µg) and high-dose (10 µg) of LPS, and after 2 h, 80 mg/kg of Quercetin was administered in 20 µl solution, once/day, for seven consecutive days. At the end of the treatment, the histopathological examination of nasal and sinus mucosa was assessed, and levels of cytokines (TNF-α, IL-1β, IL-6) and oxidative stress (malondialdehyde) in the blood, nasal mucosa, lungs and brain were also analyzed.

Results. Intranasal administration of LPS produced a local inflammatory reaction highlighted by increased levels of pro-inflammatory cytokines in nasal mucosa, and by the histopathological and immunofluorescence analysis of the nasal and sinus mucosa which revealed acute inflammatory reaction. High dose of LPS increased cytokines levels in blood, and lungs, while in brain, this effect was only on TNF-α level. There were no significant changes of malondialdehyde in the studied tissues after LPS instillation. Quercetin administration diminished the exudate and the degree of inflammation in lamina propria of nasal and sinusal areas, and also decreased local secretion of TNF-α (40.2% reduction after the low dose of LPS, and 35.4% reduction after the high dose of LPS) and IL-6 (21.4% reduction after the low dose of LPS and 35.8% reduction after the high dose of LPS). In lungs, Quercetin reduced TNF-α (43.3%) and IL-6 levels (24.5%), and in the brain, the protective effect was noticed only on TNF-α (46.5%)

Conclusions. Intranasal administration of LPS successfully induced acute rhinosinusitis in a rat model and also generated an inflammatory response in the lungs and brain. Intranasal administration of Quercetin diminished the nasal inflammation and also exerted protective effect on lungs, and partially on brain inflammatory reaction.

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Activation of drug resistance mechanisms as effect of doxorubicin treatment in triple negative breast cancer cells

Cristina Alexandra Ciocan-Cârtiță[1], Ancuța Jurj[1], Oana Zănoagă[1], Roxana Cojocneanu[1], Laura-Ancuța Pop[1], Lajos Raduly[1], Cecilia Pop-Bica[1], Liviuța Budișan[1], Alexandru Irimie[2,3], Cornelia Braicu[1], Ioana Berindan-Neagoe[1,4]

1) Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Oncological Surgery and Gynecologic Oncology, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Surgery, „Prof. Dr. Ion Chiricuță" Oncology Institute, Cluj-Napoca, Romania

4) Department of Genetics, Functional Genomics and Experimental Pathology, „Prof. Dr. Ion Chiricuță" Oncology Institute, Cluj-Napoca, Romania

Corresponding Author:
Cristina Alexandra Ciocan-Cârtiță
e-mail: crisciocan@yahoo.com

Introduction. Triple negative breast cancer (TNBC) is a heterogeneous disease with aggressive behavior promoting an unfavorable prognosis rate, particular in younger patients. Due to the lack of surface receptors, TNBC need investigated for better understanding of this complex pathology. Chemoresistance is the central cause for therapeutic failure in TNBC.

Materials and methods. The aim of this study was to assess the effect of doxorubicin in TNBC cell lines and to highlight cellular and molecular adaptation as effect of long exposure to doxorubicin, particular those linked to the activation of drug resistance mechanisms.

Results. Our data revealed that doxorubicin significantly increased the antiproliferative activity on both TNBC cell lines signifying the activation of drug resistance mechanisms. Modifications in gene expression were evaluated by microarray and detection of mutational pattern using Next Generation Sequencing. It was observed 196 upregulated and 115 downregulated genes as effect, which 15 overexpressed genes were found to be involved in drug resistance mechanisms. Furthermore, it was observed the presence of an additional TP53 pathogenic mutation.

Conclusions. The consequences of this investigation may provide novel biomarkers for drug resistance in TNBC. Furthermore, this demonstrates the complex mechanisms connected with drug resistance that can be exploited as novel targeted therapies to defeat drug resistance in TNBC.

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New strategies for reconversion of NSCLC therapy resistance using comabinatorial approaches using miRNAs associated with chemotherapy

Ancuța Jurj[1], Cornelia Braicu[1], Lajos Raduly[1], Roxana Cojocneanu[1], Anca Ghent[2], Alin Moldovan[2], Alexandru Irimie[3,4], Manuela Ferracin[5], Ioana Berindan-Neagoe[1,6]

1) Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Many targeted drugs for non-small cell lung cancer (NSCLC) have been developed in recent years in order to improve the survival rate of these patients. Nonetheless, improvements are still needed. Thus, in the present study we aimed to determine the in vitro effects of miR-21 and miR-181a modulation in

2) MEDFUTURE - Research Center for Advanced Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Surgery, „Prof. Dr. Ion Chiricuţă" Oncology Institute, Cluj-Napoca, Romania

4) Department of Oncological Surgery and Gynecologic Oncology, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

5) Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Italy

6) Department of Genetics, Functional Genomics and Experimental Pathology, The Oncology Institute „Prof. Dr. Ion Chiricuţă, Cluj-Napoca, Romania

Corresponding Author:
Ancuţa Jurj
e-mail: anca.jurj@umfcluj.ro

conjunction with vinorelbine and carboplatin to determine if these could offer new therapeutic options in NSCLC.

Materials and methods. We investigated the dual roles of miR-21 and miR-181a in NSCLC in combination with vinorelbine and carboplatin through MTT to highlight viability alterations. We have also assessed the effect on NSCLC cell lines nuclear morphology, adhesion and migratory potential. Additionally, we analysed the altered mRNA levels between the experimental conditions using microarray.

Results. Cellular viability and cytotoxicity post-therapy with above-mentioned agents, were investigated through MTT where the results showed that both chemotherapeutic agents exhibited significant effects on the viability of NSCLC cell lines. The presence of changes in morphological features, as well as inhibition of migration process could also be observed. To identify which signaling pathways were altered, we assessed the mRNA and miRNA profile of the experimental conditions using microarray. Moreover, changes in these pathways were analyzed and we observed that there were alterations present in pathways related to drug resistance.

Conclusion. In the current study we showed that NSCLC cells respond to combined therapy by altering important biological processes, including drug resistance, cell growth, migration, apoptosis and autophagy.

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SURGICAL SPECIALTIES

Ocular wounds with foreign bodies

Sorin Simion Macarie[1], Andreia Roman[2], Daniela Mariana Macarie[3]

1) Department of Ophthalmology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Ophthalmology Clinic, Cluj County Emergency Hospital, Romania

3) Ophthalmology Office, A.I. S.C.B.I. Cluj, Romania

Corresponding Author:
Sorin Simion Macarie
e-mail: sorimaca@yahoo.com

Introduction. Eye trauma with foreign bodies (FB) are sever ocular trauma, affecting the ocular structures by direct mechanical injuries, by inflammatory reactions and/or intraocular infections (inoculation of microorganisms at the intraocular level). This trauma can occur at any age and occur in both sexes, although the male sex is more commonly affected than the female sex.

Purpose. To assess the effect ocular trauma with foreign bodies (FB) on eye structures, the nature of foreign bodies, methods of investigation and treatment, sequelae and also to assess the final impact on visual function.

Methods. We performed a retrospective, cross-sectional, observational study in which we included 129 patients hospitalized at the Ophthalmology Clinic of County Emergency Hospital Cluj-Napoca, with the diagnosis of eye trauma with foreign bodies (FB) from 01.01.2014 until 31 december 2019. The diagnosis was based on objective clinical ophthalmologic examination and on imaging methods.

Results. We included 129 patients admitted in hospital with eye trauma with foreign bodies (FB). There was a significant predominance of male patients (124 patients – 96.12%) as opposed to female patients (n = 5 – 3.87%). Initial visual acuity (AV) was: nlp (no light perception) in 6 patients (4.65%), lp (light perception) to hmp (hand motion perception) in 43 patients (33.3%), 1/50-4/50 in 31 patients; (20.02%), 0.1-0.4 in 24 patients (18.60%), 0.5-1 in 25 patients (19.37%). The foreign body was more frequently located at the level of the retina in 33 patients (25.5%), in the profound layers of the cornea penetrated in anterior chambers in 30 patients (23.2%), inside the vitreous cavity in 27 patients (20.9%), in the sclera in 15 patients (11.6%), in the lens in 10 patients (7.7%), in the iris in 6 patients (4.6%) and in 7 patients (5.48%) the FB were found at the level of the orbit (the FB entered in the eye and then leaved the eye). Traumatic cataract was found in 65 patients (50.38%), vitreous cavity in 58 patients (44.9%) and 57 patients (44.1%) presented iridocyclitis and 6 patients presented retinal detachment. Endophthalmitis was found in 9 patients (6.9%). To support the diagnosis in 39 (30.2%) patients, orbital CT scan was performed, in 20 (15.5%) orbital radiography was performed, and in 6 patients (4.6%) ultrasonography in B mode. 107 foreign bodies were metallic, 16 FB (12.4%) vegetal and 6 FB (4.6%) were glass and plastic materials. Extraction of foreign bodies was performed in 124 patients (96.1%); in 5 patients, intraorbital FB were not been removed. In 44 cases the lens was extracted, posterior vitrectomy was performed in 54 cases, anterior vitrectomy in 9 cases. In one single patient with endophthalmitis evisceration had to be performed. The most frequent intraoperative complications were intraocular hemorrhages and retinal detachment. Visual acuity improved almost all patients, so at discharge the patients presented the following AV values: nlp – 3 patients, nlp-hm 32 patients (24.8%), 1/50-4/50 in 28 patients (21.7%), 0.1-0.4 in 30 patients (23.2%), 0.5-1 in 34 patients (24.3%).

Conclusions. Eye trauma with FB is very serious and produces important visual function impairment. The initial lesions are in some cases very severe and in other cases they worsen during the evolution. The location and size of the FB, the characteristics of the entrance wound, the presence of intraocular inflammation or infection, the time passed from initial trauma and treatment initiation are deciding factors for the visual prognosis. Visual functional recovery is always partial, only a quarter of patients with ocular trauma with FB recovered a visual acuity which allowed them to continue their usual activities.

Intrauterine development patterns as risk factor for cardiovascular morbidity in adult life

Gabriela Caracostea[1], Georgiana Nemeti[1], Daniel Mureșan[1], Elena Cristina Buzdugan[2]

1) Department of Obstetrics and Gynecology, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Internal Medical V, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Gabriela Caracostea
e-mail: caracostea1@yahoo.com

Introduction. Intrauterin environmental influences throughout gestation expressed as placental insufficiency, together with behavioral factors occurring in adult life (sedentarism and poor nutrition) have an impact on later development of metabolic syndrome. This "thrifty phenotype" hypothesis states that adverse intrauterine exposure affects fetal development. Fetal malnutrition leads to a down-regulation of important developmental processes increasing the risk for fetal origin of adult disease (FOAD). The aim of our research was to identify the frequency of low birthweight in a population diagnosed with metabolic syndrome.

Material and methods. A longitudinal observational study in 3 medical departments with diabetes, cardiologic and obstetric profile. Patients diagnosed with metabolic syndrome according to the National Cholesterol Education Program Adult Treatment Panel III criteria were included. A baseline questionnaire collected data regarding birthweight and gestational age at birth in order to calculate the specific birthweight centile. Pregnant women with singleton pregnancies in whom metabolic syndrome was diagnosed antepartum, were also included. The birthweight centile of their offspring was calculated. In cases with placental insufficiency, diagnosis was based on fetal biometry, Doppler velocimetry and amniotic fluid index.

Results. This multidisciplinary approach allowed enrollment of 63 patients, 24 males (38%) and 39 females (62%), including 26 pregnant women. Thirty-three patients (52.4%) had a body mass index of over 29 kg/m2. Regarding gestational age at delivery, 59 patients were born at term (93.75%), while 4 patients (6.4%) were delivered prematurely. Twenty-two cases (35%) had the birthweight below the 50th centile, from which 7 cases (11.1%) were below the 10th centile. Seventeen of the 26 pregnant women were diagnosed with placental insufficiency - 4 of these cases had primary placental insufficiency and in 13 cases it was due to severe preeclampsia. In the pregnant women subgroup, offspring birthweight below the 5th centile was recorded in 14 cases.

