

## 1. Katarina

### Abstract 1

Raman spectroscopy is a physical method with a broad spectrum of applications across multiple scientific fields. Our work here presents a possibility to differentiate between 2 important grampositive species commonly found in clinical material – *Staphylococcus aureus* and *Staphylococcus epidermidis*. We tested 87 strains, 41 of *S. aureus* and 46 of *S. epidermidis* directly from colonies grown on a Mueller-Hinton agar plate. The method showed great potential for separating these two species.

### Abstract 2

Raman spectroscopy has a broad spectrum of applications across numerous scientific fields, including microbiology. Our work here monitors the influence of culture media on Raman spectra of clinically important microorganisms (*Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Candida albicans*). Choosing an adequate medium may enhance the reproducibility of the method as well as simplifying data processing and evaluation. We tested four different media per microorganism in dependence on the nutritional requirements and clinical usage directly on a Petri dish. Some of the media have a significant influence on the microbial fingerprint (Roosevelt-Park Institute Medium, CHROMagar) and should not be used for an acquisition of Raman spectra. We found that the most suitable medium for microbiological experiments regarding these organisms was Mueller-Hinton agar.

## 2. Helena

Analysis of leukemic cells and their external supporting stimuli in relation to therapy response: my possible poster

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia found in adults in Europe and the United States. CLL is characterized by its highly variable course and outcome. This malignancy is treatable but still remains incurable. Multiple studies in recent years have shown that B-cell receptor (BCR) signaling plays an essential role in biology of CLL cells. This poster provides preliminary results from investigation of BCR signaling in three distinct groups of patients with different prognosis.

To determine the role of BCR signaling in CLL, we used samples from 30 CLL patients (10 patients with favorable prognosis, 10 patients with intermediate prognosis and 10 patients with poor prognosis). B-lymphocytes were labeled with antibodies focused on BCR signaling (p-Erk, p-Akt, p-p38, p-IKK $\gamma$ , p-NF- $\kappa$ B, p-BTK/ITK, p-PLC- $\gamma$ 2 and p-ZAP70/SYK) and were measured by flow cytometer.

The results showed different activity of BCR signaling in groups of patients with various prognoses. The response of BCR signaling after stimulation was highest in patients with poor clinical prognosis and lowest in patients with most favorable prognosis. The differences were most obvious when we focused on kinases BTK, PLC- $\gamma$ 2 and ZAP70/SYK. There was no difference observed when we focused on kinase IKK $\gamma$ .

In this study we demonstrated that the BCR signaling, which is now considered as essential pathway in CLL cells, has variable activity in CLL patients. The activity of this signaling correlates with patients' clinical prognosis and could be possibly used as an additional prognostic marker in clinical praxis.

### 3. Lasha

#### Agricultural Policy and Agricultural Cooperative Development Perspectives in Georgia

The Present study shows the relationship between agricultural policy and agricultural cooperative development in Georgia from 2013 to 2015. In terms of methodology I used quantitative and qualitative approaches to assess the research subject. In quantitative analysis I used regression model, the results of which suggests that agricultural policy implements have a significant effect on agricultural cooperatives in Georgia. In qualitative analysis I created a special questionnaire and I conducted interviews with 100 representatives from Georgian agricultural cooperatives. According to the completed questionnaire, results show that there are communication and knowledge transfer problems between the Agricultural Cooperation Development Agency and agricultural cooperatives in the country. In addition, results show that there are some significant differences and commonalities between the sectors. For example, cooperatives in the wine sector carry out a diversity of functions, however cooperatives in the hazelnut sector are mostly strong in processing. Therefore, this paper will be a step forward in scientific as well as policy-making perspectives. This will help decision makers to address the problems faced in agricultural cooperation in Georgia.

