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Abstracts

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LECTURES

L1
3S in determination of biologically active compounds and their metabolites by hyphenated separation techniques

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The implementation of scientific knowledge, as a part of applied science, should be aimed at developing practical applications for many fields of natural world (e.g. medicine, pharmacy, cosmetology, food industry etc.). Consequently, in the case of separation techniques the evaluation of separation medium comprises an essential part of analytical methods improvement. Significant efforts have been made over the last few years to achieve stationary phases imitated natural matter (e.g. biological membrane) as well as endogenous compounds (e.g. amino acids). Specificity of chemically bonded ligands in the case of new materials enables receiving a so-called dedicated stationary phases. Moreover, structural similarity of the immobilized ligands with the desired group of analytes determine the high specificity and selectivity of prepared stationary phases. Therefore, the investigations in accordance with the "3S" assumption — *similarity, selectivity, and specificity* — allow the development of a new generation of separation materials.

The chemical immobilization of amino acids and peptides — one of the most essential compounds in life science allow the preparation of materials that exhibit unique interactions with amino acids and peptides as a analytes and beyond. The amino acids- and peptides-silica stationary phases show high selectivity for other groups of biologically significant compounds, i.e. carbohydrates, nucleosides, flavonoids, optical active compounds. Furthermore, stationary phases prepared in compliance with "3S" assumption exhibit wide range of applicability in separation techniques (RP HPLC, HILIC, IC). This approach also includes the preparation of stationary phases containing in the structure specific functional groups characteristics for biological membrane. The development of this type of materials enables the modeling of the transport of substances through the biomembrane, e.g. blood/brain barrier. On the other side, stationary phases imitating membrane lipids provide unique selectivity according to lipids separation, especially phospholipids.

As a consequence, the natural biological systems provide a significant center of inspiration for development of chromatographic methods. Based on the principles that governed natural world, it is possible to obtain desired similarity, selectivity, and specificity that determine the resolution of separation methods.

L2
High-throughput NMR pipeline for metabolic phenotyping in clinical studies

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Metabolic phenotyping provides insight into biochemical pathways associated with disease pathology and thus offers great potential for diagnostic and prognostic applications. The challenge remains in translating metabolic phenotyping approaches into tangible clinical applications. The success of this translation largely depends upon the capacity of analytical platforms to produce accurate and reproducible data in a high-throughput manner. This presentation will describe an NMR-based pipeline developed and optimised at the MRC-NIHR National Phenome Centre (NPC), in Imperial College London (UK) for the analysis of large cohorts of human urine and blood plasma and serum samples [1]. The robustness and reproducibility of the pipeline has been tested through integration and combination of more than 8000 urine samples collected from 7 independent studies acquired over 4 years. I will also describe recently developed approaches for recovery of quantitative lipoprotein data from ^{1}H NMR profiles of plasma and serum samples [2] that have been assessed in a multi-laboratory, multi-spectrometer ring test trial. These results show perfect compliance with the National Cholesterol Educational Program, NCEP, criteria for lipid analysis indicating great potential for implementation of lipoprotein analysis by NMR in clinical settings. Finally, application of these approaches to heterogeneous multifactorial diseases including prediction of pneumonia in critical care ventilated patients [3] and treatment of lower urinary tract symptoms in women [4] will be presented.

References

1. Dona AC, Jiménez B, Schäfer H, Humpfer E, Spraul M, Lewis MR, Pearce JTM, Holmes E, Lindon JC and Nicholson JK. Anal Chem 2014; 86 (19): 9887–9894.
2. Budde K, Schäfer H, Boulangé CL, Kaluarachchi MR, Humpfer E, Fang F, Cannet C, Friedrich N, Pietzner M, Petersmann A, Jiménez B, Coen M, Karaman I, Pearce JTM, Holmes E, Elliot P, Lindon JC, Baumstark MW, Spraul M, Nauck M, Nicholson JK. Submitted.
3. Antcliffe D, Jiménez B, Veselkov K, Holmes E, Gordon AC. EBioMedicine 2017; 18: 244–253.
4. Bray R, Cacciatore S, Jiménez B, Cartwright R, Digesu A, Fernando R, Holmes E, Nicholson JK, Bennett PR, MacIntyre DA, Khullar V. J Proteome Res 2017 [Epub ahead of print].

L3
Protein quantitation by mass spectrometry — a tool for biomarker analysis

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The development in the end of the XX-th century of new proteomic methodology, allowing to analyze complex protein sets, brought hope for its fast application for the search for new biomarkers of a variety of diseases, including cancer. Hopes were the highest in case of protein content of human body fluids and the application of newly detected biomarkers for diagnosis, prognosis and therapy monitoring in cancer. Initial, very optimistic results of the search for new proteomics-based biomarkers in oncology, were however verified negatively quite fast, and it was pointed out that the published results were not reproducible and the reasons for that sparked a more general discussion [1]. The necessity for further improvements and more systematic basic studies preceding the application in clinics was indicated. Guidelines were formulated for plasma/serum preparative procedures as well as for proteomic procedures especially in their quantitative aspects. The most important breakthrough was the development of a new measurement method for targeted quantitative measurements of proteins, named Multiple Reaction Monitoring (MRM). It allows for the measurement of absolute values of protein concentration in the background of extremely complex protein mixtures (e.g. blood plasma/serum) using a coupled liquid chromatography — mass spectrometry (LC-MS) analysis. MRM method was named the Method of the Year 2012 by Nature Methods [2]. Moreover, in this method, a panel of 10–100 proteins, represented by hundreds of peptides, their proteolytic fragments, can be subjected to a parallel analysis in the same experiment. The foundations of the MRM method will be presented illustrated by a few applications. The applications include the assessment of the value of MRM-based cytokeratin peptide panels for diagnosis of the etiology of pleural fluid.

References

1. Check E. Proteomics and cancer: running before we can walk? Nature 2004; 429 (6991): 496–497.
2. Nature Methods 2013; 10 (1).

L4
Application of hierarchical models and Bayesian statistics in metabolomics

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The metabolomic data collected using high performance liquid chromatography coupled with mass spectrometry (LC-MS/MS or LC/TOF-MS) can provide signal intensities for a large number of compounds (peaks) present in set of samples (i.e. control and experimental). However, such large metabolomic data sets are usually difficult to interpret, due to the small sample sizes; search for effects (e.g. differences between control and experimental group) that are small; and considering a large number of hypotheses without paying enough attention to the problem of multiple comparisons. It might lead and often leads to conclusions (e.g. biomarker identification) that are exaggerated or have a wrong sign! The hierarchical modeling and Bayesian inference method can largely eliminate such problems and consequently limit the number of false positive results. Such a statistical models can be build using the existing theories, such as pharmacokinetics, and prior information available for the particular problem. The inferences are based on the Markov Chain Monte Carlo methods (JAGS/STAN) and can be run using many popular computing environments like R/Matlab. The lecture will discuss the analysis of metabolic data obtained from urine samples collected in two groups of rats (control and tumor-induced) at four time points.

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L5
Panel of serum lipids and metabolites in early detection of lung cancer

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Low-dose computed tomography (LD-CT) screening in a high-risk group is a potential strategy for early detection of lung cancer. Pre-selection of candidates for LD-CT by using the blood-based biomarkers would lower the overall costs and benefit the patients by increasing the specificity of diagnosis. In the following study, we searched for a molecular signature based on a serum lipidome and metabolome profile distinguishing individuals with early lung cancer from healthy participants of the lung cancer screening program.

Blood samples were collected during an LD-CT screening program performed in the Pomerania district by the Medical University of Gdańsk. The study involved 100 patients with early-stage lung cancer (including 31 screen-detected cases) and the matched group of 300 healthy participants of the screening program. MALDI-ToF mass spectrometry was used to analyze the molecular profile of lipid-containing fraction of serum samples in the 320–1000 Da range. The GC/MS approach was used to identify and quantify small metabolites present in serum.
Several components of the serum lipidome were detected, with abundances discriminating patients with early lung cancer from high-risk smokers. An effective cancer classifier was built with negative predictive value 98%, positive predictive value 42% and an area under the curve of 88%. The downregulation of a few lysophosphatidylcholines (LPC18:2 and LPC18:1) in samples from cancer patients was confirmed using a complementary LC-MS approach. Moreover, several metabolites were detected in the sera which abundances discriminated patients with lung cancer (31 screen-detected cases) from matched controls (92 healthy individuals). Most of differentiating components were downregulated in cancer samples, including amino acids, carboxylic acids, and tocopherols, whereas benzaldehyde was the only compound significantly upregulated. Serum signatures based on metabolome or lipidome showed potential usefulness in discriminating early lung cancer patients from healthy individuals. Although these signatures were not validated in an independent dataset, they deserve further investigation in a larger cohort study.
Acknowledgments: This work was supported by the National Science Centre, Grant 2013/11/N/NZ7/00770 and NCBiR grant PBS3/A7/29/2015 (MoltestBIS). The computation analyses were carried out using the GeCONil infrastructure (NCBiR grant POIG.02.03.01-24-099).