Conclusion. The FOAD hypothesis is supported by our study results, one third of the patients being compatible with a catch-up growth pattern. Data collected from pregnant women have implication for perpetuating the cycle of obesity in subsequent generations.

Image-guided surgery: present and future

Adrian Bartoș[1,2]

1) Department of Surgery III, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Surgery Clinic III, Regional Institute of Gastroenterology and Hepatology "Prof. Dr. Octavian Fodor", Cluj-Napoca, Romania

Introduction. Intraoperative ultrasound has an essential role in *hepato-biliary-pancreatic* surgery, being indispensable to the surgeon, for both, surgical and oncological safety. Its applications start from the diagnostic role (intraoperative confirmation of lesions, staging) and reach the effective guidance of surgeries.

Methods and results. This paper illustrates the main applications of intraoperative ultrasound for liver and pancreatic diseases, emphasizing the advantages that this technique brings in minimally invasive and oncological surgery. Also, will be

Corresponding Author:
Adrian Bartoș
e-mail: bartos.adi@gmail.com

mentioned the main areas of development in this field.

Conclusion. Together with the latest technology, they will bring a substantial benefit in the management of patients with hepato-biliary-pancreatic pathology.

Perspectives of intraoperative elastography in hepato-pancreatic surgery

Ioana Iancu[1,2], Adrian Bartoș[2,3]

1) Department of Anatomy and Embryology, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Surgery III, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Surgery Clinic III, Regional Institute of Gastroenterology and Hepatology "Prof. Dr. Octavian Fodor", Cluj-Napoca, Romania

Corresponding Author:
Adrian Bartoș
e-mail: bartos.adi@gmail.com

Introduction. Sonoelastography is a non-invasive and non-radiant ultrasound technique that allows estimating the tissue and tumors elasticity.

Methods and results. The current applications of elastography are represented by the quantification of the degree of fibrosis in patients with chronic liver disease and the differential diagnosis of liver tumors. The assesment of the liver is made using the conventional transparietal ultrasound. In the same way, can be evaluated the pancreas, but the approach is more difficult, due to the anatomical location.

Even if there are not many data in the literature about the usefulness of the intraoperative elastography, it is a promising technique. Existing research about this topic, has tried to highlight the role, that elastography could have in real-time, during the surgery, for liver and pancreatic pathology, but there are still many unknown and controversial data in the field.

Conclusion. The purpose of this paper is to illustrate the main intraoperative applications of elastography, highlighting the research field targeted on this technique.

Microarray analysis of microRNA expression patterns in male non-small cell lung cancer

Cornelia Braicu[1], Antonia Haranguș[1,2], Ancuta Jurj[1], Oana Zănoagă[1], Roxana Cojocneanu[1], Lajos Raduly[1], Cecilia Pop-Bica[1], Liviuța Budișan[1], Călin Ionescu[3,4], Mărioara Simon[2], Ioana Berindan-Neagoe[1,5]

1) Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Bronchology, Leon Daniello Pneumology Clinical Hospital, Cluj-Napoca, Romania

3) Surgical Department, Municipal Hospital, Cluj-Napoca, Romania

Introduction. Non-small cell lung cancer (NSCLC) is a major subtype in lung cancer, with high incidence and mortality rate. microRNA (miRNA) has shown to have an crucial functions in solid tumors, including NSCLC. The role of miRNAs in non-small cell lung cancer (NSCLC) has been broadly studied. This study set to evaluate miRNAs pattern in lung cancer and emphasis specific mechanisms based on behaviors risk factors in male patients.

Material and methods. In this study, we analyzed and compared the miRNAs expression profiles for 8 matched paired biopsies using miRNA microarray (Agilent technology).

4) Department of Surgery, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

5) Department of Genetics, Functional Genomics and Experimental Pathology, „Prof. Dr. Ion Chiricuță" Oncology Institute, Cluj-Napoca, Romania

Corresponding Author:
Cornelia Braicu
e-mail: cornelia.braicu@umfcluj.ro

Results. The bioinformatics analysis revealed 77 downregulated and 54 upregulated miRNAs, followed by the overlapping with the TCGA data (467 lung tumors and 19 normal adjacent tissues). We identified a common miRNAs signature composed by nine downregulated and eight upregulated miRNAs. Based on the miRNA-mRNA network using Ingenuity Pathway Analysis we identified a panel of 8 genes predicting overall survival.

Conclusion. This study provides new insights into specific miRNAs that are associated with males NSCLC, with important impact in patient prognostic.

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FUNDAMENTAL RESEARCH

The development of a new sensitive aptasensor for oxytetracycline based on innovative Au-based nanostructured platforms

Adrian Blidar[1], Oana Hosu[1], Geanina Ștefan[1,2], Diana Bogdan[3], Cecilia Cristea[1]

1) Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Physical and Chemical Analysis Department (RBRO/EQV-A), Robert Bosch SRL, Tetarom 3 Industrial Park, Jucu, Cluj, Romania

3) National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania

Corresponding Author:
Adrian Blidar
e-mail: blidar.adrian@umfcluj.ro

Introduction. Oxytetracycline (OXT) is an important with widespread use antibiotic. But, like in the case of other antibiotics, its overuse fuels the rise of the problem of antibiotic resistance. A way to combat this problem is by monitoring the antibiotic concentrations in the environment, which in turn highlights the need for new analytical methods capable of performing in field analysis. In this context, the aim of our work was the development of an aptasensor for OXT, using as a starting platform carbon-based screen printed electrodes (C-SPE), modified with Au-based nanostructures (AuNSs).

Materials and methods. C-SPE were modified with AuNSs through electrodepositon resulting in Au-based nanostructured platforms (AuNSs|C-SPE) with various structures. The obtained AuNSs|C-SPE platforms were functionalized with a thiolated deoxyribonucleic acid (DNA) aptamer, labelled with ferrocene and with mercaptohexanol (MCH) afterwards, to prevent nonspecific adsorbtion.

Results. A "signal on" aptasensor was obtained, the signal caused by the electrooxidation of the Fc rising with the increase in OXT concentration. The nanostructured platforms were evaluated based on the influence of their architecture on the analytical signal and compared also with a flat platform (Au-SPE).

Conclusion. An innovative and sensitive "signal on" aptasensor was developed for OXT using a C-SPE|AuNSs as a starting platform. The response depended linearly to the concentration of OXT in the range 0.05−1.2 µM with an estimated limit of detection of 9 nM.

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Pure instrumental methods for the simulation of hepatic metabolism of xenobiotics

Ruxandra Chira[1], Jens Fangmeyer[2], Valentin Zaharia[3], Uwe Karst[2], Ede Bodoki[1], Radu Oprean[1]

1) Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Having the most detailed picture of the underlying mechanisms of the hepatic oxidative metabolism of newly developed drug molecules, but also of those in postmarketing surveillance, is of utmost importance in reducing safety risks associated with their therapeutic exploitation.

Materials and methods. This study is focused on simulating the oxidative

2) Institute of Inorganic and Analytical Chemistry, University of Münster, Germany

3) Department of Organic Chemistry, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Ruxandra Chira
e-mail: ruxandra.chira@umfcluj.ro

metabolization processes of NK1 antagonists (aprepitant and netupitant) by applying electrochemical techniques coupled to mass spectrometry. The purely instrumental technique allows both fast and reproducible generation and subsequent detection of oxidative transformation products.

Results. The selected compounds were electrochemically oxidized in a thin-layer flow-through cell (μ-PrepCell™ 2.0, Antec Scientific) equipped with a boron-doped diamond working electrode. Experiments involving separation, structure elucidation and fragmentation were carried out on a liquid chromatography system coupled to a quadrupole-ToF-based timsToF Pro MS (Bruker Daltonik).

Such simulated biotransformation was performed for the first time for these NK1 antagonists, being able to successfully mimic N-dealkylation, hydroxylation, N-oxidation and dehydrogenation reactions. Moreover, a fast and cost-effective synthesis of the main metabolites reported in vivo for both compounds was possible. Additional information regarding the oxidative transformation pattern were obtained.

Another direction of the research intended the further development of this EC/MS biomimicking tool towards a simple, fast, high-throughput, cost-, sample- and reagent-effective experimental setup. To the best of our knowledge, coupling EC to MALDI-ToF MS (matrix-assisted laser desorption ionization) for this purpose has not been reported before.

Conclusions. The performed experiments re-endorse the possibility of using electrochemically-assisted prediction of the oxidative metabolic pathways of various xenobiotics.

Sensitive detection of cathinones and adulterants in street samples using electrochemical fingerprinting

Ana-Maria Drăgan[1], Florina Truță[1], Anca Florea[1], Jonas Schram[2], Andreea Cernat[1], Mihaela Tertiș[1], Bogdan Feier[1], Karolien De Wael[2,3], Radu Oprean[1], Cecilia Cristea[1]

1) Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) AXES Research Group, University of Antwerp, Belgium

3) NANOlab Center of Excellence, University of Antwerp, Belgium

Corresponding Author:
Ana-Maria Drăgan
e-mail: Ana.Dragan@umfcluj.ro

Introduction. The abuse of illicit drugs has become a global concern considering the widespread use of these substances and the consequences it has on societies. The current methods used to quickly detect drugs on-site, such as color-tests, are presumptive tests and they lack selectivity, giving a high number of false negative and false positive results, due to the presence of adulterants and cutting agents. Their characterization is important from a forensic point of view, in order to link different seizures to one original batch, as well as from a toxicological point of view, for the health implications some of them might have. Therefore, the purpose of the present study was the development of a sensitive electrochemical method for the detection of cathinones and their adulterants/cutting agents in street samples.

Material and methods. The electrochemical fingerprinting was performed by means of square wave voltammetry using nanomaterials-modified electrodes, such as graphene-modified electrodes and carbon-nanotubes based electrodes. Several cathinones, such as methcathinone, chloroetcathinone were tested, as well as several adulterants/cutting agents such as procaine, benzocaine, quinine, acetaminophen and lactose, starch, respectively. The effectiveness of the developed method was tested for the detection of drugs in simulated drug samples.

Results. Depending on the electrochemical signal obtained for the oxidation of

drugs, graphene and carbon-nanotubes based platforms were chosen to further test the simulated drug samples.

Conclusion. Electrochemical methods proved to be excellent techniques for the fast determination of drugs with high sensitivity and specificity, suitable for the development of miniaturized portable devices to be used in-field.

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Affinity capillary electrophoretic assessment of selectivity profile of oligomer-modified gold nanostructures intended for biosensing applications

Laura-Elena Gliga, Bogdan-Cezar Iacob, Rebeca Moldovan, Ede Bodoki, Radu Oprean

Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Laura-Elena Gliga
e-mail: gliga.laura1@gmail.com

Introduction. Affinity capillary electrophoresis occupies a key role among different non-separation and separation-based analytical methods for interaction studies. Short-chained, endogenous microRNAs play vital roles in multiple biological processes, being often targeted as biomarkers for several tumors. This explains the high interest in finding new detection strategies that overcome pitfalls as complexity or high cost of the conventional RNA detection techniques. Alternatively, oligomer-functionalized gold nanostructures are even more often tested as attractive analytical platforms for the development of fast, label-free optical or electrochemical biosensors. In our study the analytical perspectives of short ssDNA-modified gold nanorods (A2-GNRs) and their affinity towards a DNA miR-21 mimic have been assessed. The binding/selectivity and thermodynamic profile of the interaction have been interrogated by capillary gel electrophoresis (CGE) and confirmed by isothermal titration calorimetry. Because electrochemical biosensors have the advantage of rapid, simple and low-cost testing of various biomolecules, preliminary studies regarding the development of a GNRs-A2 modified platform were assessed.

Material and methods. The CGE separations, with reversed polarity, were performed in fused-silica capillaries filled with a buffered dextran-based gel (pH = 8.3) containing 190 mM TRIS, 190 mM boric acid and 0.1 mM EDTA. High pressure hydrodynamic sample injection (48s@5 bars) with DAD-detection (260 nm) was employed.

