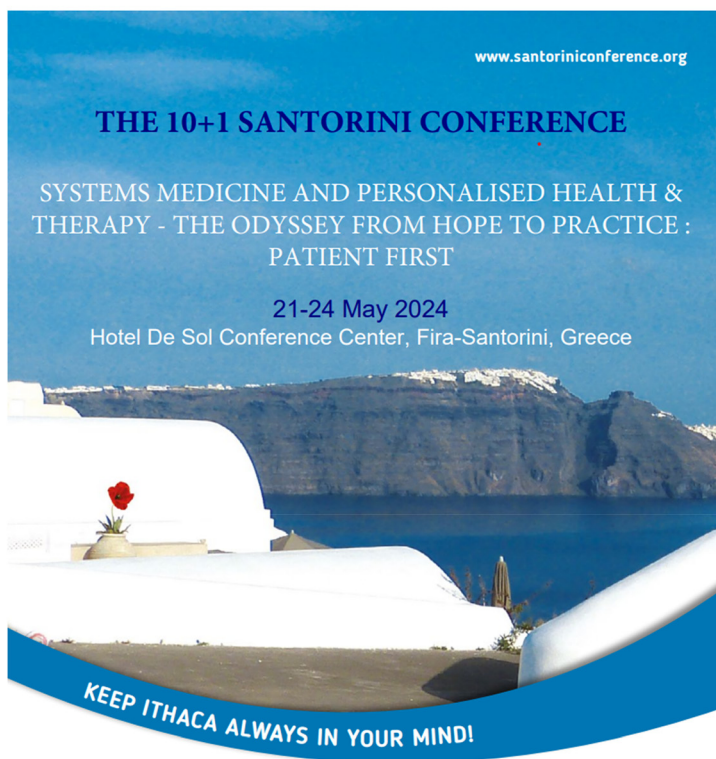




Santorini Conferences Association
For Research Innovation in Health



ABSTRACTS BOOK

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Congress Abstracts

The 10+1 Santorini Conference

TUESDAY 21 MAY, 2024

WELCOME

Chairs: Sofia Siest, Bernécourt, France / Tomris Ozben, Antalya, Turkey

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KEYNOTE LECTURE

Chairs: Sofia Siest, Bernécourt, France / Tomris Ozben, Antalya, Turkey

Reference Interval Harmonization: Harnessing the Power of Big Data Analytics to Derive Common Reference Intervals Across Populations and Testing Platforms

Khosrow Adeli

IFCC Past-President; Head and Professor, Clinical Biochemistry and Senior Scientist, Research Institute, The Hospital for Sick Children, University of Toronto, Toronto, Canada

Several national surveys have reported wide variation in reference intervals across healthcare centers in certain regions, even those using the same analytical platform and reagents for the same assay. There is a high risk of inappropriate test result interpretation when reference intervals are not appropriately harmonized. The Canadian Society for Clinical Chemistry (CSCC) Working Group on Reference Interval Harmonization was established in 2015 to develop evidence-based harmonized/common reference intervals and support their implementation in laboratories across Canada. Harnessing the power of big data, laboratory results were collected across populations and testing platforms to derive common adult RIs for 16 biochemical markers. A novel comprehensive approach was established, including: (1) analysis of big data from community laboratories across Canada; (2) statistical evaluation of age, sex, and analytical differences; (3) derivation of hRIs using the refineR method; and (4) verification of proposed harmonized reference intervals across nine laboratories with different instrumentation using serum and plasma samples collected from healthy Canadian adults. Harmonized RIs were calculated for all assays using the refineR method, except free thyroxine. Derived harmonized reference intervals met proposed verification criterion across nine laboratories and five manufacturers for alkaline phosphatase, albumin (BCG), chloride, LDH, magnesium, phosphate, potassium (serum), total protein (serum). Further investigation is needed for select analytes due to lower verification in one or more laboratory (albumin (BCP), calcium, total CO₂, total bilirubin, sodium) or concern regarding harmonized reference intervals that were considered too wide (alanine aminotransferase, creatinine, TSH). In this presentation, we will discuss the work completed by the Working Group on Reference Interval Harmonization in Canada, challenges encountered, and future plans to support implementation.

INTRODUCTION TO THE CONFERENCE

Sofia Siest, Bernécourt, France

PLENARY LECTURE

Multi-omics profiling in the era of cancer precision medicine

Tomris Ozben

IFCC President; EFLM, Past-President; Akdeniz University, Medical Faculty, Dept. of Medical Biochemistry, Antalya Turkey; University of Modena and Reggio Emilia, Medical Faculty, Clinical and Experimental Medicine, Ph.D. Program, Modena, Italy

Precision Medicine uses a deep knowledge of disease pathogenesis to tailor therapy for an individual cancer patient based on the tumour characteristics. Precision Medicine is an effective method of targeting and treating certain cancer types, providing a revolutionary treatment in oncology. Tumour heterogeneity remains a hurdle to understand tumour biology. There are variations in response to treatment among patients with the same cancers. For the same tumour type, there is a wide variation in clinical evolution of patients, highlighting differences in tumour cells' progression or mutation. These differences are barriers to develop efficient therapies. Researchers are using molecular data available to characterize tumours, identify therapeutic targets, and make precision medicine more precise. Cancer hallmarks requires molecular alterations at multiple levels including genome, epigenome, transcriptome, proteome, and metabolome. Numerous attempts using single-level OMICS approaches lack the resolving-power to establish the casual relationship between molecular signatures and the phenotypic manifestation of cancer hallmarks. The multi-OMICS approaches have the potential to uncover the intricate molecular mechanism underlying different phenotypic manifestations of cancer hallmarks. Moreover, multi-OMICS approaches can be used to dissect the cellular response to chemo- or immunotherapy as well as discover molecular candidates with diagnostic/prognostic value. Each type of omics data adds to the list of molecular differences associated with cancer, revealing useful markers of disease processes, and providing insights into biological pathways. Multi-omics and Bioinformatics are the driving forces of a revolution in diagnostic and prognostic testing. Multi-omics methods generate vast amounts and different kinds of data. Integrating them to obtain biologically meaningful insights is one of the biggest challenges. As Precision Medicine in oncology expands to include big data, omics data, and molecular imaging, there are serious challenges ahead to translate them into meaningful healthcare for patients that will enable precision medicine as an individualized approach to medicine that considers biological variation among individuals in designing treatment strategies.

WEDNESDAY 22 MAY, 2024

SESSION I - Common denominators of chronic diseases - Diagnostics, where we are

Chairs: John Lamont, Antrim, United Kingdom / Stavroula Kanoni, London, United Kingdom

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Long-term outcomes in chronic mental disorders: Relevance to personalised medicine

Behrooz Alizadeh

Unit of Personalised Medicine, Dept. Epidemiology, University Medical Centre, Groningen, Groningen, The Netherlands

This presentation discusses the complexities in the traditional classification of mental disorders specifically focusing on challenges related to the absence of objective diagnostic criteria and the intricate nature of symptoms, particularly regarding the understanding of disease course subtypes. It provides insights by focusing on deep clinical phenotyping and genetic profiling of the patients, employing data-driven approaches. This consists of a mixture distribution of positive and negative symptoms, cognitive impairments, and psychosocial functioning, aligned with a vector of patients and cohort parameters.

The identified latent subtypes exhibited diverse profiles and exhibited stable, deteriorating, relapsing, and ameliorating longitudinal naturalistic courses over time. The study comprehensively depicts the lifelong journey of patients with schizophrenia spectrum disorder, covering genetic makeup at conception, early-life premorbid adjustment and social functioning, clinical profiles and treatment modalities during the disease, social functioning and living arrangements, and quality of life post-diagnosis. While baseline symptoms, premorbid adjustment, psychotic-like experiences, and health-related quality of life are highlighted as key and strong predictors, genetic susceptibility does not play a significant role in disease outcomes. The research emphasizes the importance of Premorbid adjustment emerges as a potential source of heterogeneity and an early indicator of non-favourable long-term outcomes in individuals diagnosed outcomes in mental disorders. The presentation suggests that (pharmaco)genetic-based models lose relevance in predicting disease outcomes when robust environmental factors buffer genetic effects, altering the trajectory of affected individuals.

The findings are comprehensive, novel, and of clinical interest for precisely identifying high-risk population groups and patients with varying disease prognoses, and the selection of optimal intervention. The primary challenge in the traditional classification of mental disorders remains the understanding of disease courses and the implementation of personalised pharmacogenetic-based treatments at the individual level.

VEGF-A a potential biomarker for personalised medicine in chronic diseases

Helena Murray

Antrim, United Kingdom

Vascular endothelial growth factor–A (VEGF–A) is implicated in angiogenesis, lymphangiogenesis, vascular permeability, and haematopoiesis. It is associated with numerous pathologies including cardio-vascular diseases and cancer.

We specifically developed an integrative systems biology strategy for clinical improvement of this biomarker.

A high heritability of this trait, 60%, was estimated in the STANISLAS cohort giving us the arguments to continue for a deep characterization of its genetic component.

Therefore, we searched, by a Genome Wide Association Study (GWAS), the VEGF–A genetic variants and the inter-connexions of these biomarkers with other disease-associated molecules in healthy populations.

The GWAS was performed in 3,527 healthy participants (Framingham Heart Study) and the most significant results ($P < 5 \times 10^{-8}$) were replicated in 1,727 individuals (STANISLAS Family Study, PIVUS study).

Four polymorphisms (rs6921438, rs4416670, rs6993770, rs10738760) explaining ~50% of VEGF–A heritability were identified. A further meta-GWAS identified 6 additional rs explaining VEGF–A levels variability.

Functional transcriptomic analyses were performed in peripheral blood mononuclear cells (PBMCs). SNP rs6993770 was shown to increase VEGF₁₂₁ mRNA levels.

VEGF-A related variants, directly or via gene x gene x environment interactions had significant effects on HDL, LDL, TNF- α , IL-6, E selectin and ICAM-1 plasma levels. Furthermore, associations between VEGF–A and blood lipids were assessed in a discovery (n=1,006) and in a replication population (n=1,145) of healthy individuals.

Recently, we found that VEGF-A related polymorphisms (interaction rs7043199*rs6993770) are involved in predicting the risk of Alzheimer's disease (AD) with a protective role against AD.

Ongoing investigations focus on clinical implementation of the '–omics' determinants of this biomarker.

Our integrative strategy resulted in significant results indicating molecular links between VEGF–A cardio-vascular and cancer diseases biology and AD and the importance of epistatic and gene x environment interactions. This example illustrates an improved strategy to be applied for every biomarker with high heritability levels, consequently with potential interest in Personalised Medicine, using familial design.

Early inflammatory and fibrotic biomarkers of NAFLD and NASH

Mark Ruddock, Mary Jo Kurth, Joanne Watt, John Lamont, Peter Fitzgerald

Randox Laboratories Ltd, 55 Diamond Road, Crumlin, Co. Antrim BT29 4QY, Northern Ireland, UK

Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of excess fat in the liver (hepatic steatosis), without a history of alcohol abuse or other secondary causes of chronic liver disease. Fatty liver is a prevalent condition, affecting 25% of the global population, and has become the most common chronic liver disorder in Western countries. While liver biopsy is the established gold standard for diagnosing and staging NAFLD, its invasiveness, cost, and associated risks underscore the need for alternative approaches. In this study involving n=135 participants (n=34 controls, n=26 with simple steatosis, n=61 with NAFLD/NASH, and n=14 with alcoholic liver disease), patient serum samples were obtained, together with a detailed clinical history. Employing high-sensitivity cytokine array I, immunoassays, and ELISAs, duplicate analyses were conducted on a total of n=20 individual biomarkers. Results revealed that 13 out of the 20 biomarkers (65%) exhibited significant differences between the groups. These biomarkers included IFN γ , EGF, IL-1 β , IL-6, IL-8, IL-10, TNF α , FABP-1, PIIINP, ST2/IL-33R, albumin, AST, and ALT. Further investigation identified 5 promising biomarker candidates for additional investigation: 3 related to inflammation (IL-6, IL-8, and TNF α) and 2 were associated with fibrosis (PIIINP and ST2/IL-33R). It is acknowledged that single biomarkers may not serve as a definitive diagnostic or predictive tool given the complex heterogeneity of NAFLD. However, the combination of biomarkers holds promise for stratifying risk and staging liver disease, particularly for patients averse to undergoing biopsy. Future studies will compare the 5 identified biomarkers with existing diagnostic tests and assess their correlation with fibrotic deposition in liver tissue to further validate their clinical utility.

Use of an in vivo like 3D liver spheroid model for determining mechanisms and regulation of drug metabolism, drug toxicity and liver fibrosis under conditions of steatosis and NASH

Magnus Ingelman-Sundberg

Section of pharmacogenetics, Karolinska Institutet, Stockholm, Sweden

Significant species variations underscore the importance of conducting in vitro research using human models. We have developed a 3D liver spheroid system that closely mirrors the transcriptome, proteome, and metabolome of the corresponding donor liver. This model has proven highly effective in: i) predicting and understanding mechanisms behind drug-induced hepatotoxicity, ii) assessing the hepatic disposition and metabolite formation of low-clearance drugs, iii) elucidating the mechanisms of viral-induced hepatitis and the specific actions of viruses within the liver, iv) evaluating mechanisms of hepatocyte proliferation and v) understanding the processes by which a high-fat diet induces liver fibrosis and the molecular drivers of liver fibrosis degradation. Notably, this system facilitates the identification of novel drug targets and molecules crucial for preventing non-alcoholic steatohepatitis (NASH).

In the lecture, I will present this system, with a particular focus on recent advancements achieved through a triple culture system comprising hepatocytes, stellate cells, and liver epithelial cells (LSECs). This approach provides additional insights into the mechanisms underlying the formation and degradation of liver fibrosis, as well as the mechanisms by which steatosis and fibrosis cause clinically significant alterations in the expression of, e.g. drug-metabolizing enzymes and drug transporters.