L6
Analysis of metabolomic data obtained from designed experiments

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A general metabolomic approach to identify possible metabolites that are important for the distinction between disease entities is often based on supervised chemometric methods like linear discriminant analysis, LDA, discriminant partial least squares regression, PLS-DA, and orthogonal partial least squares regression, OPLS-DA. The main assumption in these studies is that a combination of metabolites is rather responsible for the distinction between groups than a single metabolite. In order to reduce the number of uninformative variables (metabolites), often the PLS-DA procedure is combined with the variable importance in projection score, VIP-score [1, 2] or the selectivity ratio, SR [3, 4]. However, the experiments can be designed to reveal the dynamic changes in the chemical composition of collected samples over time or to reflect the influence of drugs dosage. The analysis of the resulting highly structured data should then allow for all sources of biological variation to be considered. For this purpose, several approaches like analysis of variance-simultaneous component analysis, ASCA [5], regularised multivariate analysis of variance, rMANOVA [6], non-parametric MANOVA [7] and their extensions [8, 9] have been proposed in the literature.
In the present work, advantages and disadvantages of the methods for analysis of designed data and their extensions are discussed and illustrated with examples.

References

1. Andersen CM, Bro R. J Chemometr 2010; 24: 728–737.
2. Galindo-Prieto B, Eriksson L, Trygg J. J Chemometr 2014; 28: 623–632.
3. Kvalheim OM, Karstang TV. Chemom Intell Lab Syst 1989; 7: 39–51.
4. Za¸bek A, Stanimirova I, Deja S, Barg W, Kowal A, Korzeniewska A, Orczyk-Pawiłowicz M, Baranowski D, Gdaniec Z, Jankowska R, Młynar P. Metabolomics 2015; 11: 1563–1574.
5. Jansen JJ, Hoefsloot HCJ, Van der Geert J, Timmerman ME, Westerhuis JA, Smilde JK. J Chemometr 2005; 19: 469–481.
6. Engel J, Blanchet L, Bloemen B, van den Heuvel LP, Engelke UHF, Wevers RA, Buydens LMC. Analyt Chim Acta 205; 899: 1–12.
7. Anderson MJ. Austral Ecology 2001; 26: 32–46.
8. Stanimirova I, Michalik K, Drzazga Z, Trzeciak H, Wentzell PD, Walczak B. Anal Chim Acta 2011; 689: 1–7.
9. Marini F, de Beer D, Joubert E, Walczak B. J Chromatogr 2015; A 140: 94–102.

L7
Construction and validation of classification/discrimination models based on metabolomic data

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Classification/discrimination models have found many attractive applications in different fields of science, including diagnostic based on metabolomic profiles. Both type of models differ with respect to assignment rules, but using physico-chemical description of a sample they aim to recognize group label of a given sample.
The construction of logic classification/discrimination rules, enabling correct recognition of group labels, requires considering a few key aspects. The first one is concerned with the identification of a representative samples (a model set) used to construct a model. It should include samples that represent all present and expected sources of variability. The second aspect is related to the selection of the optimal number of components or logic rules. To simplify model and facilitate its further interpretation, elimination of irrelevant variables is carried out. Regardless

type of a model, step of model validation is crucial. Its predictive abilities are scored by different figures of merit, e.g. sensitivity, specificity, correct classification rate, etc. When it is possible, they are obtained using independent samples. If the number of available samples is limited, prediction abilities of a model are usually evaluated using a cross validation procedure [1].
In our study, we use the Monte Carlo framework [2]. In particular, it allows for obtaining distributions of figures of merit in a function of model complexity. Such a possibility helps in the selection of model complexity as well as it offers advanced approach to scoring importance of variables using their selection frequency, obtaining validation parameters and their uncertainty estimates.

References

1. Esbensen KH, Geladi P. Principles of proper validation: use and abuse of re-sampling for validation. Journal of Chemometrics 2010; 24: 168–187.
2. Krakowska B, Custers D, Deconinck E, Daszykowski M. The Monte Carlo validation framework for the discriminant partial least squares model extended with variable selection methods applied to authenticity studies of Viagra® based on chromatographic impurity profiles. The Analyst 2016; 141: 1060–1070.

L8
Metabolic flux analysis with utilization of stable isotopes — beyond static metabolite concentrations

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After a rapid rise in our understanding of transcriptional regulation of metabolic enzymes, the early 2000s saw a renaissance in metabolic research and an explosion in technology including the rise of metabolomics. The intense focus on measuring small molecules originated from the expectation that metabolites reflect the metabolic genome and respond rapidly to perturbations in physiology; therefore the metabolome should reflect the actual phenotype of a biological system. While vast numbers of metabolites can be detected, it is important to recognize that the interpretation of relative changes in metabolite levels has some limitations. A well appreciated challenge is the translation of static metabolite concentrations into functional information about the activity of metabolic pathways or individual enzymatic rates, particularly for metabolites that intersect several different pathways such as occurs in and around the tricarboxylic acid (TCA) cycle. The solution is to label substrates with stable isotopes (e.g. ^{13}C and ^{2}H) so that intracellular metabolic fluxes can be inferred from metabolite labeling patterns. As with metabolomics, NMR and MS are the main analytical platforms that are used to measure metabolite isotope enrichments. This data can then be used in the mathematical modeling of fluxes. Tracking the activity of specific metabolic pathways requires the thoughtful choice of substrate and tracer arrangement in order to estimate fluxes with high degree of confidence. Here, we discuss methods for examination of TCA cycle and oxidative fluxes that requires the combination of ^{2}H and ^{13}C isotopic labeling.

L9
A study of residual zearalenone and its metabolites in cancerous tissue

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Xenoestrogens are hormonally active substances that are common in the environment and have negative influence on living organisms. This group of compounds includes zearalenone (ZEA). This toxin is most frequently introduced into the organism as food contaminant. Shortly after ingesting it is metabolized in the alimentary track to its much more toxic metabolite, α-zearalenol (α-ZOL), and their derivative compounds, i.e. α-zearalanol, β-zearalanol and β-zearalenol. These substances contribute to endocrine disorders because the structures of ZEA and its metabolites are similar to natural estrogens, and as a consequence they have high affinity to estrogen receptors. As these compounds are present at low levels, their analysis in biological matrices requires finding selective methods of isolation and enrichment.
Our study aimed at quantitative determination of zearalenone and its main metabolite α-ZOL in human cancerous tissue. Presence of these compounds may be one of the main factors causing endometrial cancer. A necessary research stage was the development of suitable procedures for preparing such biological samples as tissue as well as selection of appropriate conditions of elution and detection in the cases when high performance liquid chromatography was used for final quantitative determination of xenoestrogens from the selected group of compounds. To isolate these substances from a biological matrix, a new technique using the QuEChERS method was proposed. which facilitated preparation of a large number of samples in a short time. Both high performance liquid chromatography with fluorescence detection and ultra-performance liquid chromatography with mass spectrometry were used for quantitative determination of zearalenone and α-zearalenol.
Acknowledgements: Our study was financed by the National Science Centre, project MAESTRO No. 2014/14/A/ST4/00641.

L10
Influence of insulin resistance on induction of metabolic disorders

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Insulin is an anabolic peptide hormone with a systemic effect. The most important stimulus for its production is the postprandial glucose uptake in the blood. Influencing the effector cells (myocytes, adipocytes, hepatocytes) increases the glucose transport to the inside of the body, reducing its levels in the blood. In addition, it controls a number of mechanisms, mainly hormonal. Our ancient genes are not prepared for nutritional changes that have occurred in the last hundreds of years, and therefore the conditions of insulin resistance and hyperinsulinemia result in non-diabetic hormone abnormalities. Hyperinsulinemia is associated with many hormonal disturbances which destructively affect homeostasis of the body. The consequence of this condition is increased lipogenesis with blockade of lipolysis, resulting in excessive accumulation of visceral adipose tissue acting pro-inflammatory. Increased cortisol levels, the same adrenergic activity and the maintenance of hyperinsulinemia by incereasing of blood sugar. Lipid disorders, hyperlipidemia, are consequence of excessive carbohydrate intake due to recurrent hypoglycaemia caused by excess insulin. Insulin interferes with the daily rhythm by blocking the melatonin, thus insufficient somatostatin discharge impairs the body's regenerative capacity. It has a bearing on the chronic feeling of fatigue. DHEA secretion also causes blockade of sex hormones (estrogens, progesterone) and fertility disorders. Hyperinsulinemia is associated with thromboembolic complications. Given the multidirectional and comprehensive nature of insulin not only for carbohydrate, it is important to keep its normal values, to regulate the whole body's hormones.

L11
Modelling high-throughput proteomics into predictive metabolomics — a novel tool for studies of *Candida spp.* biofilm infections

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Candida species are among the most common causes of fungal infection worldwide. Four species, *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis*, account for nearly 95% of infections, which are extremely difficult to treat and often deadly. *Candida* spp. form adherent communities, so called biofilms, which tolerate extremely high concentrations of antifungals in large part due to a self-produced extracellular matrix. Both the composition and function of the matrix is determined by an intricate blend of heterogeneous biologics, which behaves like an amalgam ensuing emergent properties of the biofilm. Though deciphering the functional composition of the matrix is a challenging prospect, it also presents an opportunity for identifying conserved metabolic modules in the *Candida* genus that may be useful for development of generic pan-*Candida* anti-biofilm medical therapies.