Keywords: Agricultural Policy, Agricultural Cooperatives, Qualitative and Quantitative Analysis

#### 4. Jana S.

Fruit and vegetables constitute an important source of energy and nutrients for humans. According to the World Health Organisation intake of at least five servings of fruit and vegetables (approximately 400 g) per day is recommended for prevention of noncommunicable diseases and micronutrients deficiencies. The objective of the present study was to specify eating habits, especially fruit and vegetable consumption, in the Roma population in the Southern Moravian region. Within this attitudes to fruit and vegetable consumption and knowledge about recommended daily amount and nutrient content were observed. The study was conducted across the Southern Moravian region, Czech Republic, with a sample of 102 individuals using a questionnaire method. The results revealed that fruit and vegetable consumption was insufficient. Only 16% of individuals consumed fruit and vegetables at least once a day. The recommended fruit and vegetable daily amount was unknown to 80% of individuals. According to 50% of individuals fruit and vegetables are a good source of protein. The results of this study might help promote more focused action on education in the Roma population about healthy eating habits with the health impact of sufficient daily consumption of fruit and vegetables.

Key words: nutrition, fruit, vegetable, Roma population

## 6. Pastor

### Abstract

This paper examines Brazil's fight against poverty from 2004 to 2013. The aim of the research is to test if the Conditional Cash Transfer Programs have had an impact in elementary education. Through CCTP, especially "Bolsa Familia", the government has tried to reduce poverty in two senses: short term and long term through monetary transfers and human capital investment, respectively. The strategy against poverty in this country has adopted a multidimensional approach. It has been based in education, health and standard level of life. The methodology used in this paper was Pearson X<sup>2</sup>, with a significance level of 0.05, CL=95, and the statistical programme SPSS 22. We focused mainly on testing significant statistical relationships between dependent variable "school dropout" and independent variable "stratum", namely, if being a Bolsa Family's beneficiary is related to dropout of elementary students. However, the article shows that in families with a \$R 50 income, there is a correlation between dependent variable "dropout" and independent variables such as age, region, years of study and working children, but not with gender, race, mother lives at home and stratum. Among families with a \$R 100 income, we find only a correlation with age, years of study and region. According to BID, these programs are very effective in short-term poverty reduction. However, there is no proof that demonstrates the Conditional Cash Transfer Programs' effectiveness against intergenerational transmission of poverty.

## 7. Jana F.

Mutations in ATM genes are associated with inferior prognosis in CLL patients. We noted very good response of ATM mutated CLL samples to anti-CD20 antibodies rituximab and ofatumumab in our recent study [1]. Therefore, we analyzed “in vivo destiny” of ATM mutations during the course of CLL. We selected 25 patients covering the whole spectrum of ATM defects involving mutations: missense and other types of mutations, with and without 11q-. In these patients, the presence of particular mutations was analyzed at subsequent time points during the course of the disease. Nineteen cases were monitored in a relapse (median follow-up 42 months; therapy involved anti-CD20 antibodies in 15 cases), while there was no therapeutic intervention in the period between samplings in six patients (median follow-up 18 months). Among the new samples, we detected ATM mutations in 24/25 patients. In the remaining patient (treated with ofatumumab and chemotherapy) we observed loss of a partially selected (original proportion about 15%) missense mutation. In another two patients we noted disappearance of 1 out of 2 mutations, in one case after rituximab with chemotherapy and in one case spontaneously. We also confirmed the stability of ATM mutations in another two patients analyzed retrospectively at diagnosis (median between samplings 55 months). The same mutations were again present at both analyzed time points. Altogether, our results clearly document that ATM mutations are stable during the course of CLL. Their elimination by therapy is rare, despite using most potent regimens involving monoclonal antibodies.