Results. The hybridization stoichiometry (miR21:A1/A2-GNR = 3:1) of the binary and ternary complexes were conveniently established in the given experimental conditions. Furthermore, the optimized binding conditions were readily integrated into the electrochemical detection procedure of miR21 performed on graphite-based screen-printed electrodes using methylene blue as a redox probe of the hybridization event.

Conclusion. Considering the obtained results, the developed CGE method proved to be an efficient, straightforward and cost-effective investigational tool for further development and optimization of affinity-based sensors for selective and sensitive detection of miR21 from various biological samples.

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Electrochemical DNA-based sensor for β-lactoglobulin cow milk allergen detection

Oana Hosu[1,2], Mariagrazia Lettieri[2], Alina Adumitrăchioaie[1,2], Robert Săndulescu[1], Cecilia Cristea[1], Giovanna Marrazza[2]

1) Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Chemistry, "Ugo Schiff", University of Florence, Sesto Fiorentino, Italy

Corresponding Author:
Oana Hosu
e-mail: hosu.oana@umfcluj.ro

Introduction. The most frequently allergenic protein which is responsible for cow's milk allergy is β-lactoglobulin (β-LG) mainly due to its high stability and absence in human milk. As there is no cure for cow's milk allergy yet developed but only effective care and emergency treatments available, the only viable solution is a free milk-based diet. Therefore, the development of an easy-to-use, fast, and accurate device for allergen detection is fundamental to prevent life-threatening reactions in sensitive subjects. In this work, a DNA-based sensor for β-lactoglobulin electrochemical detection was developed. Two oligonucleotide sequences labeled with amino and biotin termination, respectively, were selected using M-fold software in order to obtain a DNA sensor.

Material and methods. The aptamer-based biosensor was designed by co-electrodeposition of anthranilic acid and aniline, in order to obtain a conductive, poly(aniline-co-anthranilic acid) co-polymer at the surface of graphite screen-printed electrodes. The optimization monomers mixture was performed via cyclic voltammetry measurements.

Results. The platform with the best analytical performance was chosen as the immobilization platform for further experiments. A dose-response curve was constructed between 0.01-2.0 μg/mL β-LG concentration range and a LOD of 0.053 μg/mL was achieved.

Conclusion. An innovative and simple aptasensor for β-lactoglobulin detection based on competitive displacement was developed. The aptasensor was applied for the analysis of milk samples obtaining good recovery values which confirms the operability of the aptasensor for β-lactoglobulin detection in real samples.

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Hybrid polymer - gold nanostructured platform for lysozyme sensitive detection

Gheorghe Melinte[1,2], Oana Hosu[1], Geanina Ștefan[1,3], Diana Bogdan[4], Cecilia Cristea[1], Giovanna Marrazza[2]

1) Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Lysozyme is an enzyme present in multiple organisms where it plays various vital roles. One of the most important relies on its antibacterial activity, being also called the body's own antibiotic. Despite its proven utility, lysozyme can potentially trigger allergic reactions in sensitive individuals, even in trace amounts, thus the need of continue monitoring of lysozyme in products rich in lysozyme like wine or egg white is of high importance.

2) Department of Chemistry "Ugo Schiff", University of Florence, Sesto Fiorentino, Italy

3) Robert Bosch SRL, Physical and Chemical Analysis Department (RBRO/EQV-A), Jucu, Cluj, Romania

4) National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania

Corresponding Author:
Gheorghe Melinte
e-mail: melinte.gheorghe@umfcluj.ro

Materials and methods. An electrochemical aptasensor was designed for lysozyme analysis. Firstly, poly-L-lysine was electrodeposited at screen printed carbon electrodes in order to obtain a more structured platform with higher electroactive area. Next, gold nanostructures (nano-towers) were electrodeposited from a mixture of $HAuCl_4$ and PEG 10000 solution for enhanced electrocatalytic effect and to serve as immobilization platform for the thiolated aptamer. All steps were optimised and all platforms were electrochemically and morphologically characterized.

Results. On the best platform, the 1st aptamer was immobilized within the thiol group from its 3'-end, followed by a blocking step of the gold remaining free sites with 6-mercaptohexanol. Next, after the lysozyme solution was dropped casted on the electrode surface and the aptamer-target reaction occurred, a biotin-labelled 2nd aptamer is added to obtain a sandwich assay. Further, streptavidin-alkaline phosphatase (ALP) reacts with the biotin label of the 2nd aptamer, which further catalyses the hydrolysis of 1-naphthyl-phosphate. The 1-naphtol enzymatic product was detected by means of differential pulse voltammetry to enable lysozyme quantification.

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Aptamer selection for a glycopeptide antibiotic using innovative Magnetic beads-based SELEX technology

Geanina Ștefan[1,2], Oana Hosu[1], María Jesús Lobo Castañón[2], Noemí de-los-Santos-Álvarez[2], Cecilia Cristea[1]

1) Analytical Chemistry and Instrumental Analysis, Department I, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Departamento de Química Física y Analítica, Universidad de Oviedo, Spain

Corresponding Author:
Geanina Maria Ștefan
e-mail: Geanina.Stefan@umfcluj.ro; geanina.maria.stefan@gmail.com

Introduction. Aptamers are short single-stranded DNA, RNA, or XNA oligonucleotides that make a strong interaction with a specific target molecule, being obtained in an in vitro chemical process named SELEX technology. This poster presents our current progress in the synthesis of a new aptamer selected through Magnetic beads-based SELEX technology that will be further explored for its potential application in the detection of vancomycin from real samples.

Material and methods. Vancomycin percentage immobilized onto magnetic beads functionalized with carboxylic acid (MBs-COOH) was analyzed and optimized while testing different concentrations. UV-Vis measurements were performed to determine the quantity of vancomycin bounded onto the MBs-COOH. The electrophoretic separation provided valuable information about the interaction of the DNA pool with the MBs functionalized with the target molecule, vancomycin. Quantification by fluorescence signal confirms the amplification of the selected DNA sequences.

Results. Vancomycin shows a maximum absorbance peak at 282 nm in both HEPES buffer, pH 8.3, and HEPES, pH 8.3 with 0.01% Tween. With the increase in vancomycin concentration incubated, the surface of the magnetic beads gets saturated. The target was to maintain the equilibrium between the amount of bounded antibiotic and the surface capabilities to allow DNA sequences to interact; therefore 20 mM vancomycin was selected as the optimum concentration. Sequences of about 80 oligonucleotides are amplified through PCR, analyzed by gel electrophoresis, and quantified by fluorometric analyses.

Conclusions. Optimization of all steps involved in the Magnetic beads - based SELEX technology is envisaged to obtain an aptamer for vancomycin with a low dissociation constant (Kd in the nM range). The final goal of the study is to develop an aptasensor for the sensitive detection of vancomycin in biological and environmental samples.

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Electrochemical screen-printed sensor integrated on an intra-oral device for direct and simultaneous detection of two important advanced glycation end products in human saliva

Mihaela Tertiş[1], Cecilia Cristea[1], Anida-Maria Băbțan[2], Claudia Nicoleta Feurdean[2], Willi-Andrei Uriciuc[2], Adina Bianca Boșca[3], Aranka Ilea[2]

1) Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Oral Rehabilitation, Faculty of Dental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Histology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Mihaela Tertiș
e-mail: mihaela_tso@yahoo.com; mihaela.tertis@umfcluj.ro

Introduction. Advanced glycation end-products (AGEs), such as methylglyoxal (MGL) and N-(carboxymethyl)lysine (CML) are associated with processed food and are considered markers for the oxidative stress, atherosclerotic plaques and diabetes. The elaboration, characterization and in vivo testing of an innovative approach for the sensitive and simultaneous electrochemical detection of MGL and CML based on a lab-made printed platform were aimed in this study.

Material and methods. A lab-made printed platform using a flexible plastic foil as support, an Ag/AgCl conductive ink for the design of the reference electrode and the contacts, and a carbon ink for the working and counter electrodes. The surface of working electrode was decorated with a nanostructured platinum film generated electrochemicaly using cyclic voltammetry.

Results. The selectivity tests performed for MGL have proved that the presence of CML in the same solution does not hinder the detection. The disposable sensor was successfully tested for direct electrochemical detection of MGL and CML using differential pulse voltammetry. The employment of composite electrodes exhibits electron-mediating effect, which leads to a well-defined reduction peak associated to the MGL and a well-defined oxidation peak associated to the CML.

Conclusion. The developed assay was shown to be suitable for the simultaneous analysis of salivary MGL and CML in healthy volunteers even when tested in vivo, being promising for the elaboration of point-of-care devices for salivary analysis.

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Detection of cocaine and cathinones by electrochemical fingerprinting using nanomaterials

F. Truță[1], A. Florea[1], J. Schram[2], A. Drăgan[1], A. Cernat[1], M. de Jong[2], B. Feier[1], K. De Wael[2], C. Cristea[1]

1) Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) AXES Research Group, University of Antwerp, Belgium

3) NANOlab Center of Excellence, University of Antwerp, Belgium

Corresponding Author:
Florina Truță
e-mail: Florina.Truta@umfcluj.ro

Introduction. Illicit drugs use and abuse remains an increasing challenge for worldwide authorities and, therefore, it is important to have accurate methods to detect them in seized samples, biological fluids and wastewaters. They are recently classified as the latest group of emerging pollutants as their consumption increased tremendously in recent years. Nanomaterials have gained much attention over the last decade in the development of sensors for a myriad of applications. The applicability of these nanomaterials, functionalized or not, significantly increases and are therefore highly suitable for use in the detection of drugs of abuse.

Material and methods. We have assessed the suitability of various nanoplatforms for the electrochemical detection of illicit drugs, such as graphene, singled-walled carbon nanotubes, multi-walled carbon nanotubes functionalized with platinum nanoparticles, gold nanoparticles and platinum nanoparticles. Graphene and singled-walled carbon nanotubes were drop-casted onto graphite screen printed electrodes and left to dry at room temperature. Multi-walled carbon nanotubes were functionalized with platinum nanoparticles using a simple and efficient chemical process, in which carboxylic acid-functionalized multi-walled carbon nanotubes were mixed with chloroplatinic acid in a mixture of ethylene glycol and water to result in platinum-functionalized multi-walled carbon nanotubes. The functionalized multiwalled carbon nanotubes were drop-casted onto graphite screen printed electrodes. Gold and platinum nanoparticles were deposited by electrodeposition from a solution of chloroauric acid and chloroplatinic acid, respectively, by cyclic voltammetry.

Results. The electrochemical fingerprints of cocaine and cathinones (such as mephedrone, alpha-polyvinylpirolidone, methylmethcatinone, chloroethcatinone, chloromethcathinone, etylone) were elucidated on the above-mentioned nanoplatforms. Square wave voltammetry was performed as a high-performance electrochemical method. This allowed for the sensitive and selective (class selectivity) of the investigated illicit drugs.

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Lipophilicity evaluation of some new thiazole flavones and hydroxyflavones

Fana-Maria Coman[1], Denisa Leonte[1], Dorina Casoni[2], Valentin Zaharia[1]

1) Department of Organic Chemistry, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Many literature studies describe various pharmacological activities of natural and synthetic flavones and hydroxyflavones, these classes of compounds generating a lot of interest among medicinal chemists. The prediction of lipophilicity is very important in drug development, because lipophilicity is a physicochemical parameter that

2) Department of Chemistry, Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University, Cluj-Napoca, Romania

Corresponding Author:
Fana-Maria Coman
e-mail: Coman.Fana@umfcluj.ro

influences the pharmacokinetics of drugs and consequently their absorption, distribution, metabolization and elimination. Starting from this premise and in continuation of our research related to the synthetic analogues of natural compounds, we proposed the evaluation of lipophilicity of some new thiazole flavones and hydroxyflavones in order to determine possible structure-lipophilicity relationships.

Material and methods. The experimental lipophilicity parameters were determined by reverse-phase thin layer chromatography (RP-TLC) using isopropanol-water mixture in various proportions of isopropanol (60%, 65%, 70%, 75%, 80%) as mobile phase. Computed lipophilicity parameters generated by several software and internet websites were used for the experimental results evaluation.

Results. A statistically significant correlation has been noticed between the chromatographic and some of the computed lipophilicity parameters. Different groups of structural resemblance were obtained by generating the lipophilicity chart and the lipophilicity space for the investigated compounds.