Here, we present the application of fuzzy clustering method for the identification of conserved and unique metabolic nodes. Our novel approach integrates high-throughput proteomics and KEGG-based protein functional hierarchies along with pathway mapping. The object classification was performed on the proteomes of the isolated biofilm extracellular matrices of four *Candida* spp. The fuzzy clustering method generated a membership data matrix which represented the degree of association the *Candida* proteome components had with each cluster. Further pathway mapping of select computed protein clusters led to the identification and discrimination of conserved and unique traits responsible for amino acid, carbohydrate, lipid and RNA metabolism. This quick integrative data modelling approach offers clever data mining, which defines potential drug targets for broad-spectrum pan-*Candida* biofilm therapies.

L12
Application of metabolomics approach to study various aspects of non-small cell lung cancer

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The major histological subtypes of non–small cell lung cancer (NSCLC) include adenocarcinoma (ADC), squamous cell carcinoma (SCC) and large-cell carcinoma. Novel molecular therapies can be successfully administered to patients with advanced ADC. No specific targeted therapy is currently available for SCC patients. Consequently, a correct histologic diagnosis is important and novel therapeutic options are awaited for SCC patients. Metabolomics has potential in biomarkers discovery and may indicate novel therapeutic targets. In the present study, plasma (n = 63)

and tissue (n = 25) samples obtained from NSCLC patients were fingerprinted with LC-MS. Additionally, plasma metabolites were correlated with standardized uptake values (SUV) of 2-deoxy-2-[18F]fluoro-D-glucose by the tumor obtained from PET/MRI scan. NSCLC subtypes were well separated on PLS-DA models obtained from plasma (R2 = 0.652, Q2 = 0.408) and tissue (R2 = 0.99, Q2 = 0.7) data. Among others; sphinganine (p = 0.009), anandamide (p = 0.009), malonyl carnitine (p = 0.001), and Lyso PE 20:5 (p = 0.02) in plasma, while sphingosine (p = 0.03), four acylcarnitines (p-value 0.03–0.001), Lyso PEs 18:1 (p = 0.04) and 16:0 (p = 0.03) as well as Lyso PC 16:1 (p = 0.01) in tissue; discriminated NSCLC subtypes. Several phospholipids were found correlated with SUV. Metabolic fingerprinting allowed for selection a panel of metabolites discriminating NSCLC subtypes. Radiomics analyses have potential to indicate metabolites related to tumor activity. Although promising obtained results require further validation on larger cohort of patients.

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ORAL PRESENTATIONS

OP 1
Bioinformatic studies on infection-triggered changes in secondary metabolism of *Brachypodium distachyon*

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Purple false brome (*Brachypodium distachyon*) is a model plant for functional genomics of Poaceae as well as for studying their interaction with fungal pathogens. Nevertheless, secondary metabolism, which is a key element in the interactions of plants with environment, had been poorly known in this species. This fact prompted us to study secondary metabolite profiles in three tissues of *B. distachyon*: leaves, roots and spikes by LC-MS. Among 81 identified phytochemicals mainly poliamines, flavonoids and hydroxycinnamic acids derivatives were observed. In leaves and roots caffeoylthreonate was metabolite with the highest content whereas apigenin derivatives were the most abundant in spikes.

Two programs for pre-processing and statistical analysis of LC-MS data: MZmine and MarVis were implemented to study changes in metabolomics profiles in *B. distachyon* leaves upon inoculation with *Parastaganospora nodorum*. Raw LC-MS data in mzXML format were pre-processed by MZMine for ion detection and chromatograms building. Created chromatograms were deconvoluted into individual peaks and then isotopes and adducts were removed. Finally, alignment of all raw data was performed using Join Aligner algorithm. In addition, automated search of KEGG database allowed for preliminary identification of metabolites. Exported data table in CSV format was subjected to further statistical analysis by MarVis software. Performed PCA analysis revealed significant differences in secondary metabolite profiles between control plants and infected plants. The highest induction of most metabolites was observed at 72 hours after pathogen inoculation. Among them hydroxycinnamic acid amides (for examples *N*-*p*-coumaroylputrescine and *N*-feruloylputrescine) were significantly induced together with serotonin derivatives, which suggested their role in *B. distachyon* defense response.

OP 2
Hyphenated separation techniques in the study of in vitro and in vivo drug metabolism for targeted pharmacological therapy

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Metabolomics, the end point in the "omics" cascade, has developed rapidly because metabolites may be able to reflect physiological functions and pathological characteristics. Separation techniques coupled to mass spectrometry ($LC\text{-}MS^n$) will play a key role to obtain the metabolic profiling. The aim of this study was to develop and validate a new analytical method for the analysis of selected biologically active compounds. Combination of electrochemistry (EC) and mass spectrometry (MS) was applied for the *in vitro* determination of the studied drugs and their electrochemical products. This was one of the first applications of the EC system for generation of electrochemical products produced from selected drugs. The electrochemical results were compared to *in vivo* experiments by analyzing urine samples from patients after drugs have been administered. Overall, the comparison of electrochemistry to *in vivo* experiments shows the high potential of EC-MS as a fast analytical tool in the prediction of electrochemical conversion that could be applied to therapeutic drug monitoring and pharmacokinetic studies as well. The applied *in vitro* and *in vivo* studies bring important decisional elements for the selections of the best candidates entering clinical development and represent valuable tools to optimize future clinical studies. Thanks to it, the identification of compounds in biological samples for the purpose of a clinical diagnosing of diseases has become possible. In a pharmaceutical analysis it is an important trend to determine metabolic profiles after the administration of a drug in order to trace its lot in the organism.

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OP 3
Analysis of antisense oligonucleotides and their metabolites with the use of hyphenated separation techniques

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Phosphorothioate oligonucleotides can be used in the treatment of many diseases such as cancer, hepatitis B or C, cardiovascular disease, Alzheimer's disease. These compounds are in various phases of clinical trials and some of them are already used in the antisense therapy. For this reason there is a need for fast, cheap and effective methods of their qualitative and quantitative analysis. Liquid chromatography coupled with mass spectrometry is a relatively new analytical tool in the study of this group of compounds in various matrices.

Ion pair chromatography is the most commonly used separation technique in the analysis of modified oligonucleotides. However, hydrophilic interaction chromatography may be a good alternative. Therefore, both methods were used in the analysis of the studied group of compounds and their metabolites. The obtained results were compared in terms of separation and selectivity with particular attention paid to the composition of the mobile phase and the structure of stationary phase. Another goal of the research was to select the detection method of antisense oligonucleotides. Mass spectrometry with electrospray ionization and inductively coupled plasma were used for this purpose and compared. Comprehensive research allowed for the development of a method for the separation, qualitative and quantitative analysis of oligonucleotides and their metabolites. It was used for their determination in total RNA extracts from myotonic dystrophy cell lines.

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OP 4
Paraffin embedded tissue — new matrix for metabolomics approach

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In recent years, more attention is being paid to Prostate cancer (CaP). This type of cancer is one of the most commonly diagnosed disease among men worldwide. CaP diaganosis is challenging due to its asymptomatic development and high heterogeneity. Additionally, there is still a unsufficient knowledge in the field of CaP development. Recent reports suggest that more information may be provided by metabolomic studies, where the recognition of the organism' state is based on unique metabolic profile. As a consequence of disease development, changes in metabolic profile can be determined, which corresponds to changes occurring at the molecular level. Nowadays, paraffin-embedded tissues are proposed as a new, interesting and valuable clinical material. The main advantage of that material is its stability. On the other hand sample preparation connected with deparaffinization step is quite challenging.

The main goal of the study was to develop rapid and repeatable method of sample preparation which enables for direct extraction of metabolites from paraffin embedded tissue.

Developed method was optimized under the following parameters: type of deparaffinization procedure, type and volume of the extraction solvent, number of the extraction cycles and tissue thickness. The profile of the tissue metabolome was determined with the use of gas chromatography coupled with mass spectrometry (GC-MS/MS-TQ 8030 system, Shimadzu, Japan).

The results show that paraffin embedded specimens are an attractive matrix for metabolomic approach. In addition, citric acid and fatty acids have been found to be the metabolites mostly affected in prostate cancer development.

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OP 5
Usage of chromatographic techniques for the analysis of conjugated fatty acids in tissues of rats in cancerous process

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'Conjugated fatty acids' (CFA) is a term referring to positional and geometric isomers of polyunsaturated fatty acids with conjugated double bonds in their carbon chain. Conjugated linoleic acids (CLA) and conjugated linolenic acids (CLnA) for many years have attracted considerable attention due to numerous beneficial health effects. The purpose of this experiment was to assess the influence of pomegranate seed oil (a rich

source of CLnA) incorporation into the diet of rats on breast cancer risk and profile of CFA in their hepatic tissue. Female Sprague-Dawley rats were divided into four groups: two groups were fed for 21 weeks the basal diet (CON and CONplus) whereas diet of two other groups (PSO and PSOplus) was enriched with pomegranate seed oil. Moreover, two groups: CONplus and PSOplus received at 50th day of life 7,12-dimethtlbenz[a] anthracene in the amount 80 mg/kg of body weight for breast cancer induction. Contents of methylated fatty acids (FAME) were quantified using capillary gas chromatography with mass spectrometry (GC-MS) while concentration of CFA was determined by silver-ion liquid chromatography (Ag^+-HPLC)) with photodiode array detector (DAD) on four analytical ion-exchange columns loaded with silver ions (Chrompack ChromSpher). Applied diet modification did not reduce the breast cancer risk however significantly affected the CFA profile in liver. Inclusion of pomegranate seed oil significantly increased the content of conjugated dienes (CD), especially of *cis*,*trans* conformation, which was a result of endogenous transformation of CLnA isomers into *cis*-9, *trans*-11 CLA. However, co-existing cancerous process negatively influenced the content of CD.
This work is a result of Medical University of Warsaw and the Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences scientific collaboration (27.02.2017). It was partially supported by the Medical University of Warsaw (WUM) under Grant No. FW12/PM1/17, and by the statutory funds from the Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland.