The quest for missing heritability: Role of rare variant and gene-environment interactions

Guillaume Pare

McMaster University, Ontario, Canada

Biobank-scale genotyping has enabled the routine use of statistical genetics applications, such as Mendelian randomization, polygenic scores and heritability estimations. However, many questions remain less well explored, such as the contribution of gene-by-environment interactions (GxE) and the role of rare coding variants (RV) in complex traits. Leveraging the speed, versatility and robustness of large linear models, we sought to address these two questions. First, we introduce MonsterLM, a multiple linear regression method that does not rely on model specification and provides unbiased estimates of variance explained by GxE. Identification of GxE is crucial to understand the interplay of environmental effects on complex traits. However, current methods evaluating GxE on biobank-scale datasets have limitations. We demonstrate robustness of MonsterLM through comprehensive genome-wide simulations using real genetic data from 325K UK Biobank participants. We estimate GxE using waist-to-hip-ratio, smoking, and exercise as the environmental variables on 13 outcomes and find significant GxE for 8 environment-outcome pairs. The majority of GxE variance involves SNPs without strong marginal or interaction associations. Second, we developed a novel framework, the Rare variant heritability (RARity) estimator, to assess RV heritability (h^2_{RV}) without assuming a particular genetic architecture. We applied RARity to 31 complex traits in the UK Biobank (N=167K) and showed that gene-level RV aggregation suffers from 79% (95% CI: 68-93%) loss of h^2_{RV} . Using unaggregated variants, 27 traits had $h^2_{RV}>5\%$, with height having the highest h^2_{RV} at 21.9% (95% CI: 19.0-24.8%). The total heritability, including common and rare variants, recovered pedigree-based estimates for 11 traits. We also used RARity to reveal 11 novel gene-phenotype relationships. Finally, we demonstrated that *in silico* pathogenicity prediction (variant-level) and gene-level annotations do not generally enrich for RVs that over-contribute to complex trait variance, and thus, novel methods are needed to predict RV functionality.

SESSION II - Flash Communications

Chairs: Candan Hizel Perry, Montreal, Canada / Alexander Haushofer, Wels, Austria

Genetic diagnosis and multi-omics clustering for diabetes precision medicine

Philippe Froguel

Imperial College London and INSERM/CNRS Lille

Type 2 Diabetes (T2D) is a multifactorial disease with a strong but heterogeneous genetic background: it includes a monogenic component causing early onset diabetes, and highly polygenic forms with >500 loci increasing T2D risk. Recently, there were increasing evidence for a genetic continuum between rare and most common forms of diabetes: indeed, up to 10% of patients with regular T2D carry a “rare” pathogenic mutation in one of the genes causing monogenic diabetes, and this proportion increases to >20% in more “atypical” forms of T2D (not obese, diagnosis <40y). Dissecting both patients’ cases “oligogenic” and polygenic background is increasingly helpful for personalised diabetes care. Besides, multi-omics approaches including metabolomics, metagenome evaluation, proteomics and epigenetic analyses are assessed to better cluster patients in homogeneous etiological categories in order to foster precision medicine in a context where 1/estimated 1.3 billion people will be diabetic by 2050 and 2/ new medications are available that should dramatically decrease incidence of T2D complications (SGLT2-Inhibitors, incretin receptors agonists...) but at higher costs, which justifies precision diabetes care.

Best Practices for Earning and Maintaining Public Trust in Nutrition

Dante Preciado

Vice President, Engagement and International Affairs, American Society for Nutrition, Executive Director, National Board of Physician Nutrition Specialists

Public trust in nutrition science is the foundation on which progress in nutrition and health relies, including good public health. There is perhaps no more important issue facing nutrition science today than ensuring that the research that is conducted and disseminated is trusted by all stakeholders. These stakeholders include those who use research results to define the direction of their own research, make policy decisions such as nutritional recommendations and practice guidelines, and make funding decisions and priorities. The American Society for Nutrition (ASN) strives to work collaboratively with various stakeholders across sectors and disciplines while maintaining transparency and scientific rigor in nutrition science. In 2017, ASN commissioned an independent Advisory Committee to conduct a comprehensive review of the available literature on public trust in nutrition science and the factors that influence it and conducted outreach to stakeholders regarding to publicly available information. Seven overlapping domains were identified that can significantly influence public trust. The Committee delivered its findings and proposed best practices to support public trust, appropriate for ASN and other food and nutrition organizations motivated by the belief that public trust remains key to realizing the benefits of past, present, and future scientific advances. Its adoption by food and nutrition organizations strengthens and helps ensure that the public's continued trust in nutrition science is earned and maintained.

Personalised nutrition. The paradigm of obesity

George Dedoussis

Harokopio University of Athens, Athens, Greece

According to estimates of the World Health Organization (WHO) for 2016, a respective 40% and 13% of the global population presented overweight and obesity. The dramatic rise in obesity rates during the past decades has led WHO to reshape our perception of the disorder in a global epidemic, currently even using the term “globesity” to describe it.

Obesity is a multifactorial disorder, in its core determined by genetic makeup, cardiometabolic determinants, lifestyle habits and environmental parameters. The majority of cases of overweight or obesity are attributed to the cumulative action of a multitude of genes, usually exerting an aggravated impact in the presence of favorable contextual factors such as unbalanced diet or lack of physical activity.

Literature has focused on the combined effect of gene-environment interactions on increased weight, with special focus on the modifying or mediating effect of the obesogenic environment on the penetrance and expressivity of genes associated with obesity susceptibility. More specifically, studies have focused on the effect of dietary and physical activity habits or gene-diet interactions in obesity-associated phenotypes. The field of nutrigenetics investigates the impact of the genetic factor on the metabolic and other responses to food intake. In similar context but on the opposite side, the term nutrigenomics is used to describe the field specializing in unraveling the modifying role of nutritional components in gene expression.

The genetic architecture of increased body weight has been the subject of many genome-wide association studies (GWAS) during the past years, with significant findings allowing for the development of approaches using individualization techniques to effectively prevent and combat weight gain. The identification of multiple genetic loci associated to obesity allowed for the creation of aggravated variables named Polygenic Risk Scores (PRSs), aiming at quantifying individualized genetic risk. Such findings have allowed for the inclusion of the genetic component as contributor to prevention and treatment strategies, where the behavioral approaches adopted are not the sole determinant of successful weight management.

Current literature highlights the need for multidisciplinary attempts to further investigate causes of this purpose and propose personalised approaches as a practical means to combat excess weight. In this context, the newly-started, EU-funded BETTER4U project, consisting of 28 partners, aims at the extensive identification of all predictors related to weight gain and

the creation of an artificial intelligence (AI)-based causal model and intervention methodology for personalised lifestyle recommendations.

SESSION III - Advances in Cancer detection and treatment with Liquid biopsy

Chairs: Candan Hizel Perry, Montreal, Canada / Sanja Stankovic, Belgrade, Serbia

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Liquid biopsy: From discovery to clinical implementation

Klaus Pantel

Institute of Tumor Biology, University Cancer Center Hamburg, University Medical Center Hamburg Eppendorf, Hamburg, Germany

Liquid Biopsy has been defined as the analysis of tumor cells or products released from primary or metastatic tumor tissues into the blood or other body fluids. Over the past ten years, CTCs, ctDNA and extracellular vesicles have received enormous attention as new biomarkers and subject of translational research. In particular, CTC research has opened new avenues for a better understanding of tumor biology in cancer patients, including intra-patient heterogeneity and evolution towards resistance to therapy. Although both biomarkers are already used in numerous clinical trials, their clinical utility is still under investigation with first promising results. Clinical applications include early cancer detection, improved cancer staging, early detection of relapse, real-time monitoring of therapeutic efficacy and detection of therapeutic targets and resistance mechanisms. In particular, interventional clinical studies are required to demonstrate clinical utility of liquid biopsy as an important prerequisite for the introduction of this new diagnostic approach into clinical practice. Moreover, assay harmonization and standardization as conducted by international consortia like the European Liquid Biopsy Society (ELBS; www.elbs.eu) is essential. Here, I will discuss a conceptual framework of CTC and ctDNA assays and point out current challenges of CTC and ctDNA research with emphasis on solid tumors, which might structure this dynamic field of translational cancer research.

The utility of *Liquid Biopsy* in Immuno-Oncology: An emphasis on circulating tumor cells

Catherine Alix-Panabières

Laboratory of Rare Human Circulating Cells (LCCRH), University Medical Centre of Montpellier, Montpellier, France; CREEC/CANECEV, MIVEGEC (CREES), University of Montpellier, CNRS, IRD, Montpellier, France; European Liquid Biopsy Society (ELBS)

Cancer-related deaths are mainly caused by metastatic spread of tumor cells from the primary lesion to distant sites via the blood circulation. Understanding the mechanisms of blood-borne tumor cell dissemination by the detection and molecular characterization of circulating tumor cells (CTCs) in the blood of patients with cancer has opened a new era in cancer research.

However, blood is known to be a hostile environment for CTCs. Although the primary tumor presumably sheds thousands of cells into the bloodstream every day, only a very small percentage of these cells survive in the bloodstream and become detectable as CTCs in a blood sample. Within the immunological synapse, a multitude of inhibitory receptors have been identified. Programmed cell death protein-1 (PD-1) and its ligand, PD-L1, have been one of the most prominent examples to antagonize immune escape mechanisms employed by tumor cells.

In my talk, I will discuss about (i) CTC^{PD-L1(+)} plus extracellular vesicles expressing PD-L1 as important biomarkers in *liquid biopsy* in breast and non-small cell lung cancers as well as (ii) metastasis-competent CTCs from colon and breast cancers to discover new targetable immune checkpoint inhibitors. Indeed, these more aggressive and selected clones of CTCs have the capacity to initiate secondary tumors in distant organs; interestingly, they are not expressing PD-L1 but survived the constant immune attacks.

SESSION IV - Omics + data science: A match made in heaven

Chairs: Panos Deloukas, London, United Kingdom / Alexander Haliassos, Athens, Greece

Functional Analysis of Cardiovascular Disease Risk Variants

Panos Deloukas

William Harvey Research Institute, Faculty of Medicine and Dentistry, Queen Mary University of London, UK

We conducted a large genome-wide association studies (GWAS) meta-analysis for coronary artery disease (CAD) in 210,842 CAD cases among 1,378,170 participants of predominantly European ancestry and detected 899 conditionally independent associations of which 279 at genome-wide level of significance and the remaining 620 at 1% false discovery rate (FDR).

Similarity-based clustering suggested roles for early developmental processes, cell cycle signalling and vascular cell migration and proliferation in the pathogenesis of CAD. Focusing on the 279 CAD risk loci, we prioritized 220 candidate causal genes, combining eight complementary approaches, including 123 supported by three or more approaches.

Following the construction of ATAC-seq maps of coronary endothelial and smooth muscle cells cultured under unstimulated and VEGF stimulated conditions, we intersected the 899 CAD associated variants and all their proxies ($r^2 \geq 0.8$; ~22,000 SNPs) with ATAC-seq peaks and prioritised 653 variants for functional analysis via STARR-seq. We found 74 variants showing significant changes in regulatory activity between alleles, in human umbilical vein endothelial cells (HUVECs). Amongst the most significant of these variants, the T allele of rs76681511 was found to regulate the activity of ADAMTS7, a metalloproteinase discovered to decreased plaque stability within vasculature. Furthermore, we have generated STARR-seq data in liver cells as elevated lipid levels is an established risk factor for CAD.

Our analyses have identified many enriched loci associated with vascular CAD processes. The generation of individual isogenic cell lines will provide further understanding of the underlying mechanisms for these prioritised variant(s) and may lead to the identification of novel pathways for therapeutic intervention.

Can we predict the future? The utility of genetic risk scores for classification and prediction of autoimmune disease

Richard Oram

Exeter, United Kingdom

Autoimmune diseases are polygenic and commonly have high heritability, with a significant component of this heritable risk being conferred by a small number of HLA risk alleles present on Chromosome 6. Strong HLA associations are known for numerous autoimmune diseases such as type 1 diabetes, coeliac disease and ankylosing spondylitis, these are not widely used in clinical classification or prediction. Polygenic or genetic risk scores sum individual risk genetic risk for a disease from all associated variants and can be expressed as a single number. There is increasing interest in utilising polygenic scores in clinical care.

We recently showed that relatively small numbers of type 1 diabetes and coeliac disease associated variants can be combined to make highly discriminative polygenic or genetic risk scores for type 1 diabetes and coeliac disease. These scores can be

applied in clinical classification, for example to distinguish type 1 diabetes from type 2 diabetes or monogenic diabetes. We have now integrated these into clinical care in the UK national monogenic diabetes testing service.

Type 1 diabetes has a long latent phase of months to years before clinical presentation with high blood glucose. This latent phase can be recognised by strong biomarkers called autoantibodies, which define the pre-clinical stages of type 1 diabetes. Commonly these autoantibodies first appear in very early life. We are testing the utility of type 1 diabetes genetic risk scores for prediction of future type 1 diabetes. With the recent FDA approval of the first pre-clinical immunotherapy for type 1 diabetes, there is an urgent requirement for public health screening for type 1 diabetes, and this is likely to involve combined population screening using autoantibodies and genetics.

Artificial intelligence in External Quality Assessment - Proficiency Testing schemes (EQA-PT) in Laboratory Medicine.

Alexander Haliassos

ESEAP, Athens, Greece

External Quality Assessment (EQA) or proficiency testing (PT) schemes used to address only the analytical procedures of the medical laboratories. It is important to consider that every EQA scheme introduces some limitations and that it is not appropriate to use them as the sole means of evaluating laboratory quality. Recently schemes have been introduced to evaluate also the pre-analytical activities and post-analytical processes.

Computer science defines Artificial intelligence (AI) research as the study of "intelligent agents". The Artificial intelligence, or machine intelligence, is intelligence demonstrated by machines, in contrast to the natural intelligence displayed by humans. Intelligent agent is any device that perceives its environment and takes actions that maximize its chance of successfully achieving its goals. Alternatively, the term artificial intelligence is often used to describe machines (or computers) that *mimic "cognitive" functions* that humans associate with the human mind, such as "learning" and "problem solving".

The Artificial intelligence can be used in EQA-PT schemes mainly for the intelligent calculation of the consensus mean, the optimized exclusion of outliers, the intelligent graphs scaling and groups (methods-analyzers) classification, the expert results stratification and rating of the performance of participants, and the detection and elimination of most non-analytical errors. But also can be used for the automated and enhanced detection of Errors in EQA testing as the non-analytical errors.

Analytical errors due to EQA materials as they can exhibit a matrix effect in the examination system used by a participating laboratory (*Non commutable materials*) or any other sources of analytical problems from reagents, instruments, test methods, calibrations and calculations. Moreover, the analytical problems should be investigated to determine whether error is random or systemic.

The competency of the staff can be evaluated automatically and if human errors can be addressed and corrected as in the evaluation of limitations of analytical instruments (samples outside their linear instrument range) the non-dilution of samples, the erroneous reporting under the LOD area, the non-detection of interferences (Hemolysis-Icterus-Lipemia Index Analysis, Biotin interference).

Internet of things is the interconnection via the Internet of computing devices embedded in everyday objects, enabling them to send and receive data and can be involved in EQA schemes for the efficient Temperature tracking of control materials, the Sample Identification and the detection of Filling levels (reconstitution accuracy of samples).