Conclusion. The lipophilicity of some newly synthesized thiazole flavones and hydroxyflavones was determined by RP-TLC method. A good correlation was revealed between the experimental results and several computed log P values. Using the PCA analysis the investigated flavones and hydroxyflavones were classified according to their lipophilicity and structural similarities.

Elaboration of silica coated iron oxide magnetic nanoparticles for biomedical applications

Cristian Iacoviță[1], Ionel Fizeșan[2], Rareș Știufiuc[1,3], Constantin Mihai Lucaciu[1]

1) Department of Pharmaceutical Physics-Biophysics, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Toxicology, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Bionanoscopy, MedFuture Research Center for Advance Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Cristian Iacoviță
e-mail: cristian.iacovita@umfcluj.ro

Introduction. Cancer treatment requires stable magnetic nanoparticle (MNPs) in biological fluids (i.e., blood, interstitial, or intracellular), displaying heating properties tuned to cell destruction and a low collateral toxicity profile.

Materials and methods. Polyhedral iron oxide MNPs were synthesized at high temperature and high pressure conditions, by means of a polyol method, using chloride magnetic precursors, sodium acetate and polyethylene-glycol. Two main protocols, Stöber process and reverse micro-emulsion route, have been applied to coat the MNPs in a silica shell, which was monitored by means of transmission electron microscopy. The heating capabilities of silica coated MNPs have been investigated by magnetic hyperthermia in an external alternating magnetic field (AMF) amplitude – H – between 5 and 65 kA/m and f = 355 kHz.

Results. Both protocols allow the enveloping of MNPs in a silica shell whose thicknesses is difficult to quantify due to the ferromagnetic characters of MNPs at room temperature which results in the formation of aggregates. However, the specific absorption rate (SAR) of silica coated MNPs dispersed in water increases considerable as compared to uncoated MNPs, the difference becoming higher as the colloidal concentration of MNPs decreases. The silica coating assures a better colloidal stability of MNPs and hence their heating properties are enhanced.

Conclusion. Silica coated polyhedral iron oxide MNPs are promising candidates for in vitro destruction of cancer cells though magnetic hyperthermia therapy.

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Breast cancer discrimination by using SERS of blood plasma

Constantin Mihai Lucaciu[1], Gabriela Fabiola Știufiuc[2], Valentin Toma[3], Gabriela Morar-Bolba[4], Nicolae Leopold[2], Ioana Pavel[5], Rareș Ionuț Știufiuc[1,3]

1) Department of Pharmaceutical Physics-Biophysics, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Faculty of Physics, "Babeș-Bolyai" University, Cluj-Napoca, Romania

3) MedFuture Research Center for Advanced Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

4) Department of Senology, The Oncology Institute "Prof. Dr. Ion Chiricuță", Cluj-Napoca, Romania

5) Wright State University, Department of Chemistry, Dayton, Ohio, USA

Corresponding Author:
Constantin Mihai Lucaciu
e-mail: clucaciu@umfcluj.ro

Introduction. The screen-film mammography, the standard detection method for BC has a poor performance in women over 50 years old or with denser breast tissue. SERS represents a promising technique able to provide specific molecular information that could have a major impact on biomedical applications.

Materials and methods. SERS signals were obtained by using a solid substrate prepared by drop-casting silver nanoparticles purified by using Tangential Flow Filtration on a CaF_2 glass. The capacity of our substrates to discriminate breast cancer by SERS was tested using Multivariate Analysis of SER spectra, on blood plasma samples collected from healthy donors and breast cancer patients.

Results. The SERS signals of blood plasma samples strongly depend on the excitation wavelength. In the case of using a 532 nm excitation, the SERS is dominated by beta carotene signals. Our original solid SERS substrate, combined with an excitation at 785 nm allowed us to obtain highly reproducible spectra assigned to both proteins and lower molecular mass plasma components (uric acid, hypoxanthine). Based on a combined Principal Component - Linear Discriminant Analysis we were able to discriminate with 100% accuracy the healthy donors from breast cancer patients and with 99% accuracy the two groups of breast cancer, as can be seen from the confusion matrix presented bellow.

Actual/Prediction	Control	BC stage I and II	BC stage III and IV
Control	36	0	0
BC stage I and II	0	30	1
BC stage III and IV	0	0	28

Conclusion. Our data suggest that SERS analysis of blood serum might have a huge potential in the clinical laboratory in detecting cancer and other diseases.

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PHARMACEUTICAL SPECIALTIES

A novel strategy for the simultaneous detection of doxorubicin and simvastatin for their potential use in the treatment of cancer

Iulia Rus[1], Mihaela Tertiș[1], Cristina Barbălată[2], Alina Porfire[2], Ioan Tomuță[2], Robert Săndulescu[1], Cecilia Cristea[1]

1) Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Iulia Rus
e-mail: rus.iulia@umfcluj.ro

Introduction. Doxorubicin is an antitumor drug widely used in the treatment of cancer. It can be found on the market in different forms, alone or in combination with other molecules. Simvastatin is an inhibitor of HMG-CoA reductase, the most important enzyme from the process of cholesterol biosynthesis. There are several studies in the literature which demonstrated the decrease of cellular proliferation in the presence of high dose simvastatin and that this molecule potentiates the cytotoxic activity of antitumor drugs. Therefore, various types of drug delivery systems containing both drugs started to be investigated for the improvement of cancer therapy. The aim of this study was to develop a disposable, simple, fast and sensitive sensor for the simultaneous electrochemical detection of doxorubicin and simvastatin.

Material and methods. The electrochemical behavior of each molecule was analyzed regarding the influence of electrode material, electrolyte solution and scan rate. After each of these parameters was chosen, a LSV (Linear Sweep Voltammetry) procedure was optimized for their simultaneous detection. In the same time, two chronoamperometry procedures were tested, one for the detection of doxorubicin in the presence of simvastatin and the other one for the detection of doxorubicin and simvastatin together.

Results. Calibration curves for doxorubicin and simvastatin in the presence of each other were built using both methods, obtaining two new analytical strategies for their simultaneous detection.

Conclusion. This detection strategy represents a promising tool in the development of drug delivery systems containing both drugs whose association was proved to bring benefits in the treatment of cancer.

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Evaluation of anti-inflammatory and analgesic potential of some thiazolo[3,2-b][1,2,4]triazole derivatives

Anamaria Cristina[1,2], Bogdan Apan[3], Gabriel Marc[4], Cristina Mogoșan[2], Valentin Zaharia[1]

1) Department of Organic Chemistry, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used medicines for inflammatory diseases, however, NSAIDs are known for multiple adverse effects. This study aimed to evaluate the anti-inflammatory and analgesic properties of some thiazolo[3,2-b][1,2,4]triazole derivatives and the influences of pharmacophore moieties on the biological properties of the support molecule.

2) Department of Pharmacology, Physiology, Pathophysiology, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

4) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Anamaria Cristina
e-mail: anamaria.cristina@umfcluj.ro

Materials and methods. *In vivo* evaluation of the anti-inflammatory potential of thiazolo[3,2-b][1,2,4]triazoles 1b-12b was performed using an experimental model of acute inflammation induced with λ-carrageenan in rats, using diclofenac as the reference drug (20 mg/kg, p.o.). The studied compounds were administered in a single dose (50 mg/kg), by gavage. The Randall-Selitto test was used to evaluate the analgesic effect, measuring the pain threshold in the above-mentioned inflammatory conditions for the same compounds. At the end of the experiment, gastric mucosa was observed to identify if there were any irritations. A molecular docking study was performed to evaluate the interaction of thiazolo-triazoles with cyclooxygenase (COX-1, COX-2), to correlate the biological effects with their possible mechanism of action.

Results. Ten of the thiazolo[3,2-b][1,2,4]triazoles had a significant anti-inflammatory effect (20.84%-34.92%) at different time intervals and the pain threshold was significantly increased for most compounds, compared to the negative control group. None of the compounds produced any gastric damage. The molecular docking study highlighted the correlation between biological activity and COX selectivity of compounds.

Conclusions. The thiazolo[3,2-b][1,2,4]triazoles bearing in the C-6 position 4-bromophenyl and 4-methoxyphenyl moieties showed significant anti-inflammatory and analgesic activity and increased selectivity for COX-2. These compounds will be considered for further investigations concerning their influence on the inflammation mediators.

QbD approach for the development of oral care products containing *Vitis vinifera* L. extracts

Cătălina Bogdan[1], Sonia Iurian[2], Daniela Benedec[3], Mirela Moldovan[1]

1) Department of Dermopharmacy and Cosmetics, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Pharmacognosy, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Cătălina Bogdan
e-mail: catalina.bogdan@umfcluj.ro

Introduction. *Vitis vinifera* L. extracts represent a valuable source of bioactive compounds with potential applications in the pharmaceutical and cosmetic field. The aim of the present study was to determine the optimal conditions for the preparation of oral care products containing polyphenolic extracts from *Vitis vinifera* L. based on the QbD approach.

Material and methods. Two factorial experimental design with three factors and two levels (Modde 12.1 software, Sartorius Stedim Data Analytics AB, Umea, Sweden) were developed. Texture analysis parameters (Brookfield CT3Texture Analyzer) and viscosity measurements (Brookfield DV-III Ultra) were determined. Based on the experimental results, the optimal formulations were generated and analyzed.

Results. Regarding toothpaste analysis, it was observed that increased ratios of silicon dioxide and sodium carboxymethylcellulose led to an increase of the Consistency, Firmness, and Rigidity values while the Viscosity of the products was positively influenced by the powders ratio and the thickening agents increase.

According to the experimental data obtained for mouthwash formulations, the percentage of xanthan gum influenced the physical properties of the products, increasing the Firmness, Consistency, Cohesiveness, Resilience, and Viscosity values. The increase of stirring rate increased Firmness and Consistency values and decreased Cohesiveness values.

The optimal characteristics of two commercially available products were set as restriction criteria for the optimization and the optimal formulations were prepared and

analyzed; a good correlation between the model predicted and the experimental response was obtained.

Conclusion. The study achieved the optimum experimental conditions for the development of oral care products containing polyphenolic extracts from *Vitis vinifera* L.

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Phytochemical research and nephroprotective activity of *Cichorium Intybus* L. aerial parts tincture

Alexandra Epure[1], Alina E. Pârvu[2], Laurian Vlase[3], Daniela Benedec[1], Daniela Hanganu[1], Ana-Maria Gheldiu[4], Ilioara Oniga[1]

1) Department of Pharmacognosy, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Physiopathology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

4) Department of Pharmaceutical Botany, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Alexandra-Cristina Epure
e-mail: ale.epure@gmail.com

Introduction. The purpose of this study was to extend the current knowledge on the chemical composition of Romanian wild chicory *Cichorium Intybus* L. and to evaluate the nephroprotective activity.

Material and methods. The polyphenols from Cichorii herba were analyzed by spectrophotometric and chromatographic methods (HPLC-MS). The total polyphenols content (TPC) was assessed by Folin-Ciocâlteu method, expressed as mg gallic acid equivalents (GAE)/g d.w.; the total flavonoids content (TFC) was expressed as mg rutin equivalents (RE)/g d.w. and caffeic acid derivatives content (CADC) as mg caffeic acid equivalents (CAE)/g d.w. The evaluation of antioxidant activity was carried out *in vitro* with the DPPH· and FRAP assays. The effect of a seven-day pretreatment with chicory tincture in different concentration on the gentamycin induced acute kidney failure like lesion in rats was assessed by evaluation of serum oxidative status (TOS), oxidative stress index (OSI), nitrates (NOx), serum and urinary creatinine and urea, creatinine clearance etc.

Results. The contents of polyphenols were as follows: TPC = 14.34 ± 0.86 mg GAE/g d.w., TFC = 3.68 ± 0.16 mg RE/g d.w. and CADC = 4.65 ± 0.23 mg CAE/g d.w.. The HPLC-MS analysis revealed chlorogenic acid, caftaric acid, isoquercitrin, rutin, quercitrin, luteolin, apigenin and the major component was cichoric acid. The antioxidant capacity (IC50) was in accordance with the TPC, overall presenting a moderate activity. The tincture presented nephroprotective activity by lowering serum and urinary creatinine and urea in a concentration-dependent way and reduced oxidative stress by lowering TOS, OSI and NOx.