OP 6
Profiling of vitamin D metabolites

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Metabolism of vitamin D is a relatively well-known process. Depending on the method of administration, the vitamin is transported through the vitamin D binding protein (dermal synthesis) or it is incorporated into chylomicrons (diet), respectively. The first phase of metabolism occurs in the liver through 25-hydroxylation (CYP2R1). The resulting 25(OH)D_3 metabolite, with a relatively long half-life, is considered to be the primary parameter that determines the body's supply of vitamin D. The second stage of metabolism, with the formation of the active metabolite of vitamin D, occurs in the kidneys and target tissues via 1-hydroxylation. Calcitriol (1,25$(OH)_2D_3$) is a local metabolite and its serum concentration is not critical in diagnostics. 1,25$(OH)_2D_3$ is removed from the body via 24-hydroxylase (CYP24A1) leading to both, 1,24,25$(OH)_3D_3$ and 24,25$(OH)_2D_3$ catabolites. Apart from those mentioned, vitamin D may be subjected to a number of changes *in. al*. involving C3-epimerase or 20-hydroxylase (CYP11A1), which is still not fully understood. Experimental data indicate the presence of a number of other metabolites, especially double hydroxylated ones, in people who are taking supplements. The results of this study will focus on the extensive qualitative and quantitative analysis of vitamin D metabolites. What is more, the effect of supplementation with high doses of vitamin D on the metabolites profile over a long period of time, will be shown.

OP 7
Persistent metabolomic disturbances after an episode of hypoglycemia in children with type 1 diabetes

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Introduction: Diagnosis of hypoglycemia — low blood glucose (< 70 mg/dL) in real time is relatively easy. However, normalization of currently available parameters does not mean that metabolic pathways altered by a hypoglycemia episode revert to normal. The aim of this study was to gain broad insight into metabolic disturbances which were caused by hypoglycemia and, what is even more important, persist long after the resolution of symptoms of hypoglycemia and normalization of routinely used parameters.

Materials and methods: Three groups were recruited: study group — patients after episode of hypoglycemia (HG, n = 10) and two control groups — children with established, well-controlled diabetes (EDM, n = 25) and children with new onset diabetes without ketoacidosis group (NDM, n = 15) — representing a purely hyperglycemic state. Serum samples were collected at three time points: 0–12 h–48 h since the hospital admission for the HG group, 0–24 h–72 h for the NDM group and once in the EDM patients— during a routine hospital visit for diabetes management and education. Metabolomic fingerpriting was performed with LC-QTOF-MS (Agilent 6550 iFunnel).

Results: Metabolomic features that met the following criteria entered statistical analysis: adequate reproducibility (RSD (relative standard deviation) < 0.2) in QC samples (quality control), low detection frequency in blank samples (no more than 1/3) and detection in at least 80% of samples from each group. After filtering 402 (positive ionization) and 413 m/z (negative ionization) values were suitable for between-group comparisons, respectively to the ionization mode. Among those features we identified 14 features that were significantly higher in HG vs EDM and 16 lower in at least two out of three analyzed time points. Based on AUROC (area under the receiver operating characteristic) values feature 670.543 [RT (retention time) = 8.35] was the best biomarker of hypoglycemia [AUROC = 0.741 (95% CI 0.61–0.87) for HG vs EDM

and AUROC = 0.79 (95% CI 0.69–0.89) for HG vs NDM comparison]. We built a logistic regression model that discriminated between HG and EDM samples using three features [505.3171 (RT = 5.38), 741.5698 (RT = 9.95) and 670.543 (RT = 8.35)] with an AUROC = 0.85 (95% CI 0.74–0.96) and AUROC = 0.75 (95% CI 0.64–0.86).

Conclusions: Metabolic disturbances caused by hypoglycemia episode may be detected in the serum at least up to 48 hours after the occurrence of the episode.

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OP 8
The MALDI ionization as a tools of modern bioanalytics

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Modern microbiological diagnostics in hospitals, medical centers is based on time-consuming methods of culture. There are microbiological diagnostic laboratories that use expensive and labor-intensive molecular biology techniques to identify pathogenic microorganisms. An alternative to time-consuming and costly methods of identifying microorganisms can be the cheap and rapid electromigration analysis of microorganisms. From the point of view of the systemic infections diagnosis caused by bacterial pathogens and the identification of bacteria isolated from various biological matrices, it may be useful to use ICM (Intact-Cell Matrix-Assisted Laser Desorption Ionization with Time of Flight Mass Spectrometry (ICM, IC-MALDI TOF MS) approach. The MALDI TOF MS technique, in combination with capillary electrophoresis, is mainly recommended for biochemical and medical-clinical studies, but lack of sufficiently precise databases limits its technological capabilities and advantages. The potential of MALDI-TOF/TOF MS makes it possible to extend applications to other areas of microbiological analysis, pharmacology, nutrition technology and environmental analysis. The speed, precision and variety of information you can obtain is an undoubted advantage of this method. Therefore, the sooner it is possible to identify pathogens, the earlier preventive and preventive measures can be taken. In the case of medical analytics, this is related to the use of a suitable antibiotic. The biggest disadvantage of spectrometric analysis of microorganisms is the limited availability of databases at high cost. In addition, there is a possibility of incompatibility of the spectra of microorganisms contained in the data library with the spectra of the bacteria being investigated.

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OP 9
1H NMR- and 1H MAS NMR-based metabolomics in head and neck squamous cell carcinoma and in thyroid cancer

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1H NMR spectroscopy is a powerful tool for studying blood serum metabolites. Cancer disease as well as effects of an anticancer treatment are mirrored in morphological, functional and molecular disturbances. Our goal was to investigate these molecular disturbances in blood serum of head and neck squamous cell carcinoma (HNSCC) and in tissue samples of thyroid cancer (TC) patients. 1H NMR spectra were acquired on a Bruker 400.13 MHz spectrometer at 310 K (blood serum) as well as 277 K and rotation frequency 4000 Hz (TC tissue).

In our works we identified metabolic signature of high acute radiation sequelae (ARS) in HNSCC patients treated with chemoradiotherapy (CHRT) [1]. The preliminary results from the real-time (during the treatment) analysis of response to CHRT in HNSCC patients revealed that the increased 3-hydroxybutyrate (3HB) correlates with the episodes of severe functional and morphological ARS (strong pain and severe dysphagia, fluid diet only or refusal to eat/drink at all) and unplanned treatment brake. Furthermore we were able to distinguish the HNSCC patients with treatment failure or disease recurrence confirmed within 6 months after the treatment completion (ETF — early treatment failure). Statistical and visual analysis showed that in case of ETF the normalized integral intensities of the lipid signals around 0.9, 1.3 and at 3.24 ppm before and after treatment do not differ statistically, while in the patients with the therapy success the lipid signals are significantly lower after the treatment when compared to the pretreatment values. In TC patients we identified a metabolic profile of papillary cancer which is characterized by higher taurine, glycine, glutamate, serine, succinate, choline, lactate ascorbate and lower myo-inositol, glucose, scyllo-inositol and citrate than healthy tissue.

NMR-based metabolomics has a great potential in clinical diagnosis and prognosis. Both, liquid NMR in serum studies and MAS NMR in tissue studies provide diagnostic/prognostic information for clinicians to facilitate the personalized treatment.

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Reference

1. Boguszewicz Ł, Hajduk A, Mrochem-Kwarciak J. et al. Metabolomics 2016; 12: 102. doi:10.1007/s11306-016-1043-4.

OP 10
HPLC/MS as a tool for a functional analysis of membrane transporters in *Medicago truncatula*

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Metabolomics comprise a wide spectra of analytical techniques. Among them HPLC/MS (High Performance Liquid Chromatography/Mass Spectrometry) has been found as a tool helpful in functional characterization of plant membrane transporters. We have used HPLC/MS in the functional studies of ABC (ATP-binding cassette) proteins from the so called G subfamily in model legume plant *Medicago truncatula*. Members of the G subfamily have been showed to be engaged in numerous physiological processes including biotic and abiotic stresses response. MtABCG10 has been identified as a plasma membrane protein involved in modulation of medicarpin biosynthesis. In Medicago pterocarpan medicarpin is a main phytoalexin. HPLC/MS analyses revealed that lack of MtABCG10 results in lower accumulation level of medicarpin and its precursors, leading to an increased susceptibility of Medicago to fungal infections. Further investigation showed that metabolomic phenotype observed in *MtABCG10*-silenced roots can be averted by exogenous application of early medicarpin precursors namely: 4-coumarate and formononetin. Transport experiments conducted in BY2 cells overexpressing *MtABCG10* by using HPCL/MS revealed that MtABCG10 is responsible for the membrane translocation of 4-coumarate and liquiritigenin. The proper distribution of such a precursors and identification of transporters participating in it appears as an important step in understanding of phenylpropanoid biosynthesis in legumes. Based on obtained results, it could be proposed that the MtABCG10 participates in regulation of medicarpin biosynthesis upon biotic stress by directing of precursors from the general phenylpropanoid and flavonoid pathways to the isoflavonoid phytoalexin route.