In conclusion, the use of Artificial intelligence along with Internet of Things technologies in the field of EQA - PT schemes in Laboratory Medicine can improve the functioning of these schemes and makes them easier to use, trace and eliminate analytical and non-analytical errors, and enhance the information provided to their users, leading to the improvement of the overall quality of participating laboratories by empowering their staff and giving them a measurable way of testing their individual proficiency.

THURSDAY 23 MAY, 2024

SESSION V - Immunology versus genomics: A marriage never happened

Chairs: Sanja Stankovic, Belgrade, Serbia / Markus Paulmichl, Salzburg, Austria

Cardiac inflammation: The relative contribution of autoimmunity and genes

Silvia Fanti¹, Edward Stephenson^{1,2}, Etel Rocha-Vieira^{1,3}, Alexandros Protonotarios^{2,4}, Stavroula Kanoni¹, Perry Elliott^{2,4}, Saidi A Mohiddin^{1,2}, **Federica M Marelli-Berg**¹

¹William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, UK; ²Barts Heart Centre, Barts Health NHS Trust, St Bartholomew's Hospital, West Smithfield, London, UK; ³Federal University of Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil; ⁴Institute of Cardiovascular Science, University College London, 72 Huntley Street, London, UK

Autoimmunity is increasingly recognized as a key contributing factor in heart muscle diseases. The functional features of cardiac autoimmunity in humans remain undefined, due to the challenge of studying immune responses in-situ. We have previously described a subset of cMet-expressing memory T-lymphocytes, which preferentially migrate to cardiac tissue in mice and humans.

In-depth phenotyping of peripheral blood T-cells, including cMet+ T-cells, was undertaken in groups of patients with inflammatory and non-inflammatory cardiomyopathies, patients with non-cardiac autoimmunity and healthy controls. Validation studies were carried out using human cardiac tissue and in an experimental model of cardiac inflammation.

We show that cMet+ T-cells are selectively increased in the circulation and in the heart of patients with inflammatory cardiomyopathies. cMet+ T-cells display distinct phenotype and function compared to cMet-negative T-cells, including preferential proliferation to cardiac myosin and co-production of multiple cytokines (IL-4, IL-17 and IL-22). Further, circulating cMet+ T-cell subpopulations in different heart muscle diseases identify distinct and overlapping mechanisms of heart inflammation including in genetic cardiomyopathies.

In experimental autoimmune myocarditis, rise of autoantigen-specific cMet+ T-cells in peripheral blood marks the loss of immune tolerance to the heart. Importantly, disease development can be halted by pharmacological cMet inhibition, indicating a causative role for cMet+ T-cells.

Myocarditis: The challenge of diagnosis and the opportunities for treatment

Perry Elliott

Institute of Cardiovascular Science, University College London, UK

Myocardial injury of any kind can prompt an inflammatory response. The term *myocarditis* refers to inflammatory disease of the myocardium diagnosed by established consensus histological, immunological and immunohistochemical criteria. Somewhat confusingly, the term *inflammatory cardiomyopathy* is also used when *myocarditis occurs in association with cardiac dysfunction*.

Reported causes of myocarditis are numerous and include infection (viruses, bacteria, parasites, protozoa), toxic agents and systemic autoimmune disorders. In many cases, acute myocarditis resolves within a few weeks but in some individuals, it leads to persistent cardiac dysfunction or progression to end-stage heart failure. It may also cause cardiac arrhythmia or sudden cardiac death. However, the prevalence of myocarditis and specific aetiologies remains elusive due to (1) the heterogeneity of clinical presentations, (2) a reluctance to perform invasive and potentially hazardous endomyocardial biopsy to establish a tissue diagnosis, and (3) a poor understanding of immunotypes associated with acute and chronic

presentations of myocarditis. This diagnostic uncertainty contributes to inconsistent and often ineffective therapy for proven or suspected myocardial inflammation.

The concept of personalised, precision medicine recognises that complex diseases should no longer be considered a single entity and that different subtypes of patients within a given condition can be identified and treatment can be tailored to the underlying cause. That myocarditis plays a role in myocardial disease seems irrefutable. The corollary is that identification of patients with different sub-types of myocarditis offers an opportunity to significantly alter the course of disease.

In this presentation, I will review advances in non-invasive approaches to the diagnosis of myocardial inflammation and will consider the relevance of emerging data on the role of T-cell mediated immunity in different clinical scenarios to the development of prospective randomised studies.

SESSION VI - Immunology biomarkers in Oncology precision

Chairs: Eric Quemeneur, Strasbourg, France / Helena Murray, Antrim, United Kingdom

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Direct and indirect markers of response to anti-tumor vaccines or oncolytic virotherapy

Eric Quéméneur

TRANSGENE S.A., Parc d'innovation, 67405 Illkirch-Graffenstaden, France

Thanks to their intrinsic adjuvant properties, and amenability to genetic engineering, viral vectors represent attractive platforms for the development of immunotherapies against tumors. The demonstrated safety, and unique capacity of Vaccinia genomes to accept large genetic inserts (up to 20-25 kb) has opened the way to the design of advanced immunotherapies expressing complex recombinant payloads. Our company has designed several viral vector-based products, either cancer vaccines or oncolytic viruses. Currently five products are clinically assessed in a large diversity of solid tumor indications (www.transgene.fr/en). To support our development plans, several methods have been implemented in these trials to monitor the immune, and molecular responses to these two classes of immunotherapies, in addition to regular endpoints of clinical efficacy.

For vaccines, the main challenge was to track the kinetics of cytokine response, and the induction of adaptive immune response. Our results with TG4001 (MVA-based expressing full length membrane-anchored HPV E6-E7 protein antigens), and TG4050 (MVA expressing 30 patient-specific neoantigens) clearly demonstrated the strong activity of TG4001, as well as of TG4050 in inducing a diverse repertoire of effector CD8+ T cells within a few weeks after repeated administration of the vaccine. The potential value of circulating tumor DNA (ctDNA) as a surrogate marker for tumor lysis has also been assessed.

Oncolytic virotherapy engages replicative vectors that can be used in multiple routes of administration (i.e. intratumoral, locoregional, intravenous). Thus, the analytical challenge was more extensive, than for vaccines, combining the monitoring of viral vector biodistribution, and amplification (quantitative virometry based on qPCR), in addition to immune monitoring. Two studies involving intravenously administered OV (neoadjuvant Pexa-Vec, and TG6002) confirmed our hypotheses on ability for the vector to reach the tumor, express its recombinant payloads in the tumor microenvironment, and induce immune infiltration.

Representative examples derived from our analytical experience with both vaccines and OV will be discussed in terms of support to the mechanisms of action, as well as potential predictive markers of response.

The importance of blood biomarkers in oncology

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Blood biomarkers play a crucial role in oncology, offering valuable information for the detection, diagnosis, prognosis, and treatment monitoring of various cancers. Early detection of biomarkers can significantly improve treatment outcomes, for example prostate-specific antigen (PSA) for prostate cancer, CA-125 for ovarian cancer, and carcinoembryonic antigen (CEA) for colorectal cancer. Blood biomarkers provide insights into the aggressiveness of a cancer and help predict the likely course of the disease, for example, elevated levels of certain biomarkers may indicate a higher risk of disease progression or recurrence. Blood biomarkers are instrumental in monitoring the effectiveness of treatment response to chemotherapy, targeted therapies, and/or immunotherapy. Moreover, changes in biomarker levels over time can indicate whether the treatment is working or if adjustments are needed. Furthermore, blood biomarkers contribute to the era of personalised medicine by helping identify specific molecular characteristics of a patient's cancer. This additional information enables oncologists to tailor treatments to the unique genetic makeup of each patient's tumour, optimising therapeutic outcomes. Biomarker analysis is minimally invasive for patients. Therefore, serial blood testing allows for ongoing patient monitoring throughout the course of treatment and follow-up. In addition, blood biomarkers can help assess an individual's risk of developing specific types of cancer, influencing decisions regarding screening and preventive measures. In summary, blood biomarkers in oncology play a pivotal role across the entire cancer care continuum, from early detection and diagnosis to treatment monitoring and personalised therapeutic approaches. Blood biomarker measurements provide real-time information enabling evidence-based decision making for clinicians and improving patient outcomes.

SESSION VII - AI-facilitated integrated risk scores: From bench to bedside for personalised medicine

Chairs: Stavroula Kanoni, London, United Kingdom / Alexander Haliassos, Athens, Greece

Integrated Risk Scores (IRS) for cardiovascular disease risk prediction – Advances towards precision medicine

Stavroula Kanoni

Clinical Pharmacology and Precision Medicine, William Harvey Research Institute, Barts & the London Medical School, Queen Mary University of London, UK

Cardiovascular disease (CVD) affects 14% of adults above the age of 16 and is leading cause of death worldwide. Coronary Heart Disease (CHD) is the leading cause of CVD death in both sexes (14% men, 8% women). Controlling modifiable risk factors, like diet, exercise or smoking coupled with early prediction of genetic disease susceptibility (40-50% heritability for CHD) can play a pivotal role in the prevention of CVD. Early detection of individuals at high risk for CVD can also help prevent premature deaths. This is very important for specific populations at higher risk for CVD. South Asians have >2-fold higher prevalence for CHD compared to Europeans and tend to develop more severe CHD at a younger age including early MI.

Traditional risk stratification methods for CVD (QRISK3, pooled cohort equation, Framingham score) rely on conventional factors, yet they may not comprehensively encompass the entire atherogenic landscape. Recent studies have highlighted the importance of the genetic predisposition and have assessed the cumulative effect of genetic variants on CHD risk, in the form of Polygenic Risk Scores (PRS). The predictive power of a CHD-PRS for individuals at the top 10% of the distribution resembles that of a monogenic disease but the predictive ability drops rapidly outside this bracket, with PRSs only modestly enhancing CVD prediction. There is an urgent need for cutting-edge, artificial intelligence (AI)-driven integrated predictive tools that combine multi-modal CHD risk factors, demonstrating robust performance across diverse populations.

We have been developing an innovative integrated transcriptomics, polygenic, lifestyle and clinical risk score (IRS) for British South-Asians, with transferability to other ancestral populations. Employing state-of-the-art Machine Learning (ML) models and AI algorithms, we have integrated the PRS with transcriptome signatures, clinical factors (consistent with QRISK3 components), and lifestyle information (diet, smoking, alcohol consumption, physical activity). This comprehensive approach aims to develop a high-precision prediction tool for CVD that outperforms existing models in accuracy and reliability.

Digital Twins in Precision Medicine and Personalised Nutrition

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Department of Biology, School of Medicine of the National and Kapodistrian University of Athens (NKUA) & GENOSOPHY P.C.

Nutritional and lifestyle changes remain at the core of healthy ageing and disease prevention. Accumulating evidence underscores the impact of genetic, metabolic and host gut microbial factors on individual responses to nutrients, paving the way for the stratification of nutritional guidelines. However, technological advances that incorporate bioclinical health data at an unprecedented scale and depth conceptualize a future where preventative dietary interventions will exceed stratification and will be highly individualized. We will discuss the transformative capacity of AI to couple multi-modal bioclinical, genetic, metabolomic, lifestyle, immune and gut microbial data, enabling the implementation of a digital replica of oneself, a “virtual digital twin”, which could serve to guide nutrition in a truly personalised manner. Focusing on macronutrient tailoring, we will discuss the impact of genetic variation on responses to carbohydrate, lipid, protein, and fiber consumption. Our bioinformatic analysis of genomic variants guiding macronutrient intake reveals enriched pathways, such as circadian rhythm, melatonin metabolism, cholesterol and lipoprotein remodelling and PPAR signalling as potential targets of macronutrients for the personalised management of obesity. Notably, our *in silico* predictions hint at the potential repurposing of the SYK kinase inhibitor fostamatinib for obesity treatment in relevant genetic profiles. We propose that the application of the digital twin concept may revolutionize the management of non-communicable diseases by realizing the core principles of predictive, preventive, personalised and participatory (4P) medicine towards tangible and long-lasting effects on health.

SELECTED ABSTRACTS - ORAL COMMUNICATIONS SESSION – 1st part

Diagnostic utility of cerebrospinal fluid biomarkers in patients with Alzheimer’s disease

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Background: Alzheimer’s disease (AD) is a chronic, neurodegenerative disease that affects millions of people worldwide. It is multifactorial in nature and involves complex molecular mechanism that causes neuronal degeneration. The first neuropathological changes of the AD can be detected many years prior to the onset of clinical symptoms. Accurately diagnosing neurodegenerative dementia is often challenging due to patient heterogeneity, overlapping clinical features, etc. Numerous studies explored potential biomarkers of AD. Three cerebrospinal fluid (CSF) biomarkers (beta-amyloid 1–42 (A β 42), total tau (t-tau) and phosphorylated-tau (p-tau)) seems to have the highest diagnostic accuracy for early AD diagnosis. CSF biomarkers could be useful for diagnosing of suspected AD patients, independently of the clinical stage, by reflecting the presence of brain amyloidosis (A+) and tauopathy (T+).

Objective: The objective of this study was to examine the utility of CSF biomarkers for the early discrimination of patients in AD continuum from those with non-AD pathologic changes.

Design: The study included 72 patients presented to Neurology Clinic University Clinical Center of Serbia, Belgrade, Serbia (67% with AD). The CSF AD biomarkers levels including A β 42, t-tau and p-tau181 were determined using fully automated

electrochemiluminescence immunoassays on the automated e601 analyzer (Roche Diagnostics, Germany). Two ratios pTau/A β 42 and tTau/A β 42 were calculated. Patients were divided into ATN classes based on CSF biomarkers. The clinical diagnoses were made according to the NIA-AA recommendations.

Results: AD patients had significantly lower median value of A β 42 and higher tTau/A β 42 ($p < 0.001$) compared with non-AD patients. Although with borderline significance ($p = 0.06$), the ratio pTau/A β 42 was higher in AD compared to non-AD group. In AD patients, significant correlation ($p < 0.001$) was found between A β 42 and two ratios, as well as between pTau and tTau. 70 patients in A+ profiles showed dementia (AD, $n = 48$; non-AD, $n = 22$), compared to 2 patients in A–T–N– (non-AD).

Conclusion: Our study showed CSF A β 42 and CSF t-tau/A β 42 ratio are very robust indicators of AD. The Elecsys assays have high analytical performance that may improve CSF biomarker-based AD diagnosis.

Keywords: Alzheimer's disease, biomarker, cerebrospinal fluid.