Conclusion. *C. Intybus* is a source of valuable polyphenols with moderate *in vitro* antioxidant activity. Pretreatment with chicory tincture significantly reduced renal injury and associated oxidative stress. Further studies are needed in order to explain the complete mechanism of nephroprotective effect of chicory tincture.

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Influence of alpha lipoic acid on metabolism and cardiac function in an animal experimental study

Cristina Pop[1], Laurențiu Stoicescu[2], Georgia Ștefan[3], Dana-Maria Muntean[4], Justine Dhot[5], Valentin Adrian Bâlteanu[6], Benjamin Lauzier[5], Claudiu Morgovan[7], Bela Kiss[3], Felicia Loghin[3], Cristina Mogoșan[1], Steliana Ghibu[1]

1) Department of Pharmacology, Physiology and Pathophysiology, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Cardiology, Medical Clinic V, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Toxicology, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

4) Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

5) L'Institut du Thorax, INSERM, CNRS, UNIV Nantes, Nantes, France

6) Institute of Life Sciences, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

7) Preclinical Department, Faculty of Medicine, Lucian Blaga University, Sibiu, Romania

Corresponding Author: Cristina Pop
e-mail:
cristina.pop.farmacologie@gmail.com

Introduction. Obesity is associated with metabolic and cardiovascular disorders. Alpha lipoic acid (ALA), through complex pathways, may have beneficial effects in obesity. Our study aimed at assessing cardiac function, metabolism and ALA effects in experimental diet-induced obesity.

Material and methods. Wild type (WT) and transgenic rats overexpressing β3-adrenergic receptors (TGβ3) were either fed with standard diet (WT+SD, n=12; TGβ3+SD, n=12) or with hypercaloric diet (SD+HD, n=12; TGβ3+HD, n=12). Also, ALA treated groups doubled every group. For 35 weeks body-weight (BW), systolic blood pressure (SBP) and echocardiographic parameters were monitored. Oral glucose tolerance test (OGTT) was also performed.

Results. HD induced an increase in BW in both groups (~10.08% for WT and ~10.45% for TGβ3) since week 10 (W10), trend maintained until the end of the study. Also, in the HD groups, hyperglycemia during OGTT suggested insulin resistance. Regardless of WT or TGβ3 status, SBP (mmHg) was significantly increased by HD (~12%) at W35. Starting with W25, a decrease in early-to-late filing ratio (E/A ratio) in TGβ3+HD vs. TGβ3+SD was observed (0.97±0.04 vs 1.11±0.07), while for WT+HD vs. WT+SD, reduction occurred at W35 (0.99±0.06 vs 1.25±0.05). E'/A' ratio followed the same trend, at W25: TGβ3+HD vs. TGβ3+SD (0.93±0.05 vs 1.07±0.05), at W35: WT+HD vs. WT+SD (0.99±0.06 vs 1.19±0.04). No significant difference was observed in ejection fraction (%) between all groups (~63%). ALA treatment reduced BW and delayed the onset of diastolic dysfunction in HD fed groups.

Conclusions. HD induced metabolic and cardiovascular dysfunctions. Alteration of diastolic function occurs at 35W in obese WT rats, whereas in obese TGβ3 rats diastolic function alteration occurred earlier (W25). ALA presents multiple beneficial effects in obese rats.

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Role of pharmacists' associations in providing professional resources during the COVID pandemic

Alexandra Toma, Ofelia Crișan

Department of Pharmaceutical Legislation and Management, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Alexandra Toma
e-mail: toma.alexandra@umfcluj.ro

Introduction. Since the beginning of the COVID-19 pandemic, pharmacists have been called to fight in the front line in this health crisis. Pharmacies had to organize their activity to prevent SARS-CoV-2 infection and support their patients. International organisations created specific information hubs to help pharmacists' training. The aim of this paper was to analyse and compare the work and effort of different national pharmacists' associations in providing professional resources for their members during the COVID pandemic.

Material and methods. A documentary research was conducted on the internet to find such professional resources. Websites of national associations from seven countries were examined from COVID headings point of view. The documents found were submitted to an iterative process of reflection and discussion, then juxtaposed using the comparative method.

Results. Several resources of great quality were found on the websites of pharmacists' associations of Australia, France, Spain, and US. The common ground includes new procedures for quality and security in dispensing medicines, clinical information on COVID tests and treatments, and advice on communicating with patients. The main differences refer to providing remunerated medicines reviews for elderly in Australia, advocating for equitable vaccination and vaccine administration reimbursement for pharmacies in US, helping families with back to school first aid kit in Spain, or practical information for hospital pharmacists in France. In Finland, Hungary and Romania, the pharmacists' bodies have only published on their websites general information, if at all, but local initiatives were found in some cases.

Conclusion. Considering the main role of a professional body, pharmacists' associations should create and publish appropriate guidelines and procedures to support the best practices of their members, to protect them and to help patients during the COVID pandemic.

Doxorubicin and Simvastatin liposomes: from the concept to the optimized formulation

Cristina Barbălată, Alina Porfire, Tibor Casian, Ioan Tomuță

Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Cristina Barbălată
e-mail:
Barbalata.Cristina@umfcluj.ro

Introduction. Liposomes present a great advantage, namely they can incorporate both hydrophilic and lipophilic drugs in order to overcome the difficulties related to cancer therapy. The aim of this study was the development of an optimized liposomal co-formulation with simvastatin (SIM) and doxorubicin (DOX).

Materials and methods. For the development of liposomes, the QbD concept was applied, in accordance with ICH guideline.

Results. The quality target product profile of DOX-SIM co-formulation was set based on the quality attributes of liposomal products from the market and based on

literature review. Critical quality attributes (CQAs) of liposomes were identified as being DOX and SIM entrapped concentration, encapsulation efficiency (EE%), liposomal size, polydispersity index (PdI) and zeta potential. A screening study was first performed, in which five independent variables were selected considering the results of risk analysis, notably phospholipids and drug concentration, incubation time and the pH of the hydration medium. Statistical analysis evidenced that incubation time did not present any influence on liposomes CQAs, and thus a second risk evaluation was mandatory. An optimization process followed, in which phospholipids and drug concentration were valuated, and a design space was constructed. The optimized formulation exhibited an EE% of 78% for DOX and 41% for SIM, an average size of 116 nm with a PdI below 0.1 and a zeta potential value of -32 mV.

Conclusion. In the development of DOX-SIM co-formulation, risk evaluation was the key element, simplifying the selection of independent variables for evaluation and aiding in achieving an optimal formulation.

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QbD approach to project the performance of freeze-dried milk as a directly compressible excipient

Andreea Cornilă, Sonia Iurian, Ioan Tomuţă

Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Andreea Cornilă
e-mail: cornila.andreea@yahoo.com

Introduction. Milk is a fair candidate for becoming a popular excipient in oral pediatric formulations due to its significant presence in children's diets, increased palatability and taste-masking properties. The following approach uses previously freeze-dried milk as a directly compressible excipient, projecting and evaluating its capacities in the idea of further loading it with an API.

Materials and methods. The compression material was obtained by freeze-drying three milk samples (0.1%, 1.5% and 3.5% fat), with a freezing stage of 12 h at -55°C, primary drying at -25°C for 24 h and secondary drying at 20°C for 12 h. The dried cakes were then powdered to 800 µm and submitted to dynamic compaction analysis, using four values between 100-400 kg for the target load. The compression parameters, tensile strength, detachment and ejection stress were calculated and analyzed as responses in an experimental design in which the fat content of the milk and the target load were assigned as input variables.

Results. The quality of fit was high for all responses, with R2 values over 0.75 and response variations well explained by the model. The work of compression shows the effort required for tableting and as expected, it depends mainly on the applied load, but the effect of the fat content is also important as increasing the fat content led to a lower dependence on the load. The detachment stress becomes optimal by combining a higher fat percentage with a lower compression load, while the negative effect of higher loads on ejection stress is evened out by the lubricant capacities of the lipids.

Conclusion. The freeze-dried milk showed promising as directly compressed excipient, since it had good compressibility and the lubrication properties of the lipid content facilitated detachment and ejection.

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Implementation of Quality by Design (QbD) strategy for development of Fused Deposition Modeling (FDM) 3D printed tablets with Diclofenac

Andrea-Gabriela Crișan[1], Sonia Iurian[1], Cătălina Bogdan[2], Lucia Rus[3], Alina Porfire[1], Sebastian Porav[4], Ioan Tomuță[1]

1) Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Dermopharmacy and Cosmetics, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Drug Analysis, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

4) National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania

Corresponding Author:
Andrea-Gabriela Crișan
e-mail: Crisan.Andrea@umfcluj.ro

Introduction. Three dimensional printing (3DP) by fused deposition modeling (FDM) is rapidly advancing towards implementation in the pharmaceutical field as an innovative manufacturing technology. The purpose of this study was to explore FDM-3DP for the development of customized immediate release (IR) drug delivery systems with diclofenac sodium (DS) by applying the quality-by-design (QbD) approach.

Materials and methods. DS containing formulations were assessed for hot melt extrusion processability and fabrication of filaments for FDM-3DP. Printability of the filaments was defined by means of mechanical measurements. A systematic evaluation of the effect of independent variables (design feature, dosage form size and printing resolution) on in vitro drug release behavior of the 3D printed dosage forms was realized using a D-optimal experimental design.

Results. FDM printability of high drug loaded (50%) PVA based filaments was demonstrated. By modulating the size of the digital model via scaling, dosage forms with DS content in the range of 32-75 mg were prepared. In vitro dissolution evaluations of the custom-designed 3DP tablets showed IR patterns. The optimum formulation characterized by a concentric ring design feature and containing 50 mg of DS exhibited 78% drug release after 5 minutes and 90% drug release after 10 minutes. Investigated factors proved to have effects on the dissolution performance, high resolution printing (0.1 mm) and increased size of the tablet promoting process acceleration.

Conclusion. By utilizing QbD approach to define the influence of input variables on the characteristics of FDM-3D printed drug delivery systems, fabrication of IR tablets with customizable doses of DS was achieved, further demonstrating the relevance of the technique for personalization of medicine.

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Development of an optimized liposomal formulation by Design of Experiments, for topical delivery of tretinoin

Lucia Ruxandra Tefas[1], Cătălina Bogdan[2], Alina Porfire[1], Tryfon Digkas[3], Thomas De Beer[3], Ioan Tomuță[1]

1) Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Acne vulgaris is a chronic inflammatory condition of the pilosebaceous unit with a prevalence of 80-90% in adolescents. Topical tretinoin (TRE) is widely used for the treatment of acne vulgaris due to its high effectiveness as a comedolytic agent. However, due to its irritative and photosensitizing potential on the skin, most patients using TRE develop dermatitis. Furthermore, its use is limited by

2) Department of Dermopharmacy and Cosmetics, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Laboratory of Pharmaceutical Process Analytical Technology, Department of Pharmaceutical Analysis, Ghent University, Ghent, Belgium

Corresponding Author:
Lucia Ruxandra Tefas
e-mail: tefas.lucia@umfcluj.ro

a pronounced physicochemical instability. Therefore, this study aimed at developing a topical TRE-based liposomal gel formulation with greater local tolerability and enhanced stability.

Material and methods. TRE-loaded liposomes (L-TRE) were prepared by ethanol injection from egg phosphatidylcholine and cholesterol. Design of Experiments (DOE) was employed to study the influence of several formulation factors on the quality attributes of L-TRE. 11 formulations were prepared according to a 23 full factorial design, and characterized in terms of size, size distribution, TRE content and entrapment efficiency. An optimum liposomal formulation with desirable characteristics was evaluated in vitro regarding TRE release, and further incorporated in a Carbopol 940 gel base to a final concentration of 0.04% TRE.