OP 11
Metabolic effects of bariatric surgery

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Background and aims: Bariatric surgery (BS) has been proven as the most effective treatment of morbid obesity and purposeful therapy for associated co-morbidities. However, mechanisms responsible for metabolic gains including type 2 diabetes (T2DM) remission are not entirely clear. The aim of the study was to evaluate changes in metabolism after laparoscopic Roux-en-Y gastric bypass (LRYGB) and laparoscopic sleeve gastrectomy (LSG), including factors responsible for quicker T2DM remission.

Material and methods: Two complementary techniques (GC-MS and LC-MS) were used to perform metabolic fingerprinting of serum obese diabetic patients who underwent LSG or LRYGB. The Mann-Whitney test, a t-test, the Wilcoxon signed rank, the paired ttest and a FDR procedure were used during statistical analysis.

Results: Performed study has allowed detection of metabolites linked with numerous pathways, processes and diseases. BS induced changes in amino acids, acylcarnitines, fatty acids, phospholipids, sphingolipids and others. Interesting differences in metabolites related to modulation of gut microbiota were observed. Additionally, observed significant increase levels of carnitines can suggest the importance of fatty acid β-oxidation in T2DM remission process.

Conclusions: Critical evaluation of clinical data and obtained metabolomics results enable us to conclude that LSG and RYGB are comparable in terms of the general clinical outcome, but they strongly differ from each other in molecular mechanisms leading to the final effect. Based on detected metabolites and affected pathways we propose a "gear mechanism" showing molecular changes evoked by LSG and LRYGB. Obtained results suggest that patients with greater availability of substrates for β-oxidation before surgery are in favor of rapid T2DM remission after operation.

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References

1. Nalepa P, Piechnik A, Kiersztan A. Influence of bariatric surgery on remission of type 2 diabetes. Postepy Hig Med Dosw (online) 2011; 65: 804–818.
2. Neff KJ, Olbers T, le Roux CW. Bariatric surgery: the challenges with candidate selection, individualizing treatment and clinical outcomes. BMC Med 2013; 11: 8.

ELECTRONIC POSTERS

eP 1
Diversification of secondary metabolites in immune responses of model *Brassicaceae* species

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Pathogen recognition in plants leads to the induction of immune responses, including biosynthesis of antibiotic metabolites. This defense mechanism is common in plant kingdom, but biosynthesized phytochemicals are highly diverse and restricted to small phylogenetic clades. Model plant *Arabidopsis thaliana* biosynthesizes tryptophan derived metabolites that are crucial for immune responses of this species against number of pathogens. In a previous study, we investigated conservation of the pathogen-triggered Trp-metabolism between *A. thaliana* and its relatives (Bednarek et al., 2011). Here, we continued experiments with four *Brassicaceae* species including *Capsella rubella, Cardamine hirsuta, Thellungiella halophila* and *Arabis alpina*. LC/UV/MS analysis of samples prepared from leaves inoculated with the fungal pathogen *Plectosphaerella cucumerina* revealed number of induced metabolites, which mostly were species specific. Some of these compounds have molecular masses, which do not correspond with any so far identified *Brassicaceae* metabolites. We identified preliminary some of those compounds by LC/MS/MS analysis. As plant capacity to synthesize particular metabolites is dependent on the occurrence of corresponding genes involved in the respective biosynthesis pathways, we performed phylogenetic analysis to find orthologues and homologues of *A. thaliana* genes linked with Trp-metabolism and phenylpropanoid pathway in the tested *Brassicaceae* species. Subsequently, we performed RT-qPCR analysis to check if expression of selected genes is changing upon pathogen inoculation. This analysis showed that all tested *Brassicaceae* species are characterized by induction of genes linked with Trp-metabolism, but not of those involved in phenylpropanoid pathway.

Reference

1. Bednarek P, Piślewska-Bednarek M, Ver Loren van Themaat E et al. Conservation and clade specific diversification of pathogen-inducible tryptophan and indole glucosinolate metabolism in Arabidopsis thaliana relatives. New Phytol 2011; 192(3): 713–726.

eP 2
Molecular mechanisms of differentiation within glucosinolate biosynthetic pathway in the *Brassicaceae* family

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Glucosinolates are plant thio-glucosides commonly occurring in the *Brassicaceae* family. The loss of cellular integrity of the plant tissue as a result of wounding by insect or pathogen attack activates the binary glucosinolate-myrosinase (thio-glucosidase) system and leads to production of unstable intermediate, which rearranges spontaneously into several degradation products. The chemical nature and potential roles of these products depend on the structure and length of the glucosinolate side chain. Indolic glucosinolates (IG) deter sucking insects, while aliphatic glucosinolates (AG) protect plants from siphoning insects. However, published results suggest that some species belonging to this family can be devoid of at least one of these two glucosinolate types, what seems surprising considering their important functions. In this study, we focused on glucosinolate biosynthesis in selected species within the Camelineae tribe, particularly in close relatives of *Capsella rubella* including *Arabidopsis thaliana*, *Camelina sativa*, *Neslia paniculata*, *Capsella bursa-pastoris* and *Capsella grandiflor*a. We analysed metabolites extracts by UPLC coupled with double quadrupole mass spectrometer. Our analysis revealed that *A. thaliana* produces IG and short-chain AG in every investigated organ. In contrast, we did not detect indolic glucosinolates in *C. rubella* and these compounds were relatively low abundant in other species. Furthermore, long-chain AG accumulate to high levels in young seedlings, roots and siliques, but are low abundant in adult leaves of these species. We applied RT-qPCR to monitor expression of selected genes encoding CYP83 enzymes and MYB transcription factors linked with glucosinolate biosynthetic pathway. We also analysed genomic maps of chromosome regions containing *MAM* genes, which determine length of AG side chains.

eP 3
Lipidomics reveals molecular mechanisms leading to progression of cardiovascular disease related to chronic kidney disease

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Chronic kidney disease (CKD) is defined as progressive loss in kidney function over time. The diagnosis is based on glomerular filtration rate (GFR) which measures the standard kidney function.

Patients suffering from CKD since early stages are at strong risk of cardiovascular disease (CVD). It is known that major cause of death for all people with CKD are cardiac events nowadays. It was suggested at proteomic level that molecular mechanism of CVD related to CKD shows some kind of differences in reference to classical atherosclerosis. Nevertheless, disrupted metabolic pathways differentiating classical atherosclerosis between CVD related to CKD are not well recognized. In order to specify and to acquire knowledge about the dissimilarities in atherosclerosis development - lipid profiling of blood plasma samples was carried out.

The blood samples were taken from 24 healthy volunteers and 64 patients assigned in three groups:

1) CKD1-2 — patients at early stages of CKD and first symptoms of CVD;
2) CKD5 — patients at end-stage of CKD treated with renal replacement therapy with severe CVD symptoms;
3) CVD — patients suffering from advanced classical atherosclerosis and with normal renal function.

Extraction of lipids was performed according to protocol using MTBE extraction procedure proposed and optimized by Matyash and Shevchenko (2007) [1]. Q-Exactive Orbitrap (Thermo Fisher Scientific) coupled to TriVersa Nanomate (Advion) and UltrafleXtreme Maldi TOF/TOF (Bruker) were used as basic tools for non-targeted lipid profiling [2]. In the next step the obtained profiles were compared in order to define the quantitative and qualitative differences. Differences in lipidome of the analyzed groups were found and developed dyslipidemia was confirmed. The abnormalities in metabolism of phospholipids and triacylglycerols were observed, that can be probably related to malnutrition or systemic inflammation leading to cardiovascular disease progression and in the effect, to cardiac events in CKD. Moreover, results obtained on lipidome level demonstrated corelation whit these obtained during MS analyses of the proteomes [3].

References

1. Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. Journal of Lipid Research 2008; 49(5): 1137–1146.
2. Surma MA, Herzog R, Vasilj A, Klose C, Christinat N, Morin-Rivron D, Sampaio JL. An automated shotgun lipidomics platform for high throughput, comprehensive, and quantitative analysis of blood plasma intact lipids. European Journal of Lipid Science and Technology 2015; 117(10): 1540–1549.
3. Luczak M, Formanowicz D, Marczak Ł, Suszyńska-Zajczyk J, Pawliczak E, Wanic-Kossowska M, Stobiecki, M. iTRAQ-based proteomic analysis of plasma reveals abnormalities in lipid metabolism proteins in chronic kidney disease-related atherosclerosis. Scientific Reports 2016; 6: 32511.

eP 4
Modulation of secondary metabolism in *Arabidopsis thaliana* by silver nanoparticles and silver ion