The spectrum of founder and other novel pathogenic variants in autosomal dominant Alport syndrome in Cyprus

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Background: Autosomal dominant Alport syndrome (ADAS), previously termed thin basement membrane nephropathy (TBMN), results from heterozygous variants in the *COL4A3*/*COL4A4* genes. ADAS is distinct from the more severe autosomal recessive form leading to early kidney failure, before 30-yo, if untreated.

Methods: We assessed families with familial microscopic hematuria (MH) and/or proteinuria using sequencing techniques.

Results: Nearly every Cypriot family affected with ADAS has patients who remain with MH or low-grade proteinuria for life, while some progress to kidney failure. In 2007 & 2009, we reported on 127 patients of 11 Cypriot families with a dual diagnosis of focal segmental glomerulosclerosis and TBMN, segregating heterozygous variants in the *COL4A3*/*A4* genes (Voskarides *K et al*, *JASN* 2007). On long follow-up, up to 30% of the patients developed kidney failure by age 70yrs (mean 55yrs). Later, similar findings were published by several groups around the world. The distribution of the variants in question (*COL4A3*/*A4*) are below: a total of 49 families and 318 patients have the *COL4A3* variant, while 38 families and 165 patients have the *COL4A4* variant, both dispersed all over Cyprus. A notable concentration of 22 families with 205 patients is identified with the *COL4A3*:p.Gly1334Glu variant in Mesaoria, east of Nicosia. Additionally, the *COL4A3*:p.Gly871Cys variant affects 6 families and 36 patients, originating from villages at the northern coast occupied by Turkey since 1974. Lastly, the *COL4A4*:p.Gly1248Arg variant is found in 4 families with 7 patients from south-east Cyprus. In total, 30 mutations were identified, 11 in *COL4A3*, 19 in *COL4A4*, including Gly substitutions, 8 and 13 respectively. Others include small indels and whole exon deletions. *de novo* variants have not been identified.

In a cohort of 155 patients carrying founder *COL4A3*:p.Gly1334Glu, 47 (30%) reached ESKD at ages from 40-90yrs. Rarely do they reach ESKD before 40yrs.

Conclusion: Heterozygous *COL4A3*/*A4* variant carriers risk kidney failure at variable age, which current methods cannot predict. Family history and genetic modifiers are influential. The CYPROME project identified Pathogenic and Likely Pathogenic variants in asymptomatic individuals predisposed to chronic kidney disease (CKD).

SiMS score and siMS risk score increased in type 2 diabetes mellitus patients and development of coronary artery disease

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Background: Abdominal obesity, hyperinsulinemia, hyperglycemia, hyperlipoproteinemia and hypertension are the most evident signs of metabolic syndrome (MS). Microalbuminuria, seen in patients with DM2, CAD and MS is marker of endothelial dysfunction.

Objectives: Analyzing siMS score, as a method for quantification of MS, and siMS risk score for atherosclerosis in patients with myocardial infarction (MI) without DM2 and DM2 patients with and without CAD. Correlation of siMS score and siMS risk score with co-founding factors of MS is evaluated.

Methods: Overweight/obese individuals with Pre-MS and MS (age >45) were classified according to the presence of DM2 and CAD: I-control group- no DM2, no CAD(22), II-MI, no DM2(24), III-DM2, no CAD(25) and IV-DM2 with CAD(70). ATP-III classification was applied for diagnosing MS. Patients with less than three criteria were considered pre-MS.

Results: WC(I-76.7±8.3,II-98.2±12.3,III-96.0±15.2,IV-100.4±9.4cm), BMI(I-22.9±3.2,II-27.9±3.9,III-29.4±6.0,IV-30.6±4.4kg/m²), triglycerides(I-1.0±0.4,II-2.2±1.1,III-2.5±2.0,IV-2.7±2.1mmol/l), HOMA-IR(I-2.6±1.0,II-5.1±3.7,III-8.1±4.6,IV-13.2±4.5mmol/μU/ml), microalbuminuria(I-19.8±5.4,II-45.8±67.0,III-54.4±65.5,IV-74.4±106.2mg/24h), PAI-1(I-2.1±0.5,II-6.3±1.2,III-4.6±2.9,IV-5.3±2.4U/ml), HbA1C(I-5.2±0.2,II-5.2±0.7,III-7.6±1.6,IV-7.9±1.6%). Pre-MS(I-18.2,II-37.5,III-24,IV-36.2%) and MS(I-0,II-41.7,III-28,IV-43.5%). siMS score(I-2.18±0.49,II-3.19±1.04,III-4.5±2.04,IV-4.48±1.42). siMS score correlated with hyperinsulinemia, HOMA IR(p<0.001), PAI-1(p=0.045), microalbuminuria(p=0.05), HbA1C(p<0.01). siMS risk score(I-2.12±1.01,II-4.26±1.58,III-6.4±2.4,IV-5.88±2.11). siMS risk score correlated with hyperinsulinemia, HOMA IR(p<0.001), HbA1C(p=0.005).

Conclusion: Patients with DM2 with or without CAD were characterized by visceral obesity, hypertriglyceridemia, hyperinsulinism, PAI-1, microalbuminuria, unsatisfactory glycoregulation, higher values of siMS score, and siMS risk score compared to the control group and persons with MI and without DM2. Correlation of siMS score and siMS risk score with hyperinsulinemia, IR, PAI-1, microalbuminuria, hypertriglyceridemia and HbA1C confirms the role of MS factors on the progression coronary artery disease.

SELECTED ABSTRACTS - ORAL COMMUNICATIONS - 2nd part

Integrative exploration of metabolic syndrome in older men

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Background: Metabolic syndrome (MetS), a cluster of factors associated with risks of developing cardiovascular diseases, is a public health concern because of its growing prevalence. Considering the combination of concomitant components, their development and severity, MetS phenotypes are largely heterogeneous, inducing disparity in diagnosis.

Objective: The objective of the present work was to better characterize metabolic perturbations in MetS and define a comprehensive MetS signature stable over time in older men.

Design: A case/control study was designed within the Quebec NuAge longitudinal cohort on aging. From a 3-year follow-up of 123 stable individuals, we present a deep phenotyping approach based on a multiplatform metabolomics and lipidomics untargeted approach. A full feature selection strategy was developed to build a comprehensive molecular MetS signature, stable over time.

Results: We characterize significant changes associated with MetS, involving modulations of 476 metabolites and lipids, and representing 16% of the detected serum metabolome/lipidome. These results revealed a systemic alteration of metabolism, involving various metabolic pathways (urea cycle, amino-acid, sphingo- and glycerophospholipid, and sugar metabolisms...)

not only intrinsically interrelated, but also reflecting environmental factors (nutrition, microbiota, physical activity...). These findings allowed identifying a comprehensive MetS signature, reduced to 26 metabolites for future translation into clinical applications for better diagnosing MetS.

Conclusions: The refinement of the comprehensive signature, performed both in terms of measurement reliability, but also by showing the consistent association between the modulated metabolites/lipids and the underlying biological mechanisms, is increasing the value of the proposed biomarker combination within the reduced signature for further investigation and possible clinical application.

Acknowledgements: The NuAge Study was supported by a research grant from the Canadian Institutes of Health Research (CIHR; MOP-62842). All metabolomics and lipidomics analyses were funded and performed within the MetaboHUB French infrastructure (ANR-INBS11-0010).

Preventing Obesity Through Biologically And Behaviorally Tailored Interventions; Description Of The BETTER4U Project

Maria Kafyra on behalf of the BETTER4U Consortium Members

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Background: Obesity is an indispensable underlying cause for many non-communicable diseases. Its growing prevalence and association with adverse health outcomes renders the optimization of prevention measures a public health priority.

Objective: BETTER4U seeks to address the prevalence of obesity in Europe by leveraging modern artificial intelligence (AI) technologies and collaborating with an international, multidisciplinary group of experts.

Design: BETTER4U aims to identify and manage obesity determinants through personalised lifestyle recommendations in 4 phases: Phase 1 will identify all obesity-related determinants through meta-review and meta-analyses of data from current literature and previous projects conducted by the consortium (i.e. genetic, omics layers, microbiota, socio-economic, geographical, cultural and lifestyle features); phase 2 will focus on the creation of a robust mechanism for individualized interventions for obesity prevention, using an AI-enhanced causal model along with federated learning; phase 3 will assess effectiveness and cost-effectiveness through a pilot study and a randomized clinical trial in adults from 7 European sites, where participants will receive personalised recommendations from healthcare professionals via an AI platform and technologically-assisted, real-time monitoring tools, aspiring to result in the BETTER4U integrated methodology, and; phase 4 will focus on the communication and dissemination of said methodology.

Results: BETTER4U aims to provide three-fold results: i) the creation of an extensive dataset for identifying all obesity determinants; ii) the development and use of AI and monitoring tools to derive and implement personalised recommendations; iii) the real-world application of said recommendations through an intervention which will result in personalised methodology guidelines.

Conclusions: Overall, BETTER4U aims to unravel the complex interplay of obesity determinants using AI and monitoring tools to develop and evaluate the efficacy of the novel BETTER4ALL intervention methodology. The project aims to widely distribute the BETTER4U intervention methodology guidelines by making the latter publicly available through public health systems and via using people-centered, sustainable care approaches.

Favorable effects of semaglutides on weight reduction and reducing the risk of chronic diabetes complications

Vesna Dimitrijevic Sreckovic¹, **Ljubica Dimitrijevic¹**, Sasa Ilic¹, Ivan Soldatovic², Gordana Milic¹, Masa Ristic¹, Milica Milosevic¹, Drasko Gostiljac¹

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Background: Semaglutide, GLP-1 receptor agonist, currently occupies an important place in the treatment of diabetes mellitus type 2 (DM2). High dose semaglutide treatment of 2.4 mg/week has showed beneficial effects on body weight reduction and improvement of glycoregulation.

Objectives: The aim of this study was to examine treatment with low doses of semaglutide on anthropometric parameters and glycoregulation in obese patients with DM2.

Methods: The study included 63 obese patients (BMI>30kg/m²) with DM2, who had semaglutide s.c. in a maximum dose of 1mg/week, monitored at 3- and 6-months period. Patients were divided into two groups: I-patients with oral hypoglycemic agents (OHAs) and II- patients with OHAs+insulin in three distinct periods (0- start, 1- 3rd month, 2- 6th month). Anthropometric parameters were measured, and biochemical analysis was performed. siMS score, as a method for quantification of metabolic syndrome (MS) was used.

Results: body weight: (I-0: 119.3±9.1, I-1: 114.4±22.4, I-2: 114.0±23.9kg, II-0: 107.3±15.9, II-1: 101.1±18, II-2: 98.4±14.7kg), WC:(I-0: 122.7±14.6, I-1: 118.7±12.3, I-2: 119.3±14.0cm, II-0: 115.6±9.4, II-1: 113.5±7.2, II-2: 111.1±8.6cm), BMI:(I-0: 39.4±6.2, I-1: 37.7±5.5, I-2: 37.3±5.5kg/m², II-0: 36.7±4.1, II-1: 34.3±3.5, II-2: 33.2±3.6kg/m²), glycaemia:(I-0: 8.2±2.0, I-1: 6.2±1.3, I-2: 6.7±1.8mmol/l, II-0: 11.5±3.5, II-1: 7.5±1.5, II-2: 7.8±2.3mmol/l), HbA1c:(I-0:7.6±0.9, I-1:6.3±0.9, I-2:6.0±0.7%, II-0:8.8±1.4, II-1:7.3±1.4, II-2:7.3±1.7%), siMS score:(I-0:4.39±0.79, I-1:3.72±0.86, I-2:4.05±1.07, II-0:5.10±1.25, II-1:3.64±1.05, II-2:3.96±1.05). After 3 months weight was decreased by 5.1±4.4kg in I group and 5.8±4.1kg in II group, after 6 months by 7.7±5.3kg in I group and 7.4±5.1kg in II group. Statistical significance between groups was obtained for body weight, WC, BMI, glycaemia, HbA1c with statistical significance (p<0.05).

Conclusion: Short-term treatment with low dose semaglutide showed a significant reduction in visceral obesity, BMI, glycemia, HbA1c after 3- and 6-months period, confirmed by a reduction in the siMS score in both groups, though more pronounced in second group. HbA1c was reduced by 1.52% in both groups equally, which is an indicator of a significant reduction in the risk of vascular complications.

Development of target-guided approaches for exosome-associated miRNAs analysis standardization in the context of chronic degenerative diseases

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Background and Aim: Liquid biopsy tests have gained attention as a valuable alternative to traditional diagnostics. Exosomes are small extracellular vesicles (EVs) of 30-150 nm in diameter released by both normal and malignant cells, delivering a set of components, such as proteins or miRNAs, from parental cells to target cells, influencing the phenotype and behavior. Here, we describe a proof-of-principle application of cell-specific exosome targeting allowing the identification, isolation, and molecular characterization and miRNAs analysis of specific exosome populations based on their different antigenic reactivity.

Methods: We successfully demonstrated that tumor B cell-derived exosomes express the IgBCR of their parental B-cells, thus constituting a personal "barcode" of tumor clones that can be subsequently targeted by so-called "Id-peptides." Furthermore, we demonstrated that miRNAs-exosomes' content changes according to therapy response in the context of neurodegenerative dyscrasia. Last, we successfully validated the identification of ACE-2-expressing exosomes in patients affected by SARS-CoV2 as prognostic tools and immunomodulators.

Results: In murine myeloma multiple (MM) model 5T33MM we have specifically targeted and sorted tumor-derived exosomes characterized by the expression of the same Ig BcR of MM cells. Interesting, the analysis of serum MM-released exosomes allowed an earlier detection of MM growth compared to the conventional measure of paraprotein.

In COVID-19 disease we demonstrated an increase of ACE2-expressing exosomes directly proportional to a favorable prognosis. Of interest, these ACE2-expressing exosomes are characterized by specific miRNAs profile involved into immune modulation.

Conclusion: These new shreds of evidence show a promising and novel use of exosomes as biomarker sources for i) the early detection of minimal residual disease in hematological disease compared to conventional protocols, ii) the prediction of treatment response in neurodegenerative disease, and iii) innovative first line immune modulators in infection diseases.

Actionability in regional and private contexts

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Introduction and objectives: One major tenet of modern molecular medicine is the concept of gene/variant actionability. This concept though central to translational genomics is shrouded with limitations of outdated universal approaches.

Frequency maps between human populations and individuals, with far reaching implications to actionability particularly in genetic models of complex inheritance, and non-major genetic effects, like cancer where gene environment interactions have the upper hand in deciding the phenotype.