## 8. Barbora

In clinical practice, the KRAS mutant tumours of the colon are considered as a homogeneous group of tumours. However, currently there is direct and indirect evidence of heterogeneity not only in response to treatment, but also in survival. Understanding the cellular processes responsible for these differences is important for the research of other treatment methods. The study of gene expression in combination with clinical parameters is used for the detection of new biomarkers and is one of the techniques used to detect specific biological processes for the individual tumours. The main objective of this study was to characterize the gene expression heterogeneity of KRAS mutant colorectal cancer in an unsupervised manner and connect these findings with known clinical, histopathological and mutational markers of colorectal cancer and prognosis. A unique data set from PETACC-3 clinical study was used for this purpose, which contains information of the gene expression of 313 patients with KRAS mutations in the colon, accompanied by clinical variables, molecular markers and survival. Unsupervised analysis was used to unveil gene expression heterogeneity. By means of clustering analysis I defined 3 subtypes of patients with KRAS mutant colorectal tumours. However, there was no determination of any significant association between these subtypes and clinical variables and also there were no differences in prognosis. Heterogeneity of colon tumours with KRAS mutation was not clarified by their study on a molecular level. It seems that unsupervised analysis is not able to identify clinically and biologically meaningful subtypes.

## 9. Ivo

Amphiphilic peptides can interact with biological membranes and severely affect their barrier and signaling functions. These peptides, including antimicrobial peptides, can self-assemble into transmembrane pores that cause cell death. Despite their medical importance, the conditions required for pore formation remain elusive. Monte Carlo simulations with coarse-grained models enabled us to calculate the free energies of pore opening under various conditions. In agreement with oriented circular dichroism experiments, a high peptide-to-lipid ratio was found to be necessary for spontaneous pore assembly. The peptide length has a non-monotonic impact on pore formation, and the optimal length matches with the membrane thickness. Furthermore, the hydrophobicity of the peptide ends and the mutual positions of peptides on the membrane play a role.

This is very accurate and therefore no comments can be added.



## 10. Marketa

Interleukin 17 (IL-17) is known as a pro-inflammatory cytokine, which participates in immune response to infection by various pathogens in many animal species. However, there is still limited information about its role in the anti-infectious immunity of pigs.

In our study six piglets was intranasally infected with *Actinobacillus pleuropneumoniae*. The samples of lungs, tracheobronchial lymph nodes, BAL and blood were collected after one and three days post infection (2 x 3 pigs). The IL-17 level was measured both in protein (western blot) and mRNA (quantitative real-time PCR) level and compared with samples from six control pigs. Flowcytometry was used to detect cell populations responsible for IL-17 production in the lungs of infected pigs.

The expression of IL-17 mRNA was increased in the lungs of infected pigs at both times post infection. The increase was detectable also in protein level. Both gammadelta TCR and CD4+ T-cells isolated from pig lungs were able to produce IL-17 after in vitro stimulation with PMA. However, no significant differences in amount of IL-17 positive cells was detected in the in vitro model from control and infected pigs. Moreover, the percentage of CD4+ and gammadelta TCR+ cells in the lungs of control and infected pigs did not differ.

Therefore, interleukin 17 seems to be the player of the immune response to *Actinobacillus pleuropneumoniae* infection although it needs more experiments to elucidate the main sources and key mechanisms of response.

The work was supported by the Ministry of Agriculture of the Czech Republic (project QJ1210120).

## 11. Hana

### EMT-related miRNAs as diagnostic and prognostic markers in renal cell carcinoma

#### Background

Renal cell carcinoma (RCC) is one of the most common kidney cancers in the adult population. Because of the lack of early warning signals, a high percentage of patients with metastatic RCC occurs. Nowadays, epithelial-mesenchymal transition (EMT) is considered as a crucial event of the tumor progression resulting in metastasis. To this date, however, EMT, especially in renal cell carcinoma (RCC), remains enigmatic.

#### Methods

Immunofluorescence analysis of EMT status of formalin-fixed paraffin-embedded (FFPE) sections of 29 patients was performed using specific antibodies (E-cadherin, CK-18, CK-19, vimentin, S100A4). According to immunofluorescence analysis patients were divided into two groups, EMT(+) and EMT(-). To obtain global EMT related miRNA expression profiles TaqMan Low Density Arrays (TLDA) were performed on the same group of patients. For the first validation phase, 27 miRNAs were selected. Expression miRNAs profiles were measured using TaqMan Real Time Expression Assay (Applied Biosystem) and compared using Mann-Whitney U test and Kruskal-Wallis test. All data were normalized to RNU48.