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Nanoparticles (NPs) are investigated in industry, medicine, agriculture and biotechnology. It is frequently shown that NPs can induce an oxidative stress in plant cells and may act as elicitors of plant resistance by induction of metabolic pathway. The aim of this study was to demonstrate changes in plant secondary metabolism as a result of silver nanoparticles (AgNPs) treatment. 25-days old *Arabidopsis thaliana* seedlings were treated with different concentrations (0.5, 1.0 and 5.0 mg/L) of silver nanoparticles in sizes (10, 40 and 100 nm). Equivalent concentrations of silver ions were used as positive control. Samples were collected at 3 time-points (after 12 h, 24 h, 72 h), dried and extracted with 80% MeOH. Two chromatographic systems were used for identification and quantitation of secondary metabolites in extracts: HPLC-ESI-MS with ion trap and UPLC-UV. Contents of phenolic compounds: sinapic acid glycoconjugates (e.g. sinapoyl glucose, disinapoyl gentiobiose, sinapoyl malate ester), dihydroxybenzoic acid 3-O-pentoside, ferulic acid derivatives and flavonoids increased after AgNPs and silver ion treatment. Interestingly, phytoalexins (compounds induced by infection with pathogens) such as hydroxycamalexin glycoconjugates and 6-hydroxyindole-3-carboxylate glycoconjugates were identified in the AgNP treated seedlings. Based on these results, we can conclude that nanoparticles induce changes in the secondary metabolism in *Arabidopsis thaliana* seedlings.

eP 5
Red beetroot betalains — profile, absorption, metabolism and bioavailability

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The study aim was to characterize the profile of betalain compounds in beetroot varieties and their products, as well as to determine the absorption, metabolism and bioavailability of these compounds in experiments with *Wistar* rats and humans. Animal and human experiments were carried out after approved by the Local Ethical Committees for Experiments on Animals and the Bioethical Committee, respectively. The obtained physiological fluids were analyzed by the micro-HPLC-QTRAP-MS and micro-HPLC-QTOF-MS methods.

In red beet roots (13 varieties) and their products, 37 compounds were identified, of which 21 belonged to betacyanins, 14 to betaxanthins while 2 to betalains precursors. However, in the analyzed body fluids, a total of 26 betalains compounds were detected, including 8 native compounds as well as 18 metabolites, formed during the absorption and metabolism processes. Betanin, isobetanin, betanidin and 2,15,17-tridecarboxy neobetanin were the dominant compounds in the collected blood plasma and urine.

The obtained results suggest that each variety and product of beetroot have its own profile of betalain compounds. It was also shown that betalains are absorbed from each part of the rat's gastrointestinal tracts, however the type of betalain metabolites found in body fluids are determined by the site of absorption in the tract. Moreover, a study conducted with volunteers indicated that the long-term diet rich in betalains influence the metabolic profile of consumers. The obtained results allows to track the metabolic pathways of betalains after consuming foods rich in these compounds, which may help to determine the prophylactic potential of these pigments.

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eP 6
Utilization of metabolomics in searching for mechanism of prostate cancer development

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Cancer diseases are one of the most important civilization problems. Patient's chances for recovery depend on fast detection and availability of treatment methods. Lack of knowledge about the pathogenesis can hinder therapy process. According to worldwide statistics, prostate cancer (CaP) is the second most common and fifth lethal type of cancer among men. Its early detection is problematic due to non-specific symptoms and long period of latency. Diagnostic procedures include blood test prostate-specific antigen (PSA), digital rectal exam and transrectal ultrasonography. However, available methods are invasive and do not provide diagnosis at the early stage of the disease.

Although some factors for CaP developing can be distinguished, the pathogenesis of CaP is still unclear. Metabolomics approach seems to be useful tool in searching for markers of CaP. Researches indicate that alterations in lipid metabolism are one of the biochemical deregulation during CaP development. Because of that presented study focused on untargeted lipidomic analysis of urine, plasma and tissue samples from CaP patients and healthy volunteers.

Samples were analyzed with the use of gas chromatography coupled with triple quadrupole mass spectrometry (GC-QqQ/MS) technique. Sample preparation included optimization of liquid-liquid extraction method and utilization of derivatization procedure.

Data obtained from analytical determination undergo bioinformatics methods, including deconvolution, identification and alignment with the use of AMDIS software. As a result, 100 fatty compounds were identified in plasma samples, 40 in urine samples and 150 in tissue samples.

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eP 7
LC-MS- and GC-MS-based metabolic fingerprinting reveals similarities in urine metabolic profiles in different urinary tract cancers

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Bladder cancer (BCa) and Renal Cell Carcinoma (RCC) constitute ninth and thirteen types of cancer in terms of incidences worldwide, respectively [1]. Standard methods utilized in their diagnostics require the use of specialist equipment, may cause patients' discomfort and are adopted when disease symptoms are observed, mostly at the late stage of the disease. Therefore, specific and non-invasive diagnostic methods for early diagnosis of BCa and RCC are needed.

Urine samples obtained from BCa patients and healthy volunteers were analyzed with the use of LC-MS and GC-MS. The obtained data were subjected to deconvolution, filtration and normalization. Afterwards, statistical analysis was utilized to select metabolites that represented significant differences between studied groups. Samples obtained from RCC patients were analyzed in analogical manner. Finally, the identification of selected metabolites was performed with the use of publicly available databases allowing for creation of lists of potential metabolic indicators of BCa and RCC, that were compared for similarities (e.g. altered concentrations of hippuric acid and uridine).

The obtained results suggest that urine metabolic fingerprinting could be a powerful tool for markers' investigation and searching for explanation of BCa and RCC pathomechanisms at molecular level.

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This project was supported by the National Science Centre allocated on the basis of the decisions numbers 2015/19/N/NZ7/03397 and 2012/07/E/NZ7/04411.

Reference

1. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012, World Health Organization.

eP 8
Serum metabolic changes in chronic kidney disease

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Chronic kidney disease (CKD) constitutes a gradual loss in kidney function over months or years. CKD patients develop such complications as: high blood pressure, anaemia, renal osteodystrophy and pericarditis. The symptoms appear relatively late, mainly at the advanced state of the disease. Metabolomics, as a part of systems biology approach, can help elucidate pathology of various disorders and reveal new hypotheses about possible mechanisms of the disease.

In this pilot study, the non-targeted metabolomics approach was applied to evaluate potential metabolic differences between patients with CKD (n = 30) and healthy (n = 30) group. Serum samples were collected and subjected to extraction aiming to isolate as many metabolites as possible. Sample preparation included protein precipitation and metabolite extraction with methanol and ethanol mixture (1:1), followed by 5 min of vortexing, 60-min freezing at –20°C and 5 min of centrifugation. The obtained serum extracts were analyzed with the use of HPLC-ESI-TOF-MS technique in both positive and negative ionization modes.

Univariate and multivariate statistical analyses allowed to select significant differences between compared groups. p-value, selectivity ratio (SR) and variable importance in projection (VIP) resulted in selection of metabolites involved in discrimination between studied groups. Most of these metabolites belong to acylcarnitines; compounds that play essential role in fatty acids oxidation.

To sum up, we found that serum metabolic fingerprints of CKD patients differ from healthy group. Further investigation into statistically significant metabolites and their biochemical pathways may provide new hypotheses regarding the mechanism of CKD as well as identify potential markers of the disease.

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eP 9
Electrophoretic analysis of *Saccharomyces* cerevisiae

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Most of microorganisms are human and animal pathogens. For this reason, rapid microbiological analysis is an essential factor enabling implementation of appropriate therapy [1, 2]. Unfortunately, currently using techniques are based on the time consuming cultivation methods [3]. An alternative microbiological diagnostics are molecular biology techniques [3]. However, these methods are characterized by the reproducibility and sensitivity, they cannot be widely used due to the high cost of specialized equipment and chemicals [4]. A new approach for rapid and inexpensive microorganisms identification may constitute an electromigration techniques [3].

Electrophoretic analysis of such complicated systems as microorganisms can cause many difficulties associated with uncontrolled cell aggregation and adhesion to the inner surface of the capillary. Over the years, several strategies to eliminate the phenomenon of aggregation and adhesion, such as the addition of poly (ethylene oxide) to buffer solution or inner capillary surface modification were proposed [5, 6]. The new approach is the modification of functional groups on the microbial surface by divalent metal ions resulting in controlled aggregation of cells [7]. Physicochemical characterization of the microorganisms surface using instrumental analysis techniques is necessary to understand, yet unclear, their behavior during electrophoretic analysis and explanation the mechanisms responsible for this process.

The aim of study was to perform the control clumping of *Saccharomyces cerevisiae*. In order to identify the functional groups present on the yeast surface, the potentiometric titration and zeta potential measurements of native yeast cells was performed. The spectroscopy study in medium infrared range was carried out to identify the functional groups of yeast cells participated in calcium ions binding. Moreover, the microscopic analysis determined the impact of calcium ions for controlled yeast clumping formation at different pH. Finally, the electroanalysis of modified and unmodified yeast cells was performed and the impact of control clumping of yeast cells for the effectiveness of electrophoretic separation were examined.

References

1. Bille E, Dauphin B, Leto J, Bougnoux ME, Beretti JL, Lotz A, Suarez S, Meyer J, Join-Lambert O, Descamps P, Grall N, Mory F, Dubreuil L, Berche P, Nassif X, Ferroni A. Clin Microbiol Infect 2012; 18: 1117–1125.
2. Pomastowski P, Railean-Plugaru V, Buszewski B. In: Schmitt-Kopplin P (ed.), Capillary Electrophoresis: Methods and Protocols, Methods in Molecular Biology, Chapter 19, Microbial Analysis of Escherichia coli ATCC, Lactobacteria and Saccharomyces cerevisiae Using Capillary Electrophoresis Approach, Springer Science+Business Media, New York 2016: 393–406.
3. Dušková M, Šedo O, Kšicová K, Zdráhal Z, Karpíšková R. Int J Food Microbiol 2012; 159: 107–114.
4. Pendharkar S, Magopane T, Larsson PG, de Bruyn G, Gray GE, Hammarström L, Marcotte H. BMC Infect Dis 2013; 13: 43.