Results. L-TRE exhibited a mean size of 200-400 nm, and a narrow size distribution (polydispersity index < 0.25). TRE was successfully loaded into the liposomes with an efficiency of 70-80%. According to the DOE statistical analysis, L-TRE attributes were mainly influenced by the concentrations of phospholipid and TRE. The in vitro release of TRE from the optimum liposomal formulation was sustained, with a maximum percentage of 75% released after 48h in a mixture of PBS pH 6.5 and ethanol 1:1 (v/v) at 32°C.

Conclusion. Overall, this study reports the successful formulation and preparation of a TRE-loaded liposomal gel for topical application. L-TRE gel could be a promising candidate for effective and safe topical delivery of TRE in patients with acne vulgaris.

In vivo investigations of antitussive, antioxidant, and anti-inflammatory effects of a walnut (*Juglans regia* L.) septum extract rich in bioactive compounds

Ionel Fizeşan[1], Marius Emil Rusu[2], Carmen Georgiu[3], Anca Cherfan[1], Maria-Georgia Ştefan[1], Dana Maria Muntean[2], Simona Mirel[4], Oliviu Voştinaru[5], Daniela-Saveta Popa[1]

1) Department of Toxicology, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Department of Pathological Anatomy, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. The antitussive and anti-inflammatory effects of a walnut (*Juglans regia* L.) septum extract (WSE) rich in bioactive compounds, prepared in optimum extraction conditions, were investigated using the anti-tussive test with cough induced by citric acid aerosol inhalation.

Materials and methods. Wistar male rats divided in four groups with six animals each were used to assess the antitussive effect. For three days, the animals received by gastric gavage (1 mL/100g b.w.): (1) distilled water (control group – C); (2) codeine phosphate (Cd), 3 mg/kg b.w./day (positive control); (3) 10 mL WSE (containing 134 mg gallic acid equivalents (GAE)/kg b.w./day (WSE); (4) 5 mL WSE (containing 67 mg GAE)/kg b.w./day (WSE/2). In the 4th day, all rats were exposed to citric acid aerosols (17.5%) for 4 min and the number of coughing attacks occurring in the 8 min window frame after the beginning of exposure were counted. Each animal was sacrificed 6 hours after exposure and blood and lung tissue samples were collected for histopathological analysis and the assessment of oxidative stress (ROS, NOx, total antioxidant activity) and inflammatory (IL-6, CXC-R1, CXC-R2) biomarkers.

4) Department of Medical Devices. Pharmaceutical Practice, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

5) Department of Pharmacology, Physiology and Physiopathology, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Ionel Fizeşan
e-mail: Ionel.Fizesan@umfcluj.ro

Results. The obtained results demonstrated a better antitussive effect of WSE compared to codeine, in a dose-depending manner. The percentage of cough inhibition was 68.55% for WSE compared to 51.57% for Cd, and 23.90% for WSE/2. The anti-inflammatory effect of WSE was confirmed by his topathological analysis. Only two inflammatory biomarkers (IL-6 and CXC-R1) were significantly reduced versus control ($p < 0.05$).

Conclusion. Our study showed the antitussive effect of walnut septum extract and confirmed its *in vivo* anti-inflammatory effects. To the best of our knowledge, this is the first study to report the antitussive effect of walnut septum.

DENTAL MEDICINE

Zygomatico-orbital complex fractures – epidemiology and therapeutic algorithm

Sebastian Stoia[1], Cristian Dinu[1], Tiberiu Tamaș[1], Grigore Băciuț[1], Mihaela Băciuț[1], Simion Bran[1], Ileana Mitre[1], Horia Opriș[1], Avram Manea[1], Liana Crișan[1], Bogdan Crișan[1], Armeana Zgâia[1], Horațiu Rotaru[2], Florin Onișor[2], Rareș Roman[2], Ovidiu Mureșan[2], Mădălina Moldovan[2], Daniel Ostaș[2], Mircea Ciurea[2], Horea Almășan[2]

1) Department of Maxillofacial Surgery and Implantology, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Oral and Cranio-Maxillofacial Surgery, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Sebastian Stoia
e-mail: stoia_sebi@yahoo.com

Introduction. Zygomatico-orbital fractures (ZOF) have an increased frequency among midface traumas, occupying the second place after nasal fractures. The complexity of this anatomical region, the close relationship with the orbits and the major aesthetic and functional implications determine many therapeutic controversies in the literature like choosing the operative moment, the type of surgery and the need for orbital reconstruction.

Material and methods. The epidemiological analysis and the establishment of a therapeutic algorithm of the ZOF were the main objectives of this study. We conducted a retrospective and observational study based on the analysis of the medical records of 137 patients diagnosed and treated for ZOF at the Maxillofacial Surgery Department - Cluj-Napoca from 2015 to 2020.

Results. The mean age of patients was 41.3 years. Males were affected in 85%. Unilateral fractures were found in 55.5% of cases. Assaults and traffic accidents were the most common etiologies. Frontal and temporal zygomatic sutures were most often interested. Zygomatic fractures were associated in 40% of cases with orbital fractures. Diplopia and enophthalmia were statistically correlated with the presence of orbital fractures. Closed reduction without fixation was done in 61% of cases. Open reduction with 2 points of osteosynthesis was practiced in displaced, comminuted fractures, involving several zygomatic articulations. Twenty-one percent of the fractures required orbital reconstruction (21%).

Conclusion. ZOF require a prompt clinical and imaging diagnosis. The surgical procedure that ensures minimal morbidity must be chosen. Surgical treatment is required in 90% of the ZOF. Its choice is dictated by the displacement degree, the fracture complexity and stability after reduction. Displaced, comminuted fractures or without stability after reduction require osteosynthesis via an open approach. The fractures of the orbital walls that cause diplopia and/or enophthalmia require orbital reconstruction.

Clinical and theraupeutic aspects of the dento-maxillary anomalies

Tiberiu Tamaș[1], Cristian Dinu[1], Sebastian Stoia[1], Grigore Băciuț[1], Simion Bran[1], Ileana Mitre[1], Horațiu Rotar[2], Liana Crișan[1], Bogdan Crișan[1], Florin Onișor[2], Rareș Roman[2], Ovidiu Mureșan[2], Mădălina Moldovan[2], Daniel Ostaș[2], Avram Manea[1], Horia Opriș[1], Mircea Ciurea[2], Horea Almășan[2], Mihaela Băciuț[1]

1) Department of Maxillofacial Surgery and Implantology, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Dento-maxillary anomalies are growth and development disorders of the dento-alveolar arches and maxillary bones that induce functional and morphological problems of the dento-maxillary apparatus. The aim of this study was to evaluate the quality of life before and after treatment, distribution according the Angle classification,

2) Department of Oral and Cranio-Maxillofacial Surgery, Faculty of Dental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Tiberiu Tamaș
e-mail: tibi.tamas@yahoo.com

the main reason for referring to the doctor and the overall satisfactions of the patients regarding the results of the treatment.

Material and method. The study design was retrospective, conducted in the Cranio-Maxillofacial Surgery Department, Cluj-Napoca. We have included 103 patients with dento-maxillary anomalies, treated surgically in our department during 2015-2018. The patients have completed an anonymous online questionnaire with 21 questions using the ”Google Forms” platform. The questions were referring to the type of malocclusion, the reason for presenting to the doctor, the evolution of sleep apnea syndrome, muscles or joint pain, the masticatory efficiency and the new aesthetic aspect.

Results. The great majority of the patients were females, aged between 18-30 from urban area. Angle class type 3 was diagnosed in most of the patients. Alteration of the masticatory function and the aesthetic reasons were the most frequent causes for addressing to the doctor. After surgical treatment, most of the patients considered that they have a better masticatory function, a better aesthetic appearance, an increased respiratory function, the regression of joint and muscles pain syndrome. The sleep apnea syndrome was ameliorated or was healed in almost all the cases.

Conclusion. The main complaint of the patients are the aesthetic alteration and masticatory function disorders. Surgical treatment of these abnormalities gives patients increased satisfaction regarding the new aesthetic appearance, improved masticatory function, muscle and temporomandibular joint functionality. Also, surgical treatment can provide an improved respiratory function and a superior quality of life.

Planning fibula and mandibular osteotomy guides: the exclusive surgeon approach

Daniel Ostaș[1], Mihaela Hedeșiu[1], Simion Bran[2], Cristian Dinu[2], Gabriel Armencea[2], Avram Manea[2], Florin Onișor[2], Mădălina Anca Moldovan (Lazăr)[1], Sergiu Văcăraș[2], Ovidiu Mureșan[1], Rareș Călin Roman[1], Mircea Viorel Ciurea[1], Mihaela Băciuț[2], Grigore Baciuț[2], Horațiu Rotar[1]

1) Department of Oral and Cranio-Maxillofacial Surgery, Faculty of Dental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Maxillofacial Surgery and Implantology, Faculty of Dental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Daniel Ostaș
e-mail: daniel.ostas@gmail.com

Introduction. Applications commonly planned and printed in the hospital are anatomical models and surgical cutting guides, implantable devices being usually outsourced. Together with orthopedics, oral and maxillofacial surgery encouraged the development of medical 3D printing, one of the reasons being intensive use of surgical cutting guides. Production of devices is primarily done by third party entities; the surgeon being involved strictly for medical counseling. The aim of this paper is to illustrate an exclusively surgeon made virtual surgical planning for the reconstruction of a lower jaw defect and to give a short insight on hospital-based 3D planning and printing.

Material and methods. Planning process is described on a case of a spontaneous mandibular pathologic fracture in a patient suffering of osteonecrosis due to antiresorptive medication. Based on the computer tomographic imaging data and using a dedicated virtual planning software the surgeon designed the necessary fibula and mandibular cutting guides.

Results. By reverse engineering three cutting guides were designed - two for the mandible and one for the fibula - with the purpose of accurate positioning of the osteotomy lines ensuring optimal fibula inset at the level of the defect in order to restore continuity, soft tissue support and symmetry.

Discussions. A brief cover is given on the actual use of three-dimensional technologies at point-of-care with a stress on conveniences and constraints.

Conclusion. Having the surgeon as the main protagonist in the design process and the availability of printing infrastructure in the hospital provide adequate conditions for quick-to-produce and immediately available personalized medical devices, but whether this strategy is a better option to commercial producers remains to be further investigated.

Assesment of oral health related attitude and behavior for a population in Romania

Alexandra Vigu, Monica L Dascălu, Anca Labuneț, Adriana Objelean, Andrada Voina-Țonea, Cristina Iosif, Sorina Sava

Department of Dental Materials, Ergonomics, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Alexandra Vigu
e-mail: alexandravigu@yahoo.com

Introduction. Access to education is an earned right, guaranteed in the majority of the world's countries, and is a major interested point both at individual and also organizations level. Statistically, the level of education is a good predictor for the knowledge in both general and specific fields. The objective of the study was to evaluate to what degree the oral education level is correlated with the self-assessed Oral Health Related Attitude and Behavior scores for a population in Romania.

Material and methods. Our research used a one-time cross-sectional correlational design, and was conducted in Cluj-Napoca, Romania. Dental education level was collected and the assessment of the respondents' specific behavior and attitude with respect to their dental self-care was performed using the Romanian version of the Hiroshima University Dental Behavior Inventory (HU-DBI) questionnaire.

Results. A statistically significant correlation was identified between the oral education levels and the self-assessed attitude and behavior scores. Higher oral education levels translated to better HUDBI scores for the studied population.

Conclusion. We identified a strong support and need for efficient actions to increase awareness levels with respect to the importance of oral health related behavior and attitudes. The HU-DBI scores in relation with dental education suggest that dental education programs design and implementation should be a focal point for the individual.

Testing the antibacterial efficacy of a natural photosensitizer by electron microscopy

Laura Monica Rusu[1], Mărioara Moldovan[2], Doina Prodan[2], Rahela Carpa[3], Radu Chifor[4], Sorina Sava[1], Alexandra Vigu[1], Mîndra Eugenia Badea[4]

1) Department of Dental Materials and Ergonomics, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. The aim of this study is to test the antibacterial effect of an experimental natural photosensitizer used in photodynamic therapy.