Methods: Here we present examples of discrepancies in both regional and private contexts impacting actionability in disease like colorectal and breast cancer and furthermore propose AI algorithms such as symbolic regression to circumvent such hurdles and render the variant(s) translation in terms of actionability.

results and conclusions: This approach generates high-performing models that can both predict disease outcomes and reveal putative disease mechanisms. Due to their high performance, simplicity and explicit functional form, these biomarker signatures can be readily explained, thereby making them attractive tools for high-stakes applications in primary care, clinical decision making and patient stratification.

It explained the DNA methylation and predicted 3 biomarkers to be involved as biomarkers in Hepatocellular Carcinoma with an accuracy of 91.3%, a sensitivity of 100%, and a specificity of 87.5% on the test data.

In addition, symbolic regression identified 31 lncRNAs involved in cellular proliferation, tumor metabolism, and invasion-metastasis cascade with SNHG18 and HELLPAR as the highlights of the results.

VEGF CONSORTIUM

Moderator: Sofia Siest, Bernécourt, France – SCs President

LECTURE

Quality matters

Georges Dagher

Inserm, France, Milano-Bicocca University, Italy, Graz, Medical University, Austria, State Key Lab Stem Cells, Chinese Academy of Sciences, China

Human biospecimens provide the basis for research leading to better understanding of human disease biology and discovery of new treatments that are tailored to individual patients with cancer or other common complex diseases. The collection, processing, preservation, storage and providing access to these resources are key activities of biobanks. Biobanks must ensure proper quality of samples and data, ethical and legal compliance as well as transparent and efficient access

procedures. The standards for biobanking outlined herein are intended to be implemented in biobanks and to supply researchers with high quality samples fitted for an intended use.

FRIDAY 24 MAY, 2024

SESSION VIII - Pharmacogenomics: Clinical translation - Now and Future - Part I

Chairs: Ron Van Schaik, Rotterdam, The Netherlands / Charity Nofziger, Anif/Niederalm, Austria

Trends in precision medicine, pharmacogenetics as adjuvant in establishing a correct immunosuppressive therapy of kidney transplant

Sergio Bernardini

Dept. of Experimental Medicine, University of Tor Vergata, Rome, Italy

Allogenic transplant of solid organ is now considered the election clinical procedure in patients with organ failure. Indeed, in the last decades important milestones have been reached especially in kidney transplant. Although the transplanted organ can compensate the function of original one, the main problem remains the immune response by the host organism that can lead to a possible rejection. The management of the immune response is at the basis for a long-life successful organ transplantation. To achieve this objective, drugs targeting the key immunological players of the immunological pathways, fundamental in graft rejection mechanisms have been developed. These can be now used in clinical management of transplanted patients. It is important to note that the wide inter and intra-patient variability of both the pharmacokinetics (PK) and pharmacodynamics (PD) represent the major clinical problem. Although the plasma concentrations of drugs are constantly monitored, variation of these parameters mainly due to the polymorphic status (genetic heterogeneity) of the genes involved in xenobiotic-metabolizing enzymes, transport proteins, and in some cases drug targets, can affect metabolite concentrations in each specific patient. This can induce an important toxicity or loss of efficacy. Numerous studies have been recently conducted to examine the relationship among genetic factors, drug pharmacokinetics, and therapy outcomes. Now several polymorphic genes, influencing drug's efficacy have been catalogued, and they are now easily detected using Next generation sequencing (NGS) methods. Further research, and new investment in creating validated NGS commercial panel for the analysis of pharmacogenetics in renal transplant recipients will represent the future of precision medicine in the management of immunosuppressive therapy.

From Pharmacogenomics to Polygenic Risk Score (PRS) for Drug Response Prediction - Are We There Yet?

Candan Hizek Perry

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Clinical response to pharmacotherapy often varies among individual patients, ranging from beneficial effect to even fatal adverse drug reactions (ADRs.) Pharmacogenomics (PGs) approach, where variation in drug effects is studied as a function of genetic variation at genomic level holds the promise of precision medicine through elucidating genetic determinants responsible for drug therapy outcomes. One of the ultimate goals of genetic research is to inform genomic medicine to enable more effective strategies of disease prevention and treatment. Against this backdrop, as genotyping technology has progressed, being an agnostic approach, GWAS have matured into efficient and effective tools for interrogating the entire genome for genetic variants (most commonly single nucleotide polymorphisms, SNPs) that are associated with a specific disease or a drug response phenotype. Over the last decade personalised genotype-based approach through pharmacogenetic testing is gaining prominence and evidence-based guidelines have been developed accordingly to advise prescribers in daily clinical practice on an optimal drug regimen according to genotypes or predicted phenotypes. However, most of the

time the conventional approach of drug response prediction can not address the polygenic nature of a drug response. Recently PGx study has harnessed the power of GWAS that has enabled the construction of polygenic risk score (PRS) facilitating the field to move beyond the study of candidate genes to scanning hundreds of thousands of genetic markers for each subject. PRS which based on the cumulative effects of numerous SNPs across the genome, mostly developed in the context of predicting the risk of a specific disease and allowing for patient stratification (eg, for cancer, cardiovascular disease, and type 2 diabetes mellitus). However, due to the polygenic nature of most observable drug response outcomes arising from the interplay of multiple genetic and environmental factors, PRS can also be leveraged to assess the genetic susceptibility of an individual to drug-related adverse events, treatment outcomes, and dosage requirements. In sum, the integration of PRS into clinical practice is still evolving, but it holds great potential for enhancing precision medicine by enabling targeted interventions and risk reduction strategies.

An in vitro functional assay for determining CYP2D6 enzyme activity

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Currently, there are 163 distinct *CYP2D6* star alleles officially catalogued in PharmVar, of which 7% are normal function, 10% are decrease or severely decrease of function, 25% are no function, and a whopping 58% are unknown function. With variant testing coverage ever expanding, the latter statistic represents a serious challenge in predicting phenotype from genotype. In an attempt to bridge this knowledge gap, we have developed an in vitro functional assay using a synthetic CYP2D6 substrate and validated it with atomoxetine, a well-known non-synthetic CYP2D6 substrate.

Interindividual variability and pharmacogenomics: Possible ways forward

Munir Pirmohamed

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Interindividual variability in drug response (efficacy and/or safety) is the rule rather than the exception in clinical practice. This can be due to a number of clinical factors and genomic factors. While a genomic variant may play a major role in determining drug response, it is important not to forget about the clinical factors which also contribute. Our approach at present is unimodal (i.e. we look at one factor at a time), and we need to move to more multimodal approaches (combining genetic and clinical factors). This becomes even more important as our population grows older (with decreasing renal and hepatic function) and there is an increasing prevalence of multimorbidity and consequently polypharmacy. This puts them at higher risk of adverse drug reactions, drug interactions, and worse clinical outcomes. Tackling this will be a challenge, but genomics will add value to the usual approach of undertaking medicines reviews. In such populations, panel genotyping is likely to be the most pragmatic, and will enable us to move from reactive to pre-emptive genotyping approaches. This will be more convenient for the clinician and for the patient, as it fits in seamlessly with the current clinical pathways. The questions which need to be evaluated is when people should have panel genotyping undertaken, and which will be the most clinically-effective and cost-effective approach.

SESSION VIII - Pharmacogenomics: Clinical translation - Now and Future – Part II

Chairs: Candan Hızal Perry, Montreal, Canada / Alexander Haushofer, Wels, Austria

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Pharmacogenetics in clinical practice: Experiences and challenges

Ron H.N. van Schaik

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The discovery that the metabolism of drugs is highly variable between patients, but can be predicted by DNA analysis of genes encoding drug metabolizing enzymes or drug transporters, encouraged the field for taking pharmacogenetics into clinical care. Focus was initially on cytochrome P450 enzymes: genotyping for CYP2D6 (involved in the metabolism of 20% of all drugs, but being deficient in 5-10% of the population) and CYP2C19 (involved in metabolism of 20% of drugs, while 2-11% of the population is deficient) could benefit psychiatry, cardiology and oncology therapies. Currently, 15-30 genes for drug metabolizing enzymes and drug transporters that can be (and are) used clinically for optimizing personalization of drug therapy. The field is growing mature as a clinical diagnostic tool, in which we see a shift from reactive single-gene testing to pre-emptive panel testing. In this presentations, successes and challenges for implementing pharmacogenetics into routine health care will be highlighted.

Patient-centered drug therapy integrating Pharmacogenetics and e-health

Adrián Llerena

Pharmacogenetics and Personalised Medicine Unit, INUBE Extremadura Biosanitary Research Institute, Spain.

MedeA is a strategy of Clinical implementation of Pharmacogenetics Personalised/Precision Medicine in Extremadura Public Health Care (1.1. M inhabitants). MedeA will integrate genetic analysis with other relevant information to individualize drug prescription in regular clinical practice within the context of e-health. The main characteristics are the integration of pharmacogenetics analyses (1) plus relevant (2) clinical information for drug prescription based on electronic medical record tools (drug prescription record, physiopathology, biochemistry, hematological analyses, etc) and (3) drug-drug interactions in order to develop a Decision-Supporting Tool (Personalised Prescription System -PoPS-) that will be connected to the Electronical Medical Record System. The Implementation program will be evaluated in a large sample of (5.000 patients). As a consequence, the PPS will be updated to refine the algorithm and later extended for the whole population. To the best of our knowledge this is a very unique Project covering, not only genetics but all factors influencing drug treatment in an algorithm that allow the choice of the better drug for a given patient in the context of drug polytherapy, using AI for evaluation and integrated in the e-health system. Both health professionals and patients will be able to check what is the better drug choice using all information available in the electronical medical record. (www.proyectomedeas.es)

Pharmacogenetics and its appliance in clinical daily life – experiences from the Central Laboratory of the University Hospital of Innsbruck

Andrea Griesmacher, Christian Irsara, Lorin Loacker

Central Institute of Clinical and Chemical Laboratory Diagnostics, University Hospital of Innsbruck, Austria

Introduction. The pharmacogenetic genotype plays a major role in interindividual variability in drug response, absorption, metabolism and pharmacodynamics. Thus, pharmacogenetic testing enables optimizing drug efficacy and minimizing drug toxicity. Although there is increasing awareness for this topic, a regular appliance in clinical daily life is not the standard yet.

Methods. In the central laboratory of the University Hospital of Innsbruck, we have performed several pharmacogenetic parameters since 2012 by PCR-based methods. Among those especially genotyping of Thiopurine Methyltransferase (TPMT) for Azathioprine and 6-Mercaptopurine therapy as well as Dihydropyrimidine Dehydrogenase (DPD) for 5-Fluorouracil or Capecitabine therapy are increasingly requested by haematologists, oncologists and dermatologists. On the other hand, cytochrome P450 genotyping such as CYP3A4/A5, CYP2C9 or CYP2C19 is only rarely in demand for selected cases.

Results. Over the last years the testing requests in the central laboratory of the University Hospital of Innsbruck rose markedly for DPD (2018: 3, 2019: 8, 2020: 84, 2021: 268, 2022: 301) and to a lesser extent for the CYP P450 enzymes (6, 7, 35, 17, 14) while those for TPMT (235, 280, 214, 265, 301) essentially remained stable.

Conclusion. In conclusion, we see an increasing awareness for pharmacogenetic testing. Especially the need for DPD testing has been rising, since several clinical guidelines have recommended a general screening before 5-FU therapy. However, there still is room for improvement and education of medical staff should be continued. Regarding CYP P450 2C9 and CYP2C19 we expect a further increase in the requests due to the newly introduced drugs Siponimod and Mevacamten respectively.

PANEL DISCUSSION WITH SPEAKERS ON PHARMACOGENOMICS moderated by specialists of pharmacogenomics interpretation

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Wolfgang Schnitzel, Anif/Niederalm, Austria

Mark Ruddock, Antrim, United Kingdom

Alexander Haushofer, Wels, Austria

Urs Meyer, Basel, Switzerland

Thomas Wilckens, München, Germany

CLOSING SESSION

Sofia Siest, Bernécourt, France

28 POSTERS

1. Distribution of *CYP1A2* polymorphisms in Tunisian population

S. Chamam^{1,3}, R. Ben Ali^{1,3}, **M. Daldoul**^{1,2,3}, M. Ben Sassi^{1,2,3}, S. Ben Hammamia^{1,2,3}, H. Eljaberi^{1,3}, R. Charfi^{1,2,3}, S. Trabesli^{1,2,3}, E. Gaies^{1,2,3}

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Background: The cytochrome P450 enzyme *CYP1A2* is one of the drug-metabolizing enzymes of major importance as it catalyses the phase I metabolism of a broad range of different drugs. *CYP1A2* activity has been found to be influenced by the presence of polymorphic variants which were reported to display wide interethnic variation.

Objective: The aim of the present study was to determine the prevalence of *CYP1A2* gene polymorphisms in the Tunisian population.

Design: The study was performed in the Pharmacogenetic platform of the National Center of Pharmacovigilance of Tunisia. It included 54 Tunisian volunteers. DNA was isolated from Blood using Blood gDNA MiniPrep System Extraction Kit (PROMEGA, ReliaPREPTM, USA). *CYP1A2* gene was genotyped by Illumina Sequencing Kit using iSeq 100 Sequencer.

Results: Five polymorphisms of *CYP1A2* gene were identified by genotyping: rs762551 (*CYP1A2**1F), rs2740890 (*CYP1A2**1B), rs35694136 (*CYP1A2**1D), rs20669526 (*CYP1A2**1E) and rs2069514 (*CYP1A2**1C). Patients included in the study presented one

(9.25%) or more SNPs (72.22%) which define different allelic variants. In our population, the frequency of the wild type allele was 18.51%. Over 54 patients, *CYP1A2*1F*, *CYP1A2*1B* and *CYP1A2*1D* were the most prevalent alleles present at frequencies of 70.73%, 61.11% and 31.48% respectively while the minor alleles (*CYP1A2*1E* and *CYP1A2*1C*) were present in 18.51% and 12.96% consecutively.

Conclusion and Acknowledgement: According to PharmGKB, these results were different from those described in the African and American populations (60.50% for *CYP1A2*1F* and 45.59% for *CYP1A2*1D*). For *CYP1A2*1F*, the frequency found in the studied population was closer to the Non-Finnish European population (71.18%) while it was similar to the Latin America population (35.01%) for *CYP1A2*1D*. Concerning the minor allele (*CYP1A2*1C*), the frequency found in the Tunisian population appears to be close to that of the world population (12.66%). No prevalence of *CYP1A2*1B* and *CYP1A2*1E* has been published so far.

To our knowledge, this is the first Tunisian study related to pharmacogenetics of *CYP1A2*. On further query further studies concerning the impact of *CYP1A2* polymorphisms and pharmacokinetics for drugs should be performed in Tunisia in the future.