5. Szeliga J, Jackowski M, Kłodzińska E, Buszewski B, Kupczyk W. BMC Res Notes 2011; 4: 467.
6. Buszewski B, Kłodzińska E. Trends Anal Chem 2016; 78: 95–108.
7. Dziubakiewicz E, Buszewski B. Electrophoresis 2014; 35: 1160–1164.

eP 10
Assessment of exposure of breast-fed babies to organic impurities

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A newborn child is still developing its defense mechanisms and is vulnerable to harmful factors, which include such persistent organic pollutants as polychlorinated biphenyls (PCB). Lipophilicity of these compounds and their resistance to degradation process increases their accumulation in fat tissue PCB accumulated in mother's body pass easily to her milk because of its high lipid content. It is important to monitor the presence of toxic pollutants in milk, but due to low concentration levels of these analytes it is necessary to develop a method for isolating, enriching and purifying milk samples that is fast and minimizes sample volume.

Therefore the aim of this study was to determine the PCB content in mother's milk with gas chromatography coupled to tandem mass spectrometry. This technique provided low quantification limit (1.93 ng/mL) and linearity in the investigated range of concentration with high regression coefficients. A calibration curve was constructed from 2 to 11 ng/mL. For sample preparation, the QuEChERS method was used. The optimization of the procedure was conducted by using different types and amounts of solvents and sorbents as well as different pressure of nitrogen stream used in evaporation. The optimized method allowed us to isolate all the studied compounds with satisfactory recovery (96.5–120.0%) and with acceptable relative standard deviation (3.7–12.7%).

The obtained results confirm that the developed procedure used for isolation, purification and enrichment of the analyzed compounds as well as their determination is effective, and that it can be successfully applied to real-life samples, including breast milk.

eP 11
Determination of VOCs using a HS-SPME-GC/MS technique from saliva and related matrices

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Human saliva is a fluid produced by salivary glands and flowing into the human mouth. The oral fluid also contains gingival crevicular fluid, bacteria and their metabolites, epithelial cells, erythrocytes, leukocytes, food debris and secretions from the oropharynx, nasal cavity, upper airway and from gastrointestinal reflux. Saliva is one of many biological matrices, along with blood, urine, plasma, which chemical composition can be monitored using analytical techniques, such as gas chromatography-mass spectrometry [1]. Volatile organic compounds (VOCs) are naturally occurring products or byproducts of metabolic pathways that are found in the headspace of specimens, such as saliva and salivary bacteria. VOCs may be affected by stressing agents, such as silver nitrate, which is a well-known bactericidal agent [2].

The purpose of experiments is to register changes in volatile profiles obtained from bacteria isolated from saliva after addition of silver nitrate at three concentrations. Volatile patterns will be acquired using headspace solid-phase microextraction (HS-SPME) in conjunction with gas chromatography-mass spectrometry (GC/MS). The final goal is to evaluate changes in the distribution of functional groups of the obtained chemical compounds and to indicate which volatile components can serve as biomarkers of particular bacteria.

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References

1. Durán N, Durán M, de Jesus MB, Seabra AB, Fávaro WJ, Nakazato G. Nanomedicine 2016; 12(3): 789–799.
2. Tait E, Perry JD, Stanforth SP, Dean JR. J Chromatogr Sci 2014; 52(4): 363–373.

eP 12
Microbiology neutralization of zearalenone

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The *Fusarium* family is an important cereal pathogen, because of its ability to produce toxic secondary metabolites (mycotoxins) such as zearalenone. After infection, mycotoxins can accumulate into cereal plant, resulting in contamination of animal feed and human cereal food. Zearalenone is mostly present in corn, but it can be also found in other important crops such as wheat, barley, sorghum and rye [1]. Chemically, ZEA is a resorcyclic acid lactone and has structural similarity to the natural estrogens — it can mimic endogenous estrogens, change their mechanism of synthesis and metabolism, which contributes to change and neoplastic i.e. breast cancer [2]. There is a few methods which have been developed to control the occurrence of mycotoxins such as physical and chemical approaches, but they are non-efficient and contribute to changes in the value of food products and the occurrence of toxic substances [3]. Recently, microbiological methods has received much attention; they have been found to be safer and more effective. One of the most promising organisms able to ZEA neutralization seem to be lactic acid bacteria (LAB), which are widely used for the production of fermented foods, are part of intestinal microflora and have beneficial health effects in humans [4]. The aim of the study was to investigate a novel approach of ZEA neutralization by *Lactococcus lactis* and *Bifidobacterium* sp. The process was confirmed by identification of binding kinetics and spectroscopic studies such as FT-IR spectroscopy and MALDI-TOF-MS spectrometry. According to the obtained results, bacterial strains isolated from milk products have the ability to adsorb and neutralize the zearalenone, but biosorption processes for them are not the same. The kinetic process of zearalenone binding to *L. lactis* in comparison with *Bifidobacterium* sp. cells is not homogeneous but expressed with two main stages. The first one is quite rapid and consist of most of zearalenone biosorption (about 90%). The second stage is much slower and corresponds to the diffusion of ZEA into bacterial cells. FT-IR analysis showed that in immobilization of ZEA by LAB deprotonated carboxyl groups of bacterial proteins and peptidoglycan are mainly involved.
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References

1. Van Der Fels-Klerx et al. Food Addit Contam Part A. 2012; 29: 1581–1592.
2. Urry et al. Tetrahedron Lett 1966; 7: 3109–3114.
3. Legan JD. Int Biodeterior Biodegradation 1993; 32: 33–53.
4. Soomro et al. Pakistan J Nutr 2002; 1: 20–24.

eP 13
Amino acid profile in autism spectrum disorder

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Autism Spectrum Disorder (ASD) has raised interest among scientists around the world since the 1940s. Over the years the definition of ASD has been broadened and refined, covering an increasing number of people. Only in 2016, the problem of autism was brought up in over 4,500. scientific publications [1]. Despite the expansion of knowledge and the development of analytical and diagnostic techniques that allow the determination of compounds at very low concentrations, biomarkers of this disorder have not been identified yet [2].
The key to understanding the mechanisms of ASD is to indicate the metabolites whose abnormal presence would be characteristic of this disorder. Simultaneous monitoring of changes in the metabolic profile and the assessment of the effect of the therapy on the levels of specific compounds would allow the introduction of individual steps to address further abnormalities.
In addition to behavioral symptoms, the disorders associated with malfunctioning of the digestive tract are often observed in autistic individuals. They may adversely affect the absorption of key nutrients. Some amino acids (serotonin, dopamine and norepinephrine) act as precursors in the biosynthesis of neurotransmitters. Their disturbed levels are often associated with the manifestation of symptoms characteristic of ASD [3].
The presented poster is a summary of the knowledge of abnormalities in the amino acid profile in people affected by autism spectrum disorder.

References

1. PubMed: 1) autism or ASD autistic.
2. Naushad SM et al. Indian J Biochem Biophys. 2013; 50(5): 474–478.
3. Kałużna-Czaplińska J et al. Trends Food Sci Technol 2016; 49.

eP 14
Metabolites and Parkinson's disease

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World Health Organization (WHO) indicates that neurodegenerative disorders are one of the greatest threats to public health. It is estimated that Parkinson's disease (PD) affects up to 5% of the population worldwide [1]. Parkinson's disease is a chronic progressive neurodegenerative disorder, which manifests such symptoms like motor disorders, resting tremor, rigidity, bradykinesia, and postural disturbances [2]. Currently, there is no clear laboratory test in clinical practice used to detect PD. Therefore, in the diagnosis and treatment of Parkinson's disease identification of metabolites in body fluids may be helpful. Based on the presence of specific compounds or abnormal concentration levels we can determine the relationship between them and intensification of symptoms, and conclude about the progress of the disease. In the case of Parkinson's disease, this would serve two purposes, on the one hand, it would allow an early diagnosis, and, on the other hand, would have a preventive function. Literature reports indicate that metabolic phenotypes are closely related to the development of Parkinson's disease. In order to characterize metabolic phenotypes in body fluids of PD patients and to detect potential markers, comprehensive studies using chromatographic techniques are conducted [3]. Alarming statistics force the necessity to undertake further research relevant to etiology and assessment of the stage of Parkinson's disease in humans.

References

1. World Health Organization. 2006. Neurological disorders: Public health challenges. Geneva: World Health Organization. http://www.who.int/mental_health/neurology/neurodiso/en/.
2. Gawel M, Potulska-Chromik A. Choroby neurodegeneracyjne: choroba Alzheimera i Parkinsona. Postępy Nauk Medycznych 2015; 28(7): 468–476
3. Luan H, Liu LF, Tang Z, Zhang M, Chua KK, Song JX, Mok VC, Li M, Cai Z. Comprehensive urinary metabolomic profiling and identification of potential noninvasive marker for idiopathic Parkinson's disease. Sci Rep 2015, 5: 13888.

eP 15
Metabolomics of aqueous humor — LC-QTOF-MS method development and clinical application

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Background and aims: Aqueous humor (AH) is a transparent fluid which fills the anterior and posterior chambers of the eye. It supplies nutrients and removes metabolic wastes from avascular tissues in the eye. Proper homeostasis of AH is required to maintain adequate intraocular pressure as well as optical and refractive properties of the eye. Application of metabolomics to study human AH may improve knowledge about the molecular mechanisms of eye diseases. Among the analytical platforms utilized in metabolomics, LC-MS allows for the highest metabolome coverage. The aim of this study was development of a method for extraction and analysis of AH metabolites with LC-QTOF-MS and its subsequent application to the clinical samples.