Material and methods. The experimental gel based on oregano essential oil was used as a photosensitizing agent in antimicrobial photodynamic therapy as an adjunct treatment in the control of oral microbial biofilm. The antibacterial effect of the gel based

2) Department of Polymer Composites, Institute of Chemistry "Raluca Ripan", Babes-Bolyai University, Cluj-Napoca, Romania

3) Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania

4) Department of Prevention in Dentistry, Faculty of Dental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author: Laura Rusu
e-mail:
rusu.monica.laura@gmail.com

on oregano essential oil was tested on various bacterial cultures by the dilution method, as well as by analysis by scanning electron microscopy, in the presence of a laser light source.

Results. All samples in which the experimental gel based on oregano essential oil was used showed an antibacterial effect by inhibiting the development of bacterial colonies in the culture medium. Microscopic analysis revealed the antibacterial effect of the experimental gel by disrupting the bacterial wall and reducing the volume of bacterial colonies.

Conclusion. The results obtained are promising and essential oils should be investigated in more detail, given that they are a natural source of antibacterial compounds. The essential oil used in the experimental gel, gives antibacterial properties to the gel in which it is incorporated, a property that can be useful in antibacterial photodynamic treatment.

Preliminary prevalence data evaluating the presence of periodontitis in cerebral stroke patients

Cristina Costea, Alexandra Roman, Andrada Soancă, Andreea Ciurea, Iulia Cristina Micu, Diana Bănuţ, Alina Stanomir

Department of Periodontology, Faculty of Dental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Cristina Andrada Costea
e-mail:
Costea.cristinaandrada@yahoo.com

Introduction. Current literature data suggests a strong association between periodontitis and stroke, but the conclusions are heterogeneous.

Periodontitis is one of the most common chronic inflammatory diseases in human species. Periodontitis has an estimated prevalence among the population between 20% and 50%, and about 10% of the patients have severe forms of periodontitis, which can have a negative effect on systemic health. Stroke is the second most common cause of mortality worldwide and is the third leading cause of disability among adults.

The aim of this clinical preliminary study was to investigate the association between periodontitis and cerebral stroke and its clinical significance.

Materials and methods. Eighty patients with cerebral stroke were included in this study. The patients were examined during their hospitalization at the Recovery Hospital Cluj-Napoca. A complete specific periodontal examination was performed in order to assess the presence of periodontitis. Risk factors for both the diseases, such as smoking, were also analyzed. Demographic data and gender repartition were analyzed.

Results. The collected data showed that more severe forms of periodontitis were observed in patients with cerebral stroke. It was observed that periodontitis was more severe between patients that smoke or smoked for a long time. Periodontitis had a higher prevalence among patients who lived in rural areas than the ones living in urban areas.

Conclusion. This preliminary clinical study showed that periodontitis has a high prevalence among patients suffering from cerebral stroke.

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Evaluation of energy-dispersive X-ray analysis in assessing composite restorations adhesion

Diana Oneț (Bănuț), Alexandra Roman, Andrada Soancă, Daniela Condor, Andreea Ciurea, Iulia Cristina Micu

Department of Periodontology, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Diana Oneț (Bănuț)
e-mail: dyabanut@yahoo.ro

Introduction. Composite restorations addressed to non-carious cervical lesions (NCCLs) are frequently associated with therapeutic failure mainly due to their loss of retention. The histological and morphological features of NCCLs greatly influence the behavior of composite restorations and the quality of the adhesive interface is essential in the therapeutic success. Energy dispersive X-ray analysis (EDX) enables a semi-quantitative evaluation of the chemical elements present at the level of the different layers, including the adhesive interface, providing relative information about their structure. The aim of this study was to evaluate the quality of the adhesive interface in NCCLs restorations for two types of classical composite resins.

Materials and methods. A total of 10 extracted teeth were included in the study, divided in two groups of five teeth each. Class V cavities were prepared on the buccal and palatal surfaces of each tooth. The first group was restored with a low shrink nano-hybrid composite (Beautifil II LS®, Shofu) and the other group was restored with a micro-hybrid composite (Dynamic Plus®, President Dental), both of them associated with a 7th generation bonding agent. The prepared teeth were immersed in dye solution and they were subsequently sectioned, obtaining 20 samples, 2 samples for each tooth. To evaluate the marginal microleakage at the adhesive interface, the dental sections were analyzed using optical microscopy and scanning electron microscopy (SEM).

Results. The optical microscopy showed no dye penetration for most samples, but the SEM analysis revealed some degree of marginal microleakage for both types of the composite resins. For all the examined samples, composite restorations were associated with a well-represented hybrid layer and the continuity of the adhesive interface. EDX analyses revealed a similar chemical composition between the two materials and some differences between the sections that belonged to the same material.

Conclusions. The information resulting from the investigations on the quality of the adhesive interface suggests that the classic composite resins represent a good choice of material for the restoration of NCCLs.

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Discrepancy between maximum intercuspation-centric relation in subjects with temporomandibular disorders

Oana Almășan, Mirela Fluerașu, Mariana Constantiniuc, Smaranda Buduru

Department of Prosthetic Dentistry, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. The study of the relationship between maximum intercuspation-centric relation centric in subjects with temporomandibular disorders.

Corresponding Author:
Oana Almășan
e-mail: oana.almasan@umfcluj.ro

Material and methods. A number of thirty adolescent subjects (aged between 20-30 years) were evaluated. The presence of temporomandibular disorders was investigated by evaluating the status of the temporomandibular joint and orofacial muscles. The occlusal relationships were studied by evaluating the maximum intercuspation and the centric relation positions.

Results. Females had a higher prevalence of muscle pain. Discrepancy was enhanced in all spatial planes in subjects with temporomandibular disorders: sagittal discrepancy with 0.09 mm, transversal discrepancy with 0.66 mm and vertical discrepancy with 0.79 mm. There were no significant gender differences of discrepancy. Muscle pain was enhanced in subjects with transversal discrepancy.

Conclusions. Discrepancy was found in all subjects, at least in one spatial plane.

Assessment of temporomandibular dysfunction in subjects with Angle class II/2 malocclusion

Antonela Berar, Mariana Constantiniuc, Andreea Kui, Mirela Flueraşu, Simona Iacob, Manuela Manziuc, Smaranda Buduru

Department of Prosthetic Dentistry, Faculty of Dental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj- Napoca, Romania

Corresponding Author:
Antonela Berar
e-mail: antonela_berar@yahoo.com

Introduction. Temporomandibular dysfunction (TMD) is a pathological condition within the masticatory system, caused by various etiological factors, among which dental malocclusion. The aim of the study was to investigate the prevalence of signs and symptoms of TMD in young adults with class II/2 Angle malocclusion.

Material and methods. A number of 28 subjects, aged 20 to 25 years old, all dental students, were included based on the following criteria: Angle class II/2 malocclusion, complete dental arches, no history of systemic, musculoskeletal disorders or cervicofacial trauma. For each subject the anamnesis included filling in a questionnaire with items pertaining to medical and dental history. A clinical exooral and endooral examination was performed along with an analysis of diagnostic casts mounting in semi-adaptable articulators. The exooral examination assessed the temporomandibular joint, the masticatory and cervical muscles. The endooral examination assessed the mandibular movement, mucosae, teeth and static and dynamic occlusal relationships. The data obtained was recorded in the examination sheet and then used to set the clinical diagnosis of TMD.

Results. The most frequent subjective symptoms were muscular pain, reported in 35.7% of the subjects, and sounds in the temporomandibular joints, occurring in 67.8% of the cases. Signs and symptoms like muscular pain, articular sounds and occlusal modifications were more prevalent. Statistical analysis revealed significant differences in the distribution of occlusal interferences and premature contacts in right lateral mandibular movement ($p=0.015$) and in left lateral mandibular movement ($p=0.003$) in subjects with the clinical diagnosis of TMD.

Conclusion. Angle class II/2 malocclusion was associated with signs and symptoms of TMD by combining clinical and anamnestic data. Early identification of signs of TMD is necessary so as to apply the appropriate treatment, thus avoiding subsequent complications.

Variations in tooth preparations for metal-ceramic crowns in general dental practice

Andrea Chisnoiu[1], Simona Iacob[1], Alina Picoș[1], Andreea Kui[1], Duncea Ioana[1], Radu Chisnoiu[2], Marius Negucioiu[1], Smaranda Buduru[1]

1) Department of Prosthodontics, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Odontology, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Radu Chisnoiu
e-mail: dr.chisnoiu@yahoo.com

Introduction. The current study aimed to investigate the variations in tooth preparations for metal-ceramic crowns (MCCs) in general dental practice, by performing a laboratory-based retrospective analysis of dies.

Materials and methods. A sample of 81 tooth preparations for MCCs, featuring work from different general dental practitioners was obtained from two commercial dental laboratories. Aspects of the preparations were quantified and compared with accepted criteria defined following a review of the literature.

Results. The teeth found to be most frequently prepared for MCCs were premolars and molars (lateral group) (80.74%). Six percent of samples presented a shoulder finish line while a chamfer margin design was noticed in 62.6%. Thirty-one percent of samples had either a feathered or no clear margin design respectively. Of the cervical preparations analyzed, 86% had been underprepared, mostly on the oral and distal surfaces. A significant difference was observed between cervical preparations on molars (preparation width ≤ 0.5 mm), comparing to premolars (preparation width ≥ 0.5 mm).

Conclusion. On the evidence of this survey of this sample of general dental practitioners' work, it was found that relevant guidelines for the preparations of MCCs are not being fully adhered to.

New theories regarding the etiology, diagnosis and treatment of bruxers

Mirela Ioana Fluerașu, Simona Iacob, Silvia Balhuc, Andreea Kui, Smaranda Buduru

Department of Prosthetic Dentistry, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Mirela Ioana Fluerașu
e-mail: mfluerasu@gmail.com

Introduction. Bruxism is defined by the American Academy of Sleep Medicine as a "repetitive jaw muscle activity characterized by the clenching or grinding of teeth and/or bracing or thrusting of the mandible". Bruxism can be a common cause of dental wear, dental loss, tooth fractures, alveolar exostosis, muscle pain and the appearance of TMDs (temporomandibular dysfunctions).

Materials, methods and results. The aetiology of bruxism is still not very well established. Initially, bruxism was described as a dysfunction caused by the peripheral factors such as the type of dental occlusion and the malocclusion, but the following studies proved that multifactorial origin may also include the central nervous system. Risk factors for bruxism occurrence may include disturbances in neurotransmitters, neurological diseases, trauma, drug use, smoking, alcohol, and psychological factors including stress. Recent studies indicate different causes for the two clinical entities of the bruxism: the sleep bruxism is caused mainly by occlusal factors, and the awake bruxism is caused mainly by psychological factors such as anxiety and stress.

Diagnosing bruxism is a continuous challenge. The literature describes multiple diagnostic techniques with more or less certain results. Among them polysomnography

turned out to be the most accurate diagnostic tool, but the indication is limited due to patient discomfort. Bruxoff and Grindcare type devices counteract this discomfort, keeping the high specificity of polysomnography. Nowadays, a number of mobile application support the bruxers, facilitating the diagnosis, monitoring and quantification of his muscle activity.

Conclusion. The multifactorial etiology and the difficulties encountered during the diagnosis make the bruxers one of the permanent challenge in the dental office.

Systemic impact of dental malocclusion: the evaluation of dental practitioners' perspective

Simona M. Iacob, Andrea M. Chisnoiu, Mirela I. Fluerașu, Antonela M. Berar, Andreea Kui, Smaranda Buduru

Department of Prosthodontics, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Simona Iacob
e-mail: simona72cj@yahoo.com

Introduction. The current study underlines the degree of knowledge of the importance of restoring the functionality of the dento-maxillary apparatus (DMA) and thus a functional dental occlusion and to identify the level of knowledge of systemic implications of stomatognathic imbalances among practitioners.

Materials and methods. An online anonymous questionnaire was used in order to evaluate the level of knowledge of the participants concerning the clinical implications and management of patients with balance and occlusal disorders. The present observational study was based on 15 items for different dental specialists regarding the complex dental treatment applied or recommended for their patients and also the willingness and need of dentists to collaborate with physicians of other specialties that may be related to systemic manifestations of occlusal-articular imbalances.

