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From research to clinical practice

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Organizers

Department of Laboratory Diagnostics, Dubrovnik General Hospital, Dubrovnik, Croatia
Croatian Society of Medical Biochemistry and Laboratory Medicine

Organizing Committee:

Antonija Perović, president
Marina Njire Bratičević
Ana Milčić
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Jasna Đogić

Scientific Committee

Jerka Dumić
Tihomir Balog
Mihailo Banjanac

The path from research to practice – the Odyssey's journey

Jerka Dumić

University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia

In biomedical sciences, the path „from bench to bedside" is long, challenging, expensive and often unsuccessful. It was estimated that it usually takes 10-15 years from research finding to putting that finding into practice, while the costs exceed tens, hundreds and even a billion dollars when it comes to drugs. The failures often result from the complexity, specificities, and fragmentation by the fields of biomedical researches, non-compliance and lack of understanding of physicians (clinical scientists) and basic scientists, different goals and needs of stakeholders in the health system, and the everlasting lack of funds.

No matter how it is called (translational, precision or personalized medicine), translation of scientific discoveries into clinical benefits seeks for the bidirectional concept, namely the coordinated usage of new knowledge in clinical practice and integration of clinical observations into scientific hypotheses in the laboratory.

The path from the idea and bench research, through production, preclinical testing, and clinical trials to gaining approval by regulatory agencies is financially very demanding. In addition, once at the market, the product may fail to generate expected revenue, and furthermore, in a case of drugs, it could be withdrawn due to unexpected adverse effects. To overcome these obstacles the collaboration among basic and clinical experts from different fields should be intensified, creative, new approaches should be encouraged, and more accurate preclinical studies and inventive cost-effective approaches in clinical trials should be created. More than 10 years ago, the need for multidimensional and multidisciplinary approach, named "the concept of team science" was recognized.

Yet, the upcoming times seek for a new generation of translational scientists, trained and educated for successful tracking the path from bench to bedside and back. They should have a comprehensive perspective on science, understand how science system functions, be equipped with skills and competencies (*e.g.* critical thinking, problem-solving and communication), and at the same time be competent for successful dealing with data, legislation, bioethics, funding *etc*. Although the achievement of these demands may look too far on the horizon, according to my personal view, medical biochemists seem most appropriate and prepared to meet these demands most promptly. Their good background in molecular life sciences and clinical subjects, provides them a good platform for further development into translational scientists through lifelong learning. However, in order to well prepare future young colleagues for the upcoming challenges, it is of utmost importance to continuously update and improve the curriculum to holistically integrate concepts of translational medicine as well as to advance teaching methods that will promote skill and competencies development.

Corresponding author: jdumic@gmail.com

Procedures for implementing new biomarkers into clinical practice

Marina Njire Bratičević

Department of Laboratory Diagnostics, Dubrovnik General Hospital, Dubrovnik, Croatia

The National Institutes of Health, Biomarkers Definitions Working Group defined a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Therefore the discovery of the new biomarker is considered a successful endpoint of clinical proteomics. While a large number of biomarkers have been described through close to half a million articles in the last decade, only one per year is added to the routine repertoire of most clinical biochemistry laboratories. This disparity is a consequence of a strict,

long-lasting and expensive process aimed at demonstrating clinical validity and utility, benefit for the patient and the need for multiple collaboration between researchers, clinicians, healthcare providers, diagnostic industry, legislative bodies and patient groups.

Requirements for introducing a new biomarker, presented at the Perspectives in Proteomics Conference and also at the 12th Bergmeyer Conference, are investigations of preanalytical conditions, analytical performance, clinical validity and utility. Investigations of preanalytical variation include selection of specimen type, collection, handling, storage, biological variation assessment. Indicators of accuracy, precision and analytical measurement range, developing standard for calibration and quality control are part of analytical validity. Clinical validity represents an assessment of diagnostic accuracy and predictability, while clinical utility includes clinical studies that emphasize different performance characteristics and require different study populations. The last step is clinical impact or investigation of ethical, legal, financial or social implications after evidence-based clinical practice guidelines are formed.

Taking into account complexity of the implementation process, it is important to highlight the role of medical biochemists as important experts in above mentioned areas, all of which are essential to optimal introduction of a new biomarker.

Corresponding author: marinanjire@yahoo.com

Importance of biomarkers in drug research

Mihailo Banjanac

In vitro pharmacology, Fidelta Ltd., Zagreb, Croatia

In past several decades there is a decrease in the drug discovery productivity, best presented as a decrease of new drug approvals per dollar or euro spent in the research and development. The major cause for this decline in the drug discovery productivity is failure of new drugs in efficacy and safety in phases 2 and 3 of clinical trials. There are many different reasons that are contributing to this phenomenon (increased regulatory agencies demands, prevailing concepts in drug discovery, lack of relevant preclinical models, complex and unknown pathophysiology *etc.*).