#### Results

Using TLDA, 27 EMT related miRNAs (miR-200a, miR-200a\*, miR-200b, miR-200b\*, miR-200c, miR-429, miR-141, miR-192\*, miR-215, miR-30a-5p, miR-30a-3p, miR-30b, miR-30c, miR-30d\*, miR-30e, miR-30e-3p, miR-130\*, miR-630, miR-17\*, miR-193b, miR-26a-1\*, miR-571, miR-770-5p, miR-632) were chosen and tested as potential diagnostic and prognostic markers in RCC. Data will be presented.

#### Conclusions

Our primary data suggests that miRNA family, miR-200 and miR-30, may play an important role in RCC pathogenesis and metastatic cascade.

## 12. Ksenia

### CHANGE IN SHORT-TERM BLOOD PRESSURE REGULATION IN PATIENTS WITH RESISTANT HYPERTENSION BEFORE AND AFTER RENAL DENERVATION: A PILOT STUDY.

#### Introduction

One of the causes of hypertension is a high activity of the sympathetic nervous system, which is the reason of high secretion of the renin. This hormone has a lot of different functions which include influence of baroreflex sensitivity (BRS). The main aim of this study is therefore to detect some changes of BRS of patients with resistant hypertension before and after renal denervation.

#### Materials and Methods

We measured 10 patients with resistant hypertension (HP) before and after renal denervation (RDN). For each patient we measured beat-to-beat continuous non-invasive 5-minute recording of blood pressure by photoplethysmography. Records were processed using spectral analysis and in the medium frequency (MF), high frequency (HF) and very low frequency (VLF) region were calculated with the following parameters: normalized power spectrum of heart rate (nRRIMF, nRRIHF and nRRIVLF [n.u.]) and systolic and diastolic blood pressure (nSTKMF, nSTKHF, nSTKVLF, nDTKMF, nDTKHF and nDTKVLF [n.u.]), absolute power spectrum of heart rate (aRRIMF, aRRIHF and aRRIVLF [ms<sup>2</sup>/Hz]) and systolic and diastolic blood pressure (aSTKMF, aSTKHF, aSTKVLF, aDTKMF, aDTKHF and aDTKVLF [mmHg<sup>2</sup>/Hz]). The effectiveness of short-term regulation of blood pressure [ms/mmHg] was established in the medium frequency (BRS), HF (GainHF) and VLF (GainVLF) spectrum as the ratio of cross spectrum between RRI with STK (CrossMF, CrossHF or CrossVLF) and power spectrum STK.

#### Results

Significant difference between hypertonic before and after operation patients' results was found in BRSMF ( $4,84 \pm 2,57$  vs.  $6,09 \pm 2,96$ ;  $p < 0,05$ ) and in nDTKVLF ( $0,11 \pm 0,09$  vs.  $0,07 \pm 0,03$ ;  $p = 0,07$ ).

#### Conclusion

In MF range, we see that BRS was improved with significant difference. This means that RDN has influence not only on RAAS but also on short-term regulation of blood pressure.

### 13. Matous

This article presents results of research on NGOs educating Roma children in Brno city. The aim was to find out pedagogical strategies which are used in the process of non-institutional education of Roma children. The aim was to find out the broad educational goals of NGOs. An additional aim was also to reveal the effect of funding systems on work possibilities of NGOs. The research was based on nine semi-structured interviews with NGO representatives which is the total number of NGOs educating Roma children in Brno city. Results of the research revealed that NGOs are heavily dependent on state and European Union funding, through which they cannot be fully independent. The ideological background of their founders also has a strong influence on their pedagogical strategies and educational goals. The research revealed that the broad educational goal is not focused on school achievement, as it is usually understood, but it is focused on integration of Roma children into the wider society. The research also described pedagogical strategies used by NGOs. The research identified as main problems within the education of Roma children by NGOs in Brno city the ethnocentrism of NGO employees and the misunderstanding concerning educational goals of NGOs. This article thus provides a reflection of the non-governmental educational effort in Brno city.