2. Genetic variations of the *CYP2C9* in Tunisian population

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Background: *CYP2C9*, one of the most abundant hepatic cytochromes P450 enzymes, is involved in metabolism of 15–20% of clinically important drugs. To avoid adverse events and/or impaired drug-response, *CYP2C9* Pharmacogenetic testing is recommended.

Objective: The aim of this study was to determine the *CYP2C9* gene polymorphisms and its prevalence in the Tunisian population.

Design: This study was carried out in the Pharmacogenetic platform of the National Center of Pharmacovigilance of Tunisia (CNPV). It was performed on 54 unrelated Tunisian volunteers.

Genomic DNA was extracted from peripheral blood leukocytes using Blood gDNA MiniPrep System Extraction Kit (PROM-EGA, ReliaPREPTM, USA) according to the manufacturer's instructions. Purity and concentration of extracted DNA was determined using Thermo ScientificTM NanDropTM One Spectrophotometer. All subjects were genotyped for the *CYP2C9* by Illumina Sequencing Kit using iSeq 100 Sequencer.

Results and Discussion: Four single nucleotide polymorphisms were identified by the genotyping of *CYP2C9* gene: rs1799853 (*CYP2C9*2*), rs2256871 (*CYP2C9*9*), rs7900194 (*CYP2C9*8*) and rs2057910 (*CYP2C9*3*).

In the present study, the wild type genotype (*CYP2C9*1/*1*) was found in 64.81% of our patients. This result was in agreement with the study of Andreas performed on Caucasian populations.

Over 54 patients, the frequencies of *CYP2C9*2*, *CYP2C9*9*, *CYP2C9*8* and *CYP2C9*3* were 22.22%, 5.55%, 3.7% and 1.85% respectively. Interestingly, *CYP2C9*8* and *CYP2C9*9* are more common in Africans than in Europeans. According to pharmGKB, the frequency of *CYP2C9*2* in our population was 11x higher than the frequency in the African population while the frequencies of the 3 remaining SNPs were more or less similar (2.36% for *CYP2C9*2*, 7.48% for *CYP2C9*9*, 5.49% for *CYP2C9*8* and 1.26% for *CYP2C9*3*).

Our findings suggest that carriers of two mutant alleles (*CYP2C9*2/*2*, *CYP2C9*2/*3* and *CYP2C9*3/*3*) accounted 1.85%. This frequency was in agreement with that found by Andreas and al.

Conclusion: Several studies have shown that *CYP2C9* polymorphisms may impact enzyme activity. Thus, genotyping screening of *CYP2C9* gene prior to pharmacotherapy appear to be justified in order to enhance efficacy and minimize toxicity.

3. Genetic variations of the integrin β 3 in Tunisian population

Mouna Daldoul¹, Khoulood Berrim¹, Mouna Ben Sassi¹, Khoulood Ferchichi¹, Roua Ben Ali¹, Syrine Ben Hammamia¹, Rym Charfi¹, Hanene Jabri², Emna Gaies², Sameh Trabelsi²

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Background: Glycoprotein IIIa is coded by the the integrin β 3 (ITGB3) gene also known as antigen CD61. A platelet-specific antigen (PIA1/A2) polymorphism has been by far the most investigated GPIIIa gene polymorphism. It has been suggested that the PIA2 allelic variant causes an increased sensitivity to platelet aggregation by various agonists and an altered sensitivity to antiplatelet medications such as aspirin and clopidogrel.

Objective: The aim of this study was to determine the gene integrin β 3 polymorphisms and its prevalence in the Tunisian population.

Design: This study was carried out in the Pharmacogenetic platform of the National Center of Pharmacovigilance of Tunisia (CNPV). It was performed on 54 unrelated Tunisian volunteers. DNA was isolated from whole Blood using Blood gDNA MiniPrep System Extraction Kit (PROMEGA, ReliaPREP TM, USA). Purity and concentration of extracted DNA was quantified using Thermo Scientific TM NanDrop TM One Spectrophotometer and was genotyped for integrin β 3 polymorphisms by Illumina Sequencing Kit using iSeq 100 Sequencer.

Results: Among the 54 sequenced samples 20 were found to be carrying at least one alternative allele of the selected variants. The most common mutation in the study population were (T1565C, rs5918) with a frequency of 37%. Within this group, 25% individuals (9% of the study population) were identified as homozygous mutant genotype (c/c) genotype, while 75% individuals were heterozygous (C/T) carriers, demonstrating the presence of both alleles.

Conclusion and Acknowledgment: The homozygous mutant genotype has been rarely reported in the general population; usually less than 1%. Our results in terms of frequency of the ITGB3 rs5918 were similar to the Caucasian population in which the frequency of the (T1565C, rs5918) reported was 21.5%, with 81.9% being heterozygous and 13% homozygous. To better understand the impact of these genetic variations in coronary patients and anticipate their responses to antiplatelet treatments, it is essential to conduct further studies integrating patients' genetic profiles with clinical data.

4. Genetic variations of the methylenetetrahydrofolate reductase gene in Tunisian population

Yasmine Mahjoubi¹, **Mouna Daldoul**^{1,2,3}, Mouna Ben Sassi^{1,2,3}, Ameni Sellemi^{1,3}, Roua Ben Ali^{1,3}, Rim Charfi^{1,2,3}, Riadh Daghfous^{1,2,3}, Emna Gaies^{1,2,3}, S. Trabelsi^{1,2,3}

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Background: Methylenetetrahydrofolate reductase (MTHFR) gene encodes an essential enzyme involved in folate metabolism.

There are two common polymorphisms in the MTHFR gene: the 677 C/T and the 1298 A/C variants. Many studies have shown a significant association between these MTHFR polymorphisms and several disorders such as methotrexate toxicity and cardiovascular disease.

Objective: The aim of this study was to determine the prevalence of the genetic polymorphisms of MTHFR gene in the Tunisian population.

Design: This study was carried out in the Pharmacogenetic platform of the National Center of Pharmacovigilance of Tunisia. It was performed on 55 unrelated Tunisian volunteers. DNA was isolated from whole Blood using Blood gDNA MiniPrep System Extraction Kit (PROMEGA, ReliaPREP™, USA). Purity and concentration of extracted DNA was quantified using Thermo Scientific™ NanDrop™. Illumina Sequencing Kit using NGS technique performed polymorphisms for MTHFR gene.

Results: Two MTHFR SNPs were identified in our population: rs1801133, commonly known as the C677T allele, was present in 27 cases (49%), while rs1801131, also known as A1298C, was found in 25 cases (45.5%).

Patients included in the study presented only one (61.8%) or both SNPs (16.4%). In 21.8% of cases, no variants were found.

Conclusion and Acknowledgments: MTHFR polymorphisms are heterogeneously distributed worldwide. The 677T SNP prevalence is reported to be in Europeans and North Americans, low in East Asians and Africans. Its frequency is of 25.0%, 58% and 9% in Italian Newborns, Mexico City and West Africa, respectively.

The 1298C SNP has the highest frequency in East Asia and the lowest in Africa. Its frequency is of 25.7% and 13.9%, in South China and West Africa, respectively.

Advancements in MTHFR genotype and the introduction of personalised treatments will help to predict, prevent, and reduce the occurrence of several complications including MTX-related toxicity. Larger population-based studies are needed to provide a more comprehensive assessment of MTHFR mutation frequencies across different regions in Tunisia.

5. HLA-A*3101 polymorphism incidence in the Tunisian population

Khouloud Ferchichi^{1,2}, **Mouna Daldoul**^{1,2,3}, Mouna Ben Sassi^{1,2,3}, Khouloud Berrim^{1,2}, Ines Medini^{1,3}, Syrine Ben Hammamia^{1,2,3}, Hanene El Jebari^{1,3}, Rim Charfi^{1,2,3}, Riadh Daghfous^{1,2,3}, Emna Gaies^{1,2,3}, Sameh Trabelsi^{1,2,3}

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Background: Carbamazepine could be responsible for different hypersensitivity reactions forms. literature data demonstrated that some HLA alleles were strongly associated with carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis in the Asian populations but not in European populations.

Objective: This study aimed to describe the frequency of the HLA-A genetic polymorphisms in a Tunisian population.

Design: This study was carried out in the Pharmacogenetic Unit of the Clinical Pharmacology Department at the National Center of Pharmacovigilance in Tunisia. DNA was isolated from whole Blood for 54 unrelated Tunisian volunteers using Blood gDNA MiniPrep System Extraction Kit (PROMEGA, ReliaPREP™, USA). The purity and concentration of extracted DNA were quantified using Thermo Scientific™ NanDrop™ One Spectrophotometer and was genotyped for HLA-A*3101 polymorphisms by Illumina Sequencing Kit using iSeq 100 Sequencer.

Results: Among the 54 sequenced samples, two carried one alternative allele (heterozygous) of the HLA-A*3101 (rs1061235) mutation with a frequency of 0.04%. No other HLA-A polymorphism was detected. Our data contrasts with the prevalence of this HLA-A*3101 allele in European populations found to be between 2 and 5%. The presence of this allele showed an increase in the risk of induced carbamazepine hypersensitivity reactions from 5.0% to 26.0%. Yet, its absence reduced the risk from 5.0% to 3.8%.

Conclusions: To better determine this genetic polymorphism in the Tunisian population, multicenter studies combining a patient's genetic profile with clinical data are required.

6. N-Acetyltransferase 2 in Tunisian population: Balance between phenotyping and genotyping methods

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Background: Tuberculosis (TB) remains the major cause of death from infectious diseases. Tunisia maintains an intermediate TB endemicity. Isoniazid, a key agent in TB treatment, is primarily metabolized by the arylamine N-acetyltransferase 2 (NAT2) enzyme. The identification of NAT2 polymorphisms proves valuable in predicting both effective therapeutic doses and potential adverse effects of isoniazid. Traditionally, acetylator status have been differentiated based on phenotyping tests. More recently, genetic methods enabled the identification of NAT2 alleles. The concordance and/or discordance of NAT2 genotypes and phenotypes has always sparked discussion.

Objective: This work consists of a brief literature review focusing on the balance between phenotyping and genotyping methods of the NAT2 gene in Tunisia.

Design: A search of Pubmed, Science direct and Google scholar databases was conducted using the following keywords: N-acetyltransferase 2, Genotype-phenotype, discordance, Tunisia.

Results: In Tunisia, various studies have examined the acetylation profile of isoniazid in the adult population and revealed a high frequency of slow acetylator (SA) genotypes and phenotypes in Tunisian population.

Based on these studies, it was found that the SA phenotype had a prevalence of approximately 58%, whereas the rapid acetylator (RA) phenotype accounted for the remaining 42%.

Regarding the distribution of slow NAT2 alleles in the Tunisian population, the frequencies of NAT2*5, NAT2*7 and NAT2*14 alleles were 50%, 3.75%, and 0.39%, respectively.

N. Jebabli et al have compared acetylation phenotype with NAT2 genotype in Tunisian population and showed a concordance value of 79%. Results also show a good sensibility (98.2%) of NAT2 phenotyping for the detection of SA.

Conclusion and Acknowledgments: This work emphasizes the importance of finding an equilibrium between phenotyping and genotyping approaches when investigating the NAT2 gene in Tunisia. Integrating both methods can provide valuable insights into individual acetylator status, contributing to the optimization of drug therapy. Prospective clinical trials are essential to more evaluate the advantages of NAT2 genotyping in Tunisian population.

7. Pharmacogenetics of antiepileptic drugs in Tunisian population: A brief review

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Background: Epilepsy is a common, serious neurological disorder worldwide, with an estimated prevalence of 3.7 per 1000 in Tunisia [1]. In recent years, studies have demonstrated that genetic variation is involved in either drug response to antiepileptic drugs (AEDs) or susceptibility to adverse reactions.

Objective: This work provides a brief overview of the pharmacogenetic findings of AEDs in Tunisia.

Design: Databases were identified using PubMed, ScienceDirect and Google Scholar databases. The search strategy was applied, using the following keywords: pharmacogenetics, antiepileptics, tunisia, drug-resistant epilepsy, genes.

Results: Studies about pharmacogenetics of AEDs in tunisian population have identified multiple key genes involved in both drug metabolism and transport:

Two Tunisian studies have evaluated the association of ATP-Binding Cassette sub-family B, member1 (ABCB1) gene polymorphisms with treatment response.

M. Ajmi et al reported that the G2677T T and C3435T T alleles were associated with an increased risk of AEDs resistance, while the presence of the C1236T T allele did not seem to affect drug response. However, M. Chouchi et al demonstrated that the three polymorphisms were all involved in AED resistance.

Various cytochrome-P450 (CYP) isoenzymes play a role in AEDs metabolism. Genetic variations in CYP2C9 and CYP2C19 genes have been associated with phenytoin toxicity. Abdelhedi et al's study found no significant difference in the frequency of CYP2C19*2, CYP2C9*2, and CYP2C9*3 alleles between the Tunisian population and other populations. Notably, the CYP2C19*3 allele was absent in the Tunisian population.

Furthermore, in relation to susceptibility to adverse drug reactions, association between the HLA-A*31:01 allele and carbamazepine induced DRESS syndrome was observed within the Tunisian population.

Conclusion and Acknowledgments: Integration of pharmacogenetic testing into clinical practice has a potential to improve epilepsy management and to enhance patient care in Tunisia.

More research is needed to elucidate the role of genetic variants in influencing the response to these drugs, and to expand the current knowledge base. particularly for newer AEDs.

8. Pharmacogenetics of immunosuppressants in Tunisia: A literature review

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Background: Immunosuppressants (IS) are known for their narrow therapeutic index and a high risk of life-threatening drug- toxicity. The pharmacokinetics and dynamics of these drugs are subjects to significant inter and intra individual variability. To optimize treatment outcomes, a critical need to emphasize the impact of pharmacogenetics in clinical practice is justified, ensuring the delivery of optimal and personalised therapeutic regimens.

Objective: This work provides a brief overview of the pharmacogenetic findings of IS in Tunisia.

Design: A literature review was performed using databases including PubMed, ScienceDirect, Google Scholar. Search Terms were a combination of keywords such as: pharmacogenetics, precision medicine, Polymorphism, Tunisia, immunosuppressant. A total of 12 papers were retrieved and analyzed.

Results: 67% of the studies in Tunisia, focused on tacrolimus, predominantly in renal transplantation.