One area to improve new drugs development is biomarkers research area. There are many different definitions of biomarkers and one of those defines biomarkers as: "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention". Biomarkers are usually proteins, but are also metabolites, lipids, carbohydrates, DNA genetic variants, different types of RNA molecules, and are classified according to their utilities (disease, diagnostic, prognostic, response, predictive, pharmacodynamic, safety, surrogate markers). Biomarkers are identified using systems biology approach that enables identification of diseases and drug specific interaction networks (high throughput and high content -omics technologies, imaging technics). Much like the drug discovery process, the new biomarkers discovery process relies on a use of cell cultures that mimic disease, patient derived primary cells and fluids and transgenic animal models. After preclinical discovery, biomarkers are tested in clinical studies in order to address their utility.

Drug discovery process by itself and the role and detailed description of biomarkers in this process will be presented as well as benefits and pitfalls within these processes. Importance of early identification of biomarkers in drug discovery as well as in therapeutic regime (namely in oncology) will be addressed.

Corresponding author: mihailobanjanac@gmail.com

Oxidative stress and antioxidative enzymes, relation to diseases and ageing

Tihomir Balog

Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia

A number of age-related diseases are related to oxidative/antioxidative status. The level of oxida-

tive damage in an organism is tightly related to development of age related diseases, such as cancer, neurodegenerative diseases and diabetes. The most popular hypothesis used to explain the mechanism of ageing in relation to oxidative stress is the Free Radical Theory of Aging proposed by Denham Harman. This theory proposes that accumulation of reactive oxygen species (ROS)-induced oxidative damage of macromolecules which leads to the ageing process and development of diseases. Harmful ROS are produced as a result of normal cellular metabolism, mainly in mitochondria. The major antioxidant enzymes that eliminate excess ROS and define the potential to cope with the oxidative stress are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Numerous studies highlight the role of ROS in development of various diseases particularly developing of neurodegenerative diseases like Alzheimer's disease. On the other hand in various pathological conditions (such as atherosclerosis, apoptosis, aging, diabetes), cell ROS release-related damage can be protected by the antioxidant enzymes. In addition, the level of antioxidant enzymes (SOD-1, SOD-2, CAT, GPx-1), were often used as a marker of oxidative stress and oxidative/antioxidative status in animals. Since sexual selection can also influence lifespan and survival rates, an alternative approach to study ageing and longevity is to investigate sex differences in response to oxidative stress. In our work we explored sex differences in oxidative/antioxidative status in mice and their ability to maintain redox balance upon acute oxidative stress induced with normobaric hyperoxia as a model of oxidative stress. Oxygen toxicity in hyperoxia conditions can be controlled with factors such as sex, age, tissue and hormones. The female sex hormone estrogen (E2) has a well-established cytoprotective effect against oxidative stress, which strongly contributes to ageing. However, the mechanism by which E2 exerts its protective activity is still unclear.

Corresponding author: tihomir.balog@irb.hr

Circulating Cell-Free DNA (cfDNA): myth or reality for clinical practice

Sandra Šupraha Goreta

University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia

Circulating cell-free DNA (cfDNA) has been discovered as a potential blood-based noninvasive biomarker capable of monitoring disease progression and treatment efficacy. It has already made a great clinical impact on prenatal care, where fetal cfDNA in the circulation is now used for early diagnosis of genetic abnormalities, fetal sex, or pre-eclampsia. Regarding oncology, it is well known that circulating tumor derived DNA fragments (ctDNA) are present in the blood of cancer patients for several decades now, but it›s really only recently that technology such as digital PCR and next generation sequencing have allowed us to extract clinically useful information from these circulating DNA fragments. Despite all advantages of such biomarkers providing prognostic and predictive information in cancer, it has not been incorporated into routine clinical practice. Problems are analytical limitations concerning cfDNA and as well as a limited understanding of precisely how to interpret biomarker results across various disease stages and tumor types. The biggest challenges in these blood-based biomarker development field are focused on novel cfDNA technologies, interpretation of obtained data regarding disease evolution and heterogeneity, and logistical consideration for incorporation of cfDNA into clinical trials, and ultimately into routine clinical use. The objectives of this lecture include the presentation of the current barriers to clinical implementation and recent progress made in the field. The advances in basic and translational research will ultimately impact patient management strategies and patient outcomes, especially in oncology.

Corresponding author: ssupraha@pharma.hr

Extracellular Hsp70 - from mechanism of action to potential diagnostic marker

Lada Rumora

University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Medical Biochemistry and Haematology, Zagreb, Croatia

Heat shock proteins (Hsps) are highly conserved and ubiquitously expressed proteins that play an essential role as molecular chaperones in maintaining the homeostasis of cells and tissues. The members of the Hsp superfamily are categorised into several families on the basis of the approximate molecular weight. The best described Hsp70 family member is stress-inducible 72-kDa protein with cellular functions including preventing protein aggregation, facilitating protein refolding, and chaperoning proteins, all of which improve survival of a cell faced with stressors. During cellular stress, intracellular Hsp70 levels increase to provide cellular protection. The induction of Hsp70 has to be tightly controlled, since its persistent presence would adversely affect protein homeostasis and intracellular functions, leading to inappropriate growth control and possibly cell death.