Material and methods: AH samples were obtained from 35 (16 with type 2 diabetes and 19 controls) who underwent cataract surgery. Different solvents (acetone, acetonitrile, methanol/ethanol (1:1), and methanol/acetone/acetonitrile (1:1:1) for extraction of metabolites from AH were tested. Extracted samples were analyzed with LC-QTOF-MS.

Results: The best results were obtained when AH was mixed 1:1 (v/v) with methanol:ethanol. In the final method, 2 μL of extracted sample was analyzed by LC-RP-MS and 1 μL by LC-HILIC-MS. Using this methodology few hundreds of metabolites were putatively annotated in AH. Almost thirty metabolites discriminated AH samples from diabetic and non-diabetic patients. Significant metabolites were mainly antioxidants, amino acids and their derivatives.

Conclusions: LC-MS-based metabolomics can be useful to study aqueous humor. Increased oxidative stress and perturbations in amino acid metabolism in AH may be responsible for earlier cataract onset in diabetic patients.

eP 16
Analysis of the lung cancer proteome using an Orbitrap mass spectrometer

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Background and aims: Lung cancer is classified as small cell (SCLC) and non-small cell (NSCLC). About 70% of lung cancer patients are diagnosed with NSCLC type. The NSCLC is divided into three subtypes: squamous cell carcinoma (SCC), adenocarcinoma carcinoma (ADC), and large cell carcinoma. Currently NSCLC treatment strategy is subtype dependent. Molecular targeted therapy is effective in patients with advanced ADC, while no specific targeted therapy is currently established in SCC patients. The aim of the present study was to apply high-resolution and accurate mass orbitrap mass spectrometry to increase lung tissue proteome coverage and search for potential NSCLC protein markers.
Material and methods: Proteins were extracted from tissues, fractionated using 2D-PAGE electrophoresis, and in-gel digested. Extracted peptides were analyzed by means of a nano-LC chromatography coupled with an Orbitrap Fusion mass spectrometer (Thermo Scientific). Identification of peptides was performed on the basis of the obtained spectra and attribution to the corresponding proteins. Protein identification was performed using the Peaks Studio 8.0 software based on the current SwissPort human protein sequence database. Quantitative estimation was performed using lable-free quantification.
Results: The number of identified proteins in each sample varies in the range of 4000 protein groups. The results indicate significant differences in the protein profile between the tumor and control tissues. Altered proteins are involved in immune response, DNA synthesis, ATP production, oxidation stress, lung exchange process or tumor spread and metastasis.
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eP 17
Changes in lipids profile of erythrocyte membranes in women diagnosed with gestational diabetes mellitus (GDM)

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Gestational diabetes mellitus (GDM) is a glucose intolerance that begins or is first recognized during pregnancy. It is currently a growing health problem worldwide affecting from 1% to 14% of all pregnant women. Recent studies correlate GDM with changed levels of some metabolites. Our study aimed at investigating the erythrocyte membrane fatty acid profile of women diagnosed with gestational diabetes mellitus (GDM), with normal glucose tolerant (NGT) pregnant women as a control group.
The study group comprised 43 pregnant women, 32 of whom were diagnosed with GDM according to the WHO criteria, and 11 with normal glucose tolerance.
The analysis of fatty acids from the erythrocytes revealed important differences between GDM and NGT women in the third trimester, and the results were correlated with biochemical data. Among the 14 measured fatty acids representing the membrane lipidomic profile, the levels of three SFAs (myristic, palmitic, stearic acids) tended to decrease in GDM patients, with the percentage content of stearic acid significantly changed. The relative content of MUFA tends to increase, in particular oleic acid, and the vaccenic acid content was significantly increased in erythrocyte membranes of GDM group in comparison with NGT group. The GDM group demonstrated higher sapienic acid levels (+29%) but this change was not statistically significant. Our study showed the association between an impaired vaccenic acid concentration in erythrocyte membrane and GDM development.
Our results indicate that the SFA-MUFA families may be involved in the pathophysiology of metabolic diseases such as GDM.

eP 18
Non-targeted and targeted LC/MS analysis of the kynurenine pathway metabolites in body fluids

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The tryptophan metabolism by the kynurenine pathway leads mainly to the formation of nicotinamide adenine dinucleotide (NAD). Many intermediate metabolites with significant biological activity are also produced among others 3-hydroxykynurenine, 3-hydroxyanthranilic acid and quinolinic acid. Moreover, anthranilic, kynurenic, xanthurenic and picolinic acid are formed. Some of the compounds produced in this pathway have neurotoxic properties and have been associated with neurological disorders [1]. Research results indicate a link between the kynurenine pathway and Huntington's disease [2], AIDS dementia complex [3] and cerebral malaria [4].
Since tryptophan metabolites produced by the kynurenine pathway may be involved in the pathophysiology of other neurological diseases, it is important to conduct further studies involving these neurotoxins. The purpose of our research is to develop and optimize sensitive methods,

targeted and non-targeted, for the analysis of metabolites of the kynurenine pathway in body fluids using liquid chromatography coupled to mass spectrometry. Method optimization involved the sample preparation, chromatographic separation and parameters of the mass spectrometer operation. Various ratios of sample (body fluid) to extraction solvent were tested, and recovery and matrix effects were evaluated. The effect of the mobile phase flow rate, the parameters of ion source operation and ion emission in the mass spectrometer on the MS signal intensity was investigated. The paper presents primary research results on the development and optimization of LC/MS methods for non-targeted and targeted analysis of the kynurenine pathway metabolites in body fluids.

References

1. Smith AJ, Stone TW, Smith RA. Biochem. Soc Transactions 2007; 35: 1287.
2. Stoy N, Mackay GM, Forrest CM, Christofides J, Egerton M, Stone TW, Darlington LG. J Neurochem 2005; 93: 611.
3. Guillemin GJ, Wang L, Brew BJ. J Neuroinflammation 2005; 2: 16.
4. Medana IM, Day NPJ, Salahifar-Sabet H, Stocker R, Smythe G, Bwanaisa L, Njobvu A, Kayira K, Turner GDH, Taylor TE, Hunt NH. J Infect Dis 2003; 188: 844.

eP19
Application of rapid fire technology coupled with mass spectrometry for validation of potential markers in noninvasive diagnosis of fetal trisomy 21

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Background and aims: Trisomy 21 is one of the most common chromosomal aberration. This syndrome is routinely diagnosed by invasive (amniocentesis and chorionic villous sampling) or noninvasive (genetic, ultrasound, biochemical) prenatal tests. Invasive procedures have 1% risk of miscarriage. Noninvasive tests allow only to calculate the risk of aberration appearance and require confirmation by invasive methods. Genetic tests offer safe and noninvasive measurement with high sensitivity and specificity but are expensive. We propose measurement of new plasma marker of trisomy 21. Relatively low costs of the method can make this test commonly available.

Material and methods: Using new technology, RapidFire coupled with QQQ-MS, we developed a method for quantification of potential plasma marker of fetal trisomy 21 previously discovered by metabolic fingerprinting study. This metabolite was measured in plasma samples obtained in 16th week of gestation from 48 pregnant women (28 healthy pregnancies and 20 with confirmed fetal trisomy 21).

Results: A fast method for quantification of potential biomarker of trisomy 21 was developed. The LOQ of proposed method was established as 0.5 ng/mL. Measured metabolite was increased of 105% ($p < 0.001$) in plasma of women with Down syndrome affected pregnancies. Specificity and sensitivity of proposed marker were 85.7% and 84.2%, respectively.

Conclusion: Measurement of this metabolite in plasma of pregnant women can improve noninvasive diagnosis of fetus trisomy 21.

eP 20
QTRAP mass spectrometer in routine toxicologist's work

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Masdiag's laboratory activities include toxicological analysis of psychoactive substances and intoxicants, in accordance with the Drug Prevention Act. Toxicological analysis are divided into two groups, fast and targeted qualitative and quantitative analysis of the most popular psychotropic substances and drugs, affecting the ability to drive and the screening ones for as many psychotropic compounds as possible.

The experiments are performed using high performance liquid chromatography coupled with mass spectrometry (type QTRAP). Quantitative analysis are performed in Multiple Reaction Monitoring mode (MRM). The advantage of this mode is the ability to observe multiple compounds simultaneously, while maintaining a high sensitivity of the method. Moreover, the selectivity of the measurement is greatly increased as a result of a specific fragmentation reaction of a particular mass. Quality analysis are performed by combining the above mentioned MRM mode with Enhanced Product Ion Scan (EPI). EPI is a trap mode to observe full fragmentation spectra derived only from the selected ions. The MRM mode is used to observe the intensity of the signal of the selected fragmentation reaction. When the signal from the particular substance appears, mass spectrometer switches to EPI mode and collects the full MS/MS spectrum. During the presentation, examples of the most interesting analysis performed in Masdiag laboratory will be presented.