Tacrolimus associated gene CYP3A4 and CYP3A5 were mostly studied. For CYP3A4, the homozygous wild-type CYP3A4*1/*1 was predominant ranged from 60 to 80% the CYP3A4*1B and CYP3A4*22 were also detected. For CYP3A5, the CYP3A5*3 variant was the most frequent allele detected. (60 %)

The thiopurine drugs represented 25% of the articles, the wild-type TPMT*1(*Thiopurine S-methyltransferase*) allele was predominant. But, variations were observed in the detection of non-functional alleles across the studies. ITPA (inosine triphosphate pyrophosphatase) studies revealed a majority with the homozygous wild-type genotype. Mycophenolate Mofetil studies were centered on UDP-Glycosyltransferase(UGT), organic anion transporting polypeptides(SLCO), and Inosine monophosphate dehydrogenase IMPDH polymorphisms, associating UGT1A9 98T>C UGT1A9-275T with diarrhea and anemia, while IMPDH II 3757T>C, UGT1A9-275T and UGT1A9-2152C>T increased the risk of acute and chronic rejection. Yet, no relationship between those polymorphisms and exposure to MMF was demonstrated.

Conclusion: These studies highlight the impact of pharmacogenetics in clinical practice by making more accurate predictions on the optimal dosage, the likelihood of toxicity and adverse effects. Which could allow for more personalised and thus, more effective and safer usage of IS in Tunisia.

9. Prevalence of *CYP2C19* gene polymorphisms in Tunisian population

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Background: The *CYP2C19* gene, a member of the cytochrome P450 gene family, is highly involved in the metabolism of several drugs. It is highly polymorphic with at least 35 allelic variants. These polymorphisms result in an altered enzyme activity and thus may explain some of the interindividual differences in treatment response.

Objective: The aim of the present study was to investigate the frequency distribution of *CYP2C19* genetic polymorphisms in the Tunisian population.

Design: This study was carried out in the Pharmacogenetic platform of the National Center of Pharmacovigilance of Tunisia. It was performed on 54 Tunisian volunteers.

Peripheral blood samples were collected in EDTA tubes and stored at –20 °C until the DNA was isolated by Blood gDNA MiniPrep System Extraction Kit (PROMEGA, ReliaPREP™, USA). *CYP2C19* genotyping was performed by Next Generation Sequencing with Illumina Sequencing kit (AmpliSeq™ Custom DNA Panel for Illumina, USA).

Results: Two common allele variants of the *CYP2C19* gene were identified by genotyping: *CYP2C19**2 (rs4244285) and *CYP2C19**17 (rs12248560).

Homozygous carriers of *CYP2C19**1 allele were classified as normal metabolizers and were assigned if *CYP2C19**2 and *CYP2C19**17 variants were absent. Over 54 individuals, the wild type allele (*CYP2C19**1) was present in the majority of individuals at the frequency of 66.66%. *CYP2C19**2 allele was observed in 18.51% of which 90% were homozygous and 10% were heterozygous. *CYP2C19**17 allele was detected in 16.66% of the studied population of which homozygous genotype (*CYP2C19**17/*17) represented 88.88% and the heterozygous one represented only 11.11%. Referring to PharmGKB, these Pharmacogenetic profiles were found to be similar to the African and African American populations (17.74% for *CYP2C19**2 and 22.15% for *CYP2C19**17).

Conclusion and Acknowledgment: According to the literature, *CYP2C19* gene polymorphisms are known to affect enzyme function yielding individual differences in drug metabolism capacity from poor to ultra-rapid metabolizers. To the better understanding of the impact of these variations on our population, further studies with a larger cohort combining patient's genetic profile, clinical data and pharmacokinetic studies are required.

10. Prevalence of polymorphisms affecting clozapine metabolism in Tunisian population

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Background: Clozapine is an atypical antipsychotic used to treat resistant schizophrenia. However, due to its high inter- and intra-individual pharmacokinetic variability and its association with various side effects, clozapine is not considered as a drug of choice in first-line treatment. Several studies have shown that some genetic variations are likely to affect clozapine metabolism, consequently plasma concentration and clinical response. These variations include polymorphisms of the CYP1A2, CYP2D6, MTHFR, DRD1, HTR2C and ABCG2 genes.

Objective: This present study aims to determine the prevalence of genetic polymorphisms of these genes in the Tunisian population.

Design: This study was carried out at the pharmacogenetics platform of Tunisia's National Pharmacovigilance Center (CNPV). DNA was isolated from 78 unrelated Tunisian volunteers using the Blood gDNA MiniPrep System extraction kit (PROMEGA, ReliaPREP TM, USA). Genotyping was performed with the Illumina Sequencing Kit using the iSeq 100 sequencer. The Pharmacogenetics panel includes 16 variations (SNPs and InDels) from genes associated with clozapine metabolism, including: six for CYP1A2 (rs2069514, rs35694136, rs762551, rs72547516, rs56107638, rs72547517); six for CYP2D6 (rs1135840, rs28371725, rs16947, rs5030655, rs1058164, rs1065852); two for MTHFR (rs1801131, rs1801133); one for DRD1 (rs4532); one for HTR2C (rs3813929) and one for ABCG2 (rs2231142).

Results: Among the 78 sequenced samples, 75 were found to be carrying at least one alternative allele and 72 had more than one polymorphism of selected variants. The most common mutations in the study population were rs4532 DRD1 and CYP2D6*2 (rs16947) CYP2D6 with a frequency of 67.94% for both. Three CYP1A2 variants (rs72547516, rs56107638, rs72547517) and one CYP2D6 variant (rs5030655) were not detected in any sample.

Conclusion: In order to assess the effect of the two most occurring polymorphisms on clozapine metabolism in the Tunisian population, it remains essential to integrate pharmacokinetic and clinical factors. This multidisciplinary study can potentially facilitate individualized dosing in clinical practice and reduce adverse effects.

11. Human leukocyte antigens alleles and cutaneous adverse drug reactions to carbamazepine: A Literature Review

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Background: Recent years have witnessed the emergence of genetic polymorphism from Human leukocyte antigens (HLA) genes as a powerful tool in predicting cutaneous adverse drug reactions (cADRs) to carbamazepine. Of all the reported HLA variants, HLA-A*31:01, HLA-B*15:02 and HLA-A*24:02 have been most commonly reported for their associations with cADRs.

Objectif: The aim of our review was to summarize the heterogeneity of common HLA alleles to susceptibility to cADRs in patients treated with carbamazepine.

Design: A search of the literature in pubmed, Medline and the Cochrane database was carried out for all the articles investigating the association of HLA variants with cADRs in patients treated with carbamazepine. Databases were searched up to 31 January 2024.

Results: In north Africa, one study was performed to investigate the genetic predisposition of this population to carbamazepine-DRESS syndrome. This study identified the HLA-A*31: 01 allele in 57.1% of cases of carbamazepine induced DRESS syndrome, nevertheless, HLA-B*15:02 was not detected in this population.

In the European population, Cormack and al showed the presence of the HLA-A * 31: 01 in 40% of cases This association was also notified in the Korean and the North American population.

Some authors have suggested another association between HLA-B*15: 02 and DRESS syndrome, however studies performed on Chinese and Vietnamese populations didn't find a significant association and HLA-B*15:02 was associated with the occurrence of stevens Johnson syndrome and toxic epidermal necrolysis. Finally, Shi YW et al confirmed in 2017 a strong association between HLA-A*24:02 and SJS induced by the aromatic antiepileptic drugs in the southern Han Chinese . In the north African study HLA-A*24:02 was not present in any patients. This may be related to the small sample size.

Conclusion: A considerable heterogeneity exists in the literature concerning the role of different HLA alleles in carbamazepine induced cADRs among diverse ethnic groups.

HLA-A*31:01 allele was significantly associated with carbamazepine-DRESS syndrome in various populations and recently in north African population. This discovery is beneficial to forecasting carbamazepine-DRESS syndrome in these populations.

12. Evaluation of the analytical performance of hormone on the Alinity i analyzer and the application of the “six sigma” principle

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Background: Six Sigma provides a quantitative framework for process evaluation and more objective evidence for process improvement in the medical laboratory.

Objective and design: The aim of the work was to evaluate the analytical characteristics of the determination of sex hormones on the Alinity i analyzer (Abbott Laboratories, Abbott Park, Illinois, U.S.A.), by calculating the sigma value. For all examined parameters, sigma values were calculated according to the formula $\text{Sigma} = (\text{TEa} - \text{bias}) / \text{Kv}$, where all values in the formula are expressed as percentages (%). TEa values were taken from three sources: Clinical Laboratory Improvement Amendment (CLIA), EuBIVAS (European Federation of Laboratory Medicine, EFLM) and Ricos database of desirable specifications of analytical quality based on biological variation (Desirable Biological Variation Database Specifications, DBVDS). Kv values for all hormones were calculated from internal quality control data on the Alinity i immunochemistry analyzer. Bias values for each analyte were calculated based on the results of the monthly external quality control program in which the laboratory participates (Randox International Quality Assessment Scheme, RIQAS).

Results: Based on the results obtained according to the CLIA preferred specifications, 25% of the tests have a mean value of sigma 3, 33% of the tests have a mean value of sigma 4, 17% of the tests have a mean value of sigma 5, 25% of the tests have a mean value of sigma 6. Based on the results obtained according to EuBIVAS preferred specifications, 13% of tests have mean sigma < 3, 31% of tests have mean sigma 3, 19% of tests have mean sigma 4, 6% of tests have mean sigma 5, 31% of tests have mean sigma 6. Based on the results obtained according to the DBVDB preferred specifications, 14% of the tests have a mean sigma value < 3, 14% of the tests have a mean sigma value of 3, 29% of the tests have a mean sigma value of 4, 7% of the tests have a mean sigma value of 5, 36% of the tests have mean sigma value 6.

Conclusion: The analytical performance of the Alinity i analyzer (Abbott Laboratories, Abbott Park, Illinois, U.S.A.) cannot reach the current requirements of the preferred performance specifications (EuBIVAS and DBVDB), while the CLIA specifications are more adequate for use.

13. Diagnostic potential of microRNAs miR-19a, miR-92a, miR-193a and miR-210 in colorectal cancer

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Background: Colorectal cancer (CRC), whose previous stage is colorectal adenoma (preCRC), is one of the most common cancers in Croatia. MicroRNAs (miRs) are non-coding RNAs involved in cancer malignancy, and they are stable in blood and tissues. The aim of this study was to assess diagnostic potential of miRs (miR-19a-3p, miR-92a-3p, miR-193a-3p and miR-210-3p)

in CRC by comparing their levels of expression in formalin-fixed paraffin-embedded (FFPE) tissue samples between CRC and pre-CRC patients. Also, we determined the difference in expressions of chosen miRs between FFPE tissue samples and exosomes obtained by liquid biopsy.

Objective and Design: Blood samples of 19 CRC patients were collected by liquid biopsy in CellSave tubes (Menarini Silicon Biosystems). MiRNeasy Serum/Plasma Advanced Kit (Qiagen) was used for exosomal miRs isolations. MiRs from tissue samples of 44 preCRC and 25 CRC patients were isolated with miRNeasy FFPE Kit (Qiagen). Reverse transcription of miRs was performed with miRCURY RT LNA kit (Qiagen). Expression levels were determined on 7500 Real-Time PCR System (Applied Biosystems) with miRCURY LNA SYBR GREEN PCR Kit (Qiagen). MiR-103a was used as a reference and UniSp6 as an internal control. The Mann-Whitney test was used for statistical analysis (MedCalc).

Results: Expression levels of miR-19a-3p ($P=0.002$), miR-92a-3p ($P=0.001$), miR-193a-3p ($P<0.0001$) and miR-210-3p ($P=0.002$) showed statistically significant difference between CRC and preCRC tissue samples. Exosomal miR-19a-3p and miR-210-3p were significantly elevated compared to their tissue levels ($P=0.023$ and $P=0.003$, respectively), while there was no significant difference between miR-92a-3p ($P=0.544$) and miR-193a-3p ($P=0.120$) levels.

Conclusions: Four chosen miRs represent a promising tool for distinguishing CRC and preCRC. Possible biomarkers for CRC diagnosis using liquid biopsy could be miR-19a-3p and miR-210-3p but their potential should be examined on larger number of samples.

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14. Influence of alcohol consumption on biochemical and hematological parameters in abstinent chronic alcoholics

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Background: Chronic intake of alcohol leads to tissue and organ damage. The degree of damage is directly related to the amount and length of alcohol consumption, type and drinking pattern. This leads to a progressive disruption of metabolic activity, structure and function of liver cells and blood tissues, and chronic inflammation.

Objective: The study aim was to examine the influence of chronic alcohol consumption on these parameters, relative to the presence and absence of liver cirrhosis, anemia, inflammation, length, and consumption pattern, in chronic alcoholics in abstinence.

Design: The research included 70 chronic alcoholics, 35 of whom had alcoholic liver cirrhosis. Concentrations of biochemical and hematological parameters were determined by routine laboratory methods and shown as median (Me) and inter-quartile Range (IQR).

Results: It was found that patients with liver cirrhosis have significantly higher values of direct bilirubin (Me: 13.8 $\mu\text{mol/L}$, IQR 8.24-28.8), AST (Me: 40 U/L, IQR 29-53), γ -GT (Me: 61 U/L, IQR 46-116), ALP (Me: 94 U/L, IQR 84-125) ($P<0.05$) due to liver damage and lower albumin value (Me: 36 g/L, IQR 33-38), transferrin (Me: 1.8 g/L, IQR 1.4-2.8) and fibrinogen (Me: 2.4 g/L, IQR 1.7-3.1) ($P<0.05$), indicating reduced synthetic function of the liver. Reduced triglyceride values (Me: 0.93 mmol/L, IQR 0.75-0.99) with reduced cholesterol (Me: 3.12 mmol/L, IQR 2.62-3.92), HDL (Me: 0.97 mmol/L, IQR 0.81-1.12) and LDL (Me: 1.56 mmol/L, IQR 1.45-2.31), can be used for differential diagnosis of cirrhosis from early stages of liver damage. The presence of inflammation was shown, CRP (Me: 7.7 mg/L, IQR 4.2-13.4) and sedimentation (Me: 28 mm/h, IQR 18-45) were elevated ($P<0.05$). In patients with cirrhosis, the presence of anemia was confirmed (83%), namely macrocytic anemia (23%), normocytic anemia (51%) and sideropenic anemia (9%). In patients without liver cirrhosis, the presence of macrocytosis (26%) without signs of anemia was found. In patients with cirrhosis, thrombocytopenia (Me: $88 \times 10^9/\text{L}$, IQR 67-119) and lymphopenia (Me: $1.42 \times 10^9/\text{L}$, IQR 1.27-1.69) were confirmed.

Conclusion: The results of this study confirm that long-term alcohol abuse leads to the progression of metabolic disorders, alcoholic liver disease and cirrhosis, the development of anemia and thrombocytopenia.