To date, Hsp70 has mainly been studied as intracellular chaperone that is released from cells only after severe damage that causes cell lysis. It is now also known that Hsp70 can be secreted from a variety of cell types, even when these cells are completely viable, acting as a damage-associated molecular pattern (DAMP).

People suffering from a variety of inflammatory and infective diseases have chronically elevated basal levels of extracellular Hsp70 (eHsp70) relative to healthy, aged-matched controls. In addition, an increase of eHsp70 in the blood occurs in healthy organisms after exposure to acute stressors.

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease with many comorbid and confounding conditions, and markers of disease may vary from one phenotype to another. Therefore, exploration of systemic inflammatory parameters will provide new information on the systemic inflammatory COPD phenotype that might need more specific and more personalized therapeutic strategy. This might improve relevant clinical outcomes of the disease.

The lecture will discuss the mechanism of action of eHsp70 and its potential diagnostic significance, especially in COPD.

Corresponding author: lrumora@pharma.hr

Fucosylated plasma N-glycans as biomarkers of HNF1A-MODY in young adults

Olga Gornik

University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia

HNF1A-MODY is often misdiagnosed disease due to lack of diagnostic protocols and limited availability of genetic testing. Benefits of making the diagnosis include possible treatment with derivatives of sulphonylurea as well as screening of relatives.

In our previous work we showed that defects in processes regulated by *HNF1A* result in an altered plasma N-glycan profile in HNF1A-MODY and that this gene is a key regulator of protein fucosylation in plasma. Thus, we aimed to evaluate the diagnostic potential of glycosylation in an unselected population of young adults. Included were individuals in UK and Croatia (N = 1032), with diabetes onset below 45 years, who were antibody negative and C-peptide positive, which mostly excluded individuals with type 1 diabetes.

N-glycans were analysed using Hydrophilic Interaction Liquid Chromatography-UPLC and profiles were divided into 38 glycan groups (GP1-GP38) with assigned structures. Sanger sequencing of *HNF1A* was performed and rare *HNF1A* variants (MAF < 1%) underwent a systematic assessment of their functional effect.

Twenty-five rare *HNF1A* variants were found in 29 probands. After collecting previous knowledge, performing of *in vitro* functional studies and using

of bioinformatic tools, we assigned 13 variant alleles, found in 18 individuals, as HNF1A-MODY-causing.

The glycan groups GP30, GP36 and GP38, all containing fucosylated glycans, showed good discriminative power between HNF1A-MODY and those without variants (C-statistics of 0.86-0.90). Glycan group GP30 had the best performance, with sensitivity of 89% and specificity of 76%. Using cut-off of 0.73, GP30 detected 16 out of 18 patients with damaging *HNF1A* alleles.

Our results show that fucosylated glycans are good discriminative markers for HNF1A-MODY among young adults and that their use could significantly improve current diagnostic protocols for HNF1A-MODY.

Corresponding author: ogornik@pharma.hr

Translational medicine - possibilities and challenges for medical biochemists

Antonija Perović

Department of Laboratory Diagnostics, Dubrovnik General Hospital, Dubrovnik, Croatia

Translational medicine as multidirectional and multidisciplinary integration of basic research, clinical research, and population-based research is strongly oriented to the patient, who is in the centre of events, partner in research. The term "multidirectional" implies that a key step toward improvement in the treatment or prevention of disease may begin in each segment of translational medicine, and new knowledge and hypotheses are generated at each of the next steps. Medical biochemists competences, acquired through years of education and professional training, that lie in the knowledge and understanding of biochemical and molecular mechanisms as well as physiological and pathological processes play an important role in multidisciplinary integration.

The application of research findings from genes, proteins, cells, tissues, in animal and human model, can be a path to the clinical research in patient populations. Additionally, medical biochemists competences through the knowledge and dissemination of new methods can play a key role in the discovery and evaluation of various biomarkers as an indicator for screening, monitoring and progression of the disease or response to therapy. Assessing the linkage between biomarker response and clinical end-points in patients requires strong partnership between patient and clinician, but also medical biochemists and pharmacological industry. The role of medical biochemists in educating medical staff about pre-analytic factors that can influence the results is also important, as well as the implementation of the guidelines through a minimum retesting interval. Taking into account the financial limits of laboratory diagnostics, this is all part of the translation medicine, since reducing unnecessary analysis can lead to the introduction of new promising biomarkers that can be used for patients. Although most often characterized as „the path from bench to bedside", according to Steven H. Woolf: „Translational research means different things to different people, but it seems important to almost everyone. "

Corresponding author: perovic.antonija@gmail.com