## 14. Shahla

### Accentual types of Modern English words.

This paper deals with accent types in Modern English. It has been acknowledged that second language learners and even some native speakers have difficulties in pronouncing words with correct stress. So pronunciation words play an important role in communication. This research has been investigated by different phoneticians like Torsuyev and Gimson. However, after analyzing some pronunciation dictionaries we think that the notion of accentual structure opposes the latest experiment. We tested it by phonetic experiment. The corpus material of the experiment consists of five hundred words which are classified according to different accentual types and recorded by two speakers with original British accents. The purpose of the experiment is to check whether British English speakers will show the accentual types of words in Modern English. According to the results of the phonetic experiment we defined accentual types of words in Modern English. The variability of the accentual structure of English words presents great difficulty for English learners. They should be well acquainted with the four most widely spread accentual types of words and be aware of the modification of the words' accentual patterns influenced by rhythm and tempo in connected speech. The practical value of this text is that the results can be used in seminars on theoretical and practical phonetics.

## 15. Michaela

### Chronic Lymphocytic Leukemia Cells with Mutation in NOTCH1 Gene Respond Poorly to Ofatumumab

Several recurrent mutations represent important prognostic and/or predictive factors in chronic lymphocytic leukemia (CLL). However, the response of respective patients to immunotherapy targeting CD20 is only poorly understood. The recent report on the outcomes of CLL8 trials noted an association between the presence of NOTCH1 mutations in CLL patients and none benefit from rituximab added to fludarabine and cyclophosphamide (Stilgenbauer et al. 2014). Another study similarly showed a poor effect of anti-CD20 immunotherapy in NOTCH1-mutated CLL patients, specifically for rituximab-based induction and consolidation treatment (Bo et al. 2014). These two observations prompted us to determine whether NOTCH1-mutated CLL cells exhibit higher primary resistance to an anti-CD20 antibody compared to samples without mutation. The hot-spot mutation c.7544\_7545delCT in NOTCH1 gene was detected by Sanger sequencing of a part of exon 34. The CLL cells (samples from CLL patients monitored and/or treated at the University Hospital Brno) were cultivated in the presence of 15% active human serum and 20 µg/ml of ofatumumab. The viability in comparison with untreated control cells was assessed by a metabolic WST-1 assay. We analyzed the level of CD20 and CD55/CD59 using flow-cytometry detection. The impact of ofatumumab on cell viability was assessed in 45 CLL samples. The median viability after 24 hrs of ofatumumab treatment in the genetic groups was as following: 70% in NOTCH1-mutated samples (n = 12), 35% in ATM-mutated samples (n = 12) and 48% in wt group (n = 21). Thus, the NOTCH1-mutated samples were substantially more resistant to ofatumumab than ATM-mutated samples (P = 0,002) and wt samples (P= 0,024). In line with the response to ofatumumab, the highest CD20 level was observed in ATM-mutated samples (median 72 798 of ABC units), intermediate in wt-samples (62 633) and the lowest in NOTCH1-mutated samples (46 638) (ATM-mut vs. NOTCH1-mut P = 0,094). The distribution of the sum of CD55 and CD59 densities assessed on the same samples then emerged – also in line with the response to ofatumumab – in the opposite manner: median level of 38 914 of ABC units in ATM-mutated samples, 48 861 in wt group and 53 566 in NOTCH1-mutated samples (ATM-mut vs. NOTCH1-mut P = 0,095). Our results more specifically indicate that this poor response may be accounted also to a higher primary resistance of CLL cells to anti-CD20 MAbs. In addition, our study demonstrates the usefulness of recurrent mutation identification in studies evaluating response of CLL patients to monoclonal antibodies. It is supported by XX and YY projects.