Results. A total of 116 dentists participated in the present study , most of them working in urban areas (85.3%); as a specialty, most respondents are specialists in „general dentistry" (64.7%), the rest of dental specialties being in lower percentages. A strong statistical correlation was observed between Orthodontics/Pediatric Dentistry specialists and complex clinical occlusal examination of the patients ($p=0.012$). Fourty-four percent of the dental practitioners consider that "sometimes" the untreated pathological dental occlusion may have a negative impact on the muscular-skeletal system, while only 30.2% of them consider as "frequent" an association between the pathologies. However, when associated these two pathologies, almost 40% of the Orthodontists/ Pediatric Dentistry specialists consider there is "always" a correlation ($p=0.025$). Restoring the functional occlusion based on a correct prosthetic treatment is "frequently" or "always" mandatory for over 80% of the practitioners with a professional experience between 5-20 years ($p>0.05$).

Conclusions. The study confirms that dental practitioners are aware of the interrelationship between the dento-maxillary apparatus and other systems, thus observing their availability and at the same time their desire, regardless of specialty, to collaborate with doctors from other specialties to achieve a complete and complex dental treatment, leading to appropriate systemic balancing.

Students' Perception of E-Learning during Covid-19 pandemic

Andreea Kui, Silvia Balhuc, Marius Negucioiu, Mariana Constantiniuc, Smaranda Buduru

Department of Prosthetic Dentistry, Faculty of Dentistry, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author: Andreea Kui
e-mail: andreeakui@gmail.com

Introduction. The e-learning process during Covid-19 pandemic had an impact on both teachers and students; as a result, for the students who were assigned to staying home there were limited opportunities to practice in the clinic. The aim of the research was to assess medical and dental students' perceptions about the efficiency of the remote teaching method and to investigate their opinion about implementing some online teaching features even after the pandemic period would pass.

Material and methods. A survey, providing questions related to social-demographic factors as well as 20 statements specific related to the topic was distributed via email, for assessing the opinion of the respondents regarding the online educational process implemented during the pandemic period.

Results. The answers of 667 respondents were statistically analyzed. We noticed that students accepted remote lectures and other learning activities due to the higher risk of contamination. But an important number of students would like to restart the classic activities as soon as possible, especially for the labs or clinical practice. We observed also a tendency to accept remote lectures, or a combination of online and traditional lectures, even after the pandemic period is over.

Conclusion. Considering the results of the present survey, we can state that the participants in our research had an overall positive opinion about the acceptability and usability of online medical learning. The students viewed online activities as helpful, but as a supplement to their learning rather than a replacement for traditional teaching methods.

Possibilities of polymethyl methacrylate denture resins improvement using nanomaterials

Cecilia Bacali[1], Mariana Constantiniuc[1], Mîndra Badea[2], Mărioara Moldovan[3], Alex Bacali[4], Ana Ispas[1], Daniela Popa[1], Andreea Kui[1], Smaranda Buduru[1]

1) Department of Prosthetic Dentistry, Faculty of Dental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Prevention in Dentistry, Faculty of Dental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) "Raluca Ripan" Chemistry Research Institute, Cluj-Napoca, Romania

Introduction. Acrylic resins, and especially the PMMA (polymethyl methacrylate) based resins, are the most used materials for fabricating removable dentures due to their many favorable characteristics. However their drawbacks regarding the mechanical properties are associated with denture fractures. The addition of nanomaterials with high strength and flexibility could improve the mechanical properties of denture base resins by reinforcing their matrix.

Materials and methods. Different specimens were fabricated using a PMMA based denture resin (Castavaria, New York Dental), with and without graphene (in different concentrations) addition.

Results. The specimens - subjected to different mechanical tests - showed improved strength when using the commercial resin with graphene addition, the effects depending on the concentration.

4) Private Dental Practice, Cluj-Napoca, Romania

Corresponding Author:
Cecilia Bacali
e-mail: Cecilia.Bacali@yahoo.com

Conclusion. The results showed that the nanoparticles added to the PMMA based resin improved the mechanical properties of the material.

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Applicability of CAD CAM materials in the prosthetic superstructure

Silvia Balhuc, Smaranda Buduru, Daniela Popa, Mirela Fluerașu, Andreea Kui, Marius Negucioiu

Department of Prosthetic Dentistry, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Silvia Balhuc
e-mail: syylvya@yahoo.com

Introduction. The main concepts that led to the digital manufacture of prosthetic restorations were the shortening of the working stages and the gradual removal of less aesthetic materials (those with a metal frame such as metal-ceramic).

Materials, methods and results. Over time, scientific research has focused on the discovery of new types of materials with increasing strength to ensure long-term clinical success while providing superior aesthetics.

The boundary of the preparation at the cervical finish line level is defined as an edge between the intact, untouched portion of the tooth and the apical point of the preparation itself.

Currently in the literature there are many cervical finish line designs, that are also influenced by the type of the future dental crown's material.

Conclusions. It is important to know the indications, contraindications but also failures, before choosing the right type of the material suitable for a certain clinical situation, in order to obtain lasting results.

A clinical comparison of all ceramic and metal-ceramic fixed partial prostheses - review

Ana Ispas, Andreas Binder, Mariana Constantiniuc, Antarinia Crăciun, Manuela Manziuc, Daniela Popa, Corina Tișler, Smaranda Buduru

Department of Prosthetic Dentistry, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author: Ana Ispas
e-mail: ana24ispas@yahoo.com

Introduction. The metal-ceramic fixed partial prostheses (FPDs) are still perceived as the golden standard for posterior tooth restorations as they present excellent mechanical properties and little aesthetic function. On the other hand, all-ceramic materials offer an adequate alternative with better optical qualities. They are more tooth resembling in terms of colour and display increased translucency. The aim of this systematic review was to assess the survival rate and complications of all ceramic and metal-ceramic fixed partial prostheses.

Material and methods. We performed an electronic search for specialty literature articles in English by using Pub Med. This systematic review focused on research articles published between 2010 and 2020. All the studies, which analyzed the survival rate and complications of tooth-supported fixed partial dentures, were included in the present article.

Results. The electronic searches resulted in 9720 articles, of which 43 articles were included as full text articles, and 14 articles met the inclusion criteria. The incidence of caries and loss of vitality were found to be higher in all ceramic prostheses when compared to the metal-ceramic ones. A significant framework fracture was reported in zirconia fixed partial dentures in comparison to metal-ceramic fixed partial dentures.

Conclusion. The clinical comparison of all ceramic restorations revealed a lower survival rate and complications than in metal-ceramic restorations.

Orthopantomography - evaluation of the technical errors encountered in post-traumatic and post-operative context

Bianca Petric[1], Raluca Roman[2], Alina Coclici[2], Sara Roman[1], Emil Crăsnean[2], Mihaela Hedeşiu[2]

1) Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Dental Radiology, Faculty of Dental Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Raluca A. Roman
e-mail: roman.raluca@umfcluj.ro

Objective. Considering that orthopantomography is performed as the first imaging step in mandibular traumas or surgical context of the mandible, the objective was to evaluate the technical errors occurrence in these specific situations. Due to soft tissue and bone morphology changes in these cases, the performance of the panoramic radiography meets certain quality limitations, the positioning being more difficult and generating errors.

Material and methods. A number of 62 panoramic images performed in patients with fractures and orthognathic surgery, were retrospectively evaluated. The technical errors, regarding positioning and exposure, were assessed by 2 examiners, a 6th year student and a radiologist.

Results. All cases presented at least 2 errors, with a maximum of 5 errors in cases with orthognathic surgery. The most common error was the incorrect positioning in the focal through due to the absence of the mouthpiece, and the difficulty of assessing the mid-sagital line (the pipe is not used in 73% of cases in the context of bi-maxillary immobilization). Incorrect positioning in the horizontal plane and outside the focal plane had an increased incidence. The position in flexion was detected in 45% of patients, 29% being in extension. The difficulty of correctly assessing the amount of radiation, taking in consideration the tumefaction and metallic devices, generated exposure errors in 12% of situations, metal artifacts being also present.

Conclusions. The post-traumatic or post-operative status of the patient, through changes in symmetry, swelling and immobilization devices, makes it difficult to position in the focal plane of the orthopantomography device, generating a multitude of errors. Due to the increased incidence of technical errors in these particular situations, the radiographer must carefully approximate the alignment plans in order to obtain a qualitative image for diagnostic.

Evaluation of aerosol generating events during the nasopharyngeal swabbing for SARS-CoV-2 detection. The reach from a lottery to a Russian roulette

Cosmin Andrei Cismaru[1], Sergiu Chira[1], Gabriel Cismaru[2], Andreea Nuțu[1], Ioana Berindan-Neagoe[1]

1) The Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Internal Medicine, Cardiology - Rehabilitation, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Corresponding Author:
Cosmin Andrei Cismaru
e-mail: Cosmin.Cismaru@umfcluj.ro

Introduction. During specimen collection, the contact of the nasopharyngeal swab with the respiratory mucosa often triggers defense reflexes such as sneezing and coughing, which generate airborne particles. The persistence of infectious aerosols for several hours after their generation can represent a threat for susceptible individuals in the vicinity of their emission point.

Methods. We conducted a prospective observational cohort study from July to October 2020 on the general population which addressed our facility for RT-PCR SARS-CoV-2 testing. Participants were evaluated for the occurrence of aerosol generating events (AGEs) such as coughing and sneezing during the nasopharyngeal swabbing.

Results. Of the 1239 individuals, we reported 264 in which AGEs occurred during the specimen collection. 97 individuals tested positive for SARS-CoV-2, of which 20 presented AGEs during the nasopharyngeal swabbing.

Conclusion. The risk of coughing or sneezing during the nasopharyngeal swabbing was increased among the tested individuals. Additional strategies of limiting the risk of contamination of other participants to the testing session from potentially infectious and persistent aerosols should be ensured in SARS-CoV-2 testing units.

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Antiproliferative and apoptotic effects of caffeic acid phenethyl ester (CAPE) on oral squamous cell carcinoma. A preliminary study

Ciprian Ioan Oșan[1,2], Oana Zanoaga[2], Ancuta Jurj[2], Livia Budișan[2], Lajos Raduly[2], Cornelia Braicu[2], Roxana Cojocneanu[2], Ioana Berindan-Neagoe[2,3]

1) Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction. Oral squamous cell carcinoma (OSCC) is the most common type of oral malignancy. Nowadays, it is an increase in the need to identify new methods for prevention and treatment for this disease. The purpose of this study was to evaluate the effects of caffeic acid phenethyl ester (CAPE) on human oral cancer cells and to further determine its therapeutic value and anticancer activity.

2) Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Haţieganu University of Medicine and Pharmacy- Cluj-Napoca, Romania

3) Department of Genetics, Functional Genomics and Experimental Pathology - The Oncology Institute "Prof. Dr. Ion Chiricuţă", Cluj-Napoca, Romania

Corresponding Author:
Ciprian Ioan Oşan
e-mail: cipri.osan@yahoo.com

Material and methods. The antiproliferative effects of CAPE were assessed on PCI-9 and PCI-13 cell lines and included the following methods: MTT assay; scratch assay for cell migration/invasion determination; fluorescence microscopy for apoptosis and autophagy evaluation, flow cytometry for the different phases of the cell cycle evaluation, and reverse transcriptase quantitative PCR (qRT-PCR) for gene expression analysis.

Results. Our data showed that CAPE exhibits an important antiproliferative effect which is corelated with a minimal dose used for the cell treatment, 8,434 μM for PCI-9 cells and 4,384 μM for PCI-13 cells, showing the potential of this natural compound in impairing in vitro cancer spreading. The effect was significantly observed in cellular morphology for both cell lines, autophagy function being observed as a pre-existing state before apoptosis. Evaluation of lncRNA expression by qRT-PCR found that MALAT1, GAS5 and HOTAIR expression was influenced by CAPE in dose and time-dependent manner.

Conclusion. This study demonstrated that CAPE has the potential to be an excellent therapeutic compound for patients with OSCC due to its ability to modulate biological processes and signaling pathways involved in apoptosis, cell cycle, migration and tumor progression.

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