15. Genetic variations associated with thrombophilia risk: A study on F2(rs1799963), F5(rs6025) and MTHFR (rs1801133, rs1801131) polymorphisms in a South-Eastern Caucasian population

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Background: This study investigates Single Nucleotide Polymorphisms (SNPs) of genes F2(rs1799963), F5(rs6025), and MTHFR (rs1801133, rs1801131) associated with thrombophilia risk.

Objective and Design: The objective is to document the polymorphism frequency distribution in a sample of South-Eastern European Caucasians, examining potential associations with gender and comparing results with other European populations and global data.

A total of 860 volunteers from the South-Eastern Caucasian population, spanning both genders and ages 18 to 89 years, participated in the study. DNA was extracted from buccal swab samples, and genotyping was conducted using KASP-PCR. The study focused on analyzing genotype and allele distribution of the specified polymorphisms, followed by a comprehensive statistical analysis of the results.

Results: Contrary to gender-based expectations, the findings revealed no statistically significant difference in the distribution of any studied polymorphisms between male and female volunteers. These results align with previous research on polymorphism frequency distribution. Overall, this study contributes valuable insights into the genetic landscape of thrombophilia risk in the South-Eastern Caucasian population.

16. Ensuring harmonized laboratory diagnostics: Stability of pooled sera for proficiency testing programs

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Background: Proficiency testing programs are essential to ensure the accuracy of laboratory values and, thus, standardized diagnostics. Widely available pooled sera can be a viable option to provide a standardized matrix that closely mimics actual patient samples while ensuring individual sample anonymity. Given the delay between pooling and analysis in participating laboratories, evaluation of storage-related variability is critical to ensure test accuracy and material suitability for specific markers.

Objectives: We tested ten serum pools over two weeks to assess their stability for clinical chemistry and endocrinology parameters.

Design: Ten pools were generated by the Biobank of the Medical University of Vienna from residual sera collected at the Department of Laboratory Medicine, Medical University of Vienna. At the time of pooling, the material was stored for three days to ensure that all routine tests were completed. Pools were analyzed immediately (baseline) and at 1, 2, 3, 4, 7, and 14 days at room temperature (RT) or refrigerated (2–10°C). All analyses were performed on either Roche cobas or DiaSorin Liaison instruments.

Results: In the refrigerated sample, the mean deviation from baseline values remained <5% for 36/50 analytes over the 14 days. It was between 5 and 10% for six analytes (ASAT, GIDH, CRP, FT3, FT4, beta-HCG) and >10% for the remaining eight (ALAT, LDH, HBDH, PTHi, Progesterone, Folate, 25-OH and 1,25-OH2-Vitamin D). At RT, 26/50 markers were stable (<5% deviation). Notably, LDH, HBDH, and FT3, which were unstable in the refrigerated sample, were robust at RT. In addition to the other markers, which were also unstable under refrigeration, only phosphate, total bilirubin, CK, triglycerides, fructosamine, prolactin, and insulin (all >10%) and, to a lesser extent, alkaline phosphatase, creatinine, LDL, HDL, oestradiol and vitamin B12 (all 5–10%) showed relevant deviations at RT.

Conclusions: Pooling of sera provides a homogeneous sample that is stable for two weeks and allows laboratory performance evaluation in different diagnostic tests while maintaining patient confidentiality.

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17. Plasma nucleic acids as potential predictors of statin associated muscle symptoms

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Background: Statin-associated muscle symptoms (SAMS) are the most common undesirable side effect (USE) of widely indicated statin therapy. So far, appropriate biochemical markers allowing early prediction and confirmation of the causal association between SAMS and statin use are missing.

Objective: To examine if plasma cell-free nucleic acids (miRNAs, nucleic and mitochondrial DNAs) could be new markers of muscle damage.

Design: Using quantitative PCR, we analyzed plasma concentrations of three muscle-specific miRNAs (133a-3p, 1-3p, and 23a-5), nucleic DNA (markers at IL-6 and FTO genes) and mitochondrial DNA (two independent markers) in a total of seventeen adult subjects (13 men and 4 women) with acute coronary events who were treated with statins after (but not before) the event. One sample was available before statin treatment and three samples during the first year of statin treatment. Because of the variance in cell-free nucleic acid concentrations, values were compared to the untreated sample (arbitrary standardised values of 1.00 for each subject/marker analyzed).

Results: Seven of seventeen (i.e. 41%) subjects reported different degrees of SAMS on treatment. The relative change of unadjusted concentrations (significant at $P < 0.05$ in two cases) of all three miRNAs analyzed was (when compared with the first examination) different in subjects with SAMS (e.g., miRNA 1-3p; $1.00 \rightarrow 1.06 \pm 0.28 \rightarrow 1.07 \pm 0.25 \rightarrow 1.10 \pm 0.40$) compared with subjects without ($1.00 \rightarrow 1.43 \pm 0.52 \rightarrow 1.54 \pm 0.74 \rightarrow 1.52 \pm 0.69$). Changes of cell free DNAs in time were independent on SAMS presence.

Conclusions: Concentrations of regulatory miRNAs (but probably not cfDNA) may be markers of muscle damage induced by statin treatment.

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18. Sex-differences in triglyceridemic genetic risk scores and risk of myocardial infarction

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Background: Increased plasma triglyceride (TG) levels are an independent risk factor for the development of cardiovascular disease, including myocardial infarction (MI). Final TG levels are influenced by both environmental (such as dietary habits or physical activity) and genetic factors.

Objective: To focus on potential sex differences in the genetic determination of MI using the genetic risk score (GRS).

Design: Single nucleotide polymorphisms (SNP) within 10 genes (*GCKR*, *APOE*, *APOA5*, *CAPN*, *NAT2*, *FRMD5*, *TYW1B*, *LPL*, *CYP26A1* and *CILP*) have been genotyped in controls (890 males and 1341 females) and MI patients (913 males and 680 females). Only adults aged between 18 and 65 years at the time of examinations have been included. Based on the comparisons of individual SNPs, male-specific (mGRS) and female-specific (fGRS) GRSs have been created.

Results: With two exceptions (*APOA5* and *GCKR*), SNPs were associated with an increased risk of MI with *P* between 0.01 and 0.1 in either males, females or both. However, for five genes (*CAPN*, *FRMD5*, *TYW1B*, *LPL*, and *CILP*), the risk alleles differed between males and females. Regardless of the type of comparison, fGRS was not associated with an increased risk of MI in females and was not informative in males. Males with mGRS values greater than 6 were under increased risk of MI (OR; 95% CI = 1.85; 1.34-2.56; *P* < 0.0005) when compared with subjects with mGRS 3 and less. mGRS was not informative for females (OR; 95% CI = 0.84; 0.57-1.25; *P* = 0.40).

Conclusions: The genetic risk of increased TG seems to be associated with an increased risk of MI only in males. Sex differences need to be taken into account and sex-specific GRSs need to be created to estimate the genetic risk of MI associated with triglycerides.

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19. *SLC2A9* interacts with thiazides to moderate hyperuricaemia risk: A UK Biobank study of 109,140 hypertensive participants

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Background: The solute carrier family 2 member 9 (*SLC2A9*) gene has been previously reported to moderate hyperuricaemia risk in hypertensive participants treated with thiazide diuretics.

Objective: We aimed to replicate the urate *SLC2A9*-thiazide interaction and also identify additional loci that interact with thiazide-use to increase the risk of hyperuricaemia and other thiazide-related disturbances such as hyperglycaemia.

Design: Following approval from the UK biobank (application ID: 56653), we obtained two analysis cohorts both comprising unrelated hypertensive participants of ‘White British’ ancestry: a) a discovery cohort (*N*=95,493; 25% on self-reported Bendroflumethiazide treatment at recruitment), and b) a validation cohort (*N*=13,647; 25% on self-reported thiazides other than Bendroflumethiazide). In the discovery cohort, we performed genome-wide variance quantitative trait locus (vQTL) analysis of ~6.2 million SNPs (including those located in/near *SLC2A9*) and four relevant outcomes (blood glucose, serum urate, urine potassium and urine sodium) that were measured by the UK Biobank. Using a stringent significance threshold (*P*<2.0E-9), vQTLs enriched for thiazide-interacting loci were identified and these underwent gene-environment interaction (GEI) tests with a Bonferroni-adjustment of the 0.05 statistical significance threshold.

Results: During vQTL analysis, eight loci were identified for the two blood-related outcomes (urate: 3 loci; glucose: 5 loci) and none for the urine-related outcomes. When GEI testing was undertaken for the serum urate outcome, only the previously reported loci (*SLC2A9*) had significant GEI effects in the discovery, but not validation cohort. For blood glucose, only one (*CDKAL1*) of the five loci was significant, again in the discovery, but not validation cohort.

Conclusion: In conclusion, we conducted vQTL analysis followed by GEI testing to replicate a previously known locus (*SLC2A9*:thiazide interaction for serum urate/hyperglycaemia) and a novel biologically plausible locus (*CDKAL1*:thiazide interaction for blood glucose/hypoglycaemia). Understanding gene-environmental interactions is an important step towards precision medicine as it can help identify patients who may require enhanced monitoring for thiazide-related adverse effects.

20. Adherence to dietary patterns modifies the effect of genetic predisposition for increased BMI levels on body fat levels of Greek adults with overweight or obesity

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Background: Current research highlights the significance of gene-diet interactions on measurements of body composition indices such as body fat (kg). Hereby, results are presented for participants of the iMPROVE study, who participated in a dietary intervention following a hypocaloric high-protein or high-carbohydrate diet, for 3 months.

Objective: The present study sought to explore potential associations between gene-diet interactions and baseline body fat (kg) in adult participants of the Greek iMPROVE study.

Design: We used baseline data from 202 participants of the iMPROVE cohort. We tested for associations between logBodyFat levels (kg) at baseline and the interaction of previously extracted dietary patterns (i.e. the Mixed, Med-proxy, Eating-out, Traditional, vegetarian-alike and High in unsaturated fats and fruit juice consumption patterns) with 10 candidate variants known for their association with BMI (namely rs6548238, rs1801282, rs2241766, rs925946, rs1421085, rs1121980, rs17817449, rs3751812, rs9939609, rs17782313). The level of statistical significance was corrected based on the Bonferroni method. All associations were examined through linear regression models adjusted for age and sex, using the PLINK version 1.9.

Results: Carriers of the BMI-raising rs3751812-T and rs9939609-A alleles who adhered to the “Traditional, vegetarian-alike” pattern displayed nominally significantly lower levels of logBodyFat (kg) ($\beta_{\text{interaction}} = -0.026$, $p_{\text{interaction}} = 0.023$ and $\beta_{\text{interaction}} = -0.030$, $p_{\text{interaction}} = 0.008$, respectively). On the contrary, carriers of the BMI-raising rs3751812-T allele who adhered to the “High in unsaturated fats and fruit juice consumption” pattern showed nominally higher levels of logBodyFat (kg) ($\beta_{\text{interaction}} = 0.046$, $p_{\text{interaction}} = 0.026$).

Conclusions: Adherence to certain dietary patterns appears to modify the effect of genetic predisposition on body fat levels of Greek adults. Such findings could potentially denote a mitigating effect of balanced diets on the genetic risk for obesity.

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21. Generation of a polygenic risk score for MASLD prediction based on MRI-PDFF in UK Biobank

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Background/Objectives: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the leading cause of liver-related morbidity and mortality. Although, the invasive liver biopsy remains the golden standard for MASLD diagnosis, magnetic resonance imaging-derived proton density fat fraction (MRI-PDFF) is an accurate, non-invasive method for the assessment of treatment response. The scope of this study was to develop a Polygenic Risk Score (PRS) to improve MRI-PDFF prediction using MRI-PDFF data derived from the UK Biobank, in order to assess an individual's genetic liability to MASLD.

Methods: We iteratively sequestered 10% of MRI-PDFF samples as a validation set and split the dataset into base and target partitions, containing Genome-Wide Association Studies summary statistics and raw genotype data, respectively. We used PRSice2 in order to extract PRS candidates. Based on the frequency of each SNP appearance, we generated different SNP sets according to variable frequency cutoffs. By applying the PRSs to the validation set, we identified the optimal SNP set, which was then applied to the Hellenic Nonalcoholic fatty liver disease (NAFLD) study.

Results: Implementation of the designed pipeline, using data from 3,553 UK Biobank participants resulted in 49 different SNP sets. After calculating the PRS on the validation set for every SNP set, an optimal PRS with 75 SNPs was selected (incremental $R^2=0.025$, p -value=0.00145). Interestingly, 43 out of 75 SNPs were successfully mapped to MASLD-related known genes. The selected PRS could predict traits, like LDL cholesterol and diastolic blood pressure in the UK Biobank, as also MASLD outcome in the Hellenic NAFLD study.

Conclusion: Our findings provide strong evidence that PRS is a powerful prediction model for MASLD and strengthens the application of such methodology in order to enhance PRS integration in clinical practice. In addition, we showed that the optimal PRS can be applied on populations of different ethnicity.

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22. Frequency distribution of rs1801133 and rs1801131 polymorphisms of the MTHFR gene in patients with epilepsy

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Background: Epilepsy, a prevalent neurological disorder with a substantial genetic component, affects over 50 million individuals worldwide. The methylenetetrahydrofolate reductase (MTHFR) enzyme, essential in homocysteine metabolism and influencing diverse biological processes, has been implicated in regulating cellular functions. Notably, MTHFR gene mutations, specifically C677T and A1298C, have been reported to reduce enzyme activity. Previous studies suggested a potential correlation between decreased MTHFR activity and epilepsy susceptibility.

Design: In this study, blood samples from 214 patients with generalized and/or focal epilepsy were collected and subjected to DNA isolation and genotyping for MTHFR C677T and A1298C polymorphisms using the KASP assay.

Results: Results were compared with a control group of 1411 individuals. Statistical analysis, employing the chi-square test via MedCalc software, revealed no strong correlation between MTHFR polymorphisms and epilepsy susceptibility overall. However, intriguingly, an analysis of MTHFR C677T polymorphism indicated a potential association with early-onset epilepsy, particularly in patients aged 0-5 years. The T allele exhibited higher frequency in this subgroup compared to the control group (30% vs. 18%, OR=2.5, p -value=0.02).

Conclusion: This study suggests that, on a general scale, MTHFR polymorphisms may not significantly impact epilepsy susceptibility. Nevertheless, the observed association with early-onset epilepsy warrants further investigation. Future

research could elucidate the specific role of the MTHFR C677T polymorphism in this subgroup, providing valuable insights into the genetic underpinnings of early-onset epilepsy.

23. Investigating the Role of Inflammation-Related Genetic Polymorphisms in the Occurrence of Multiple Sclerosis: A Study in the Greek Population

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Background: The etiology of multiple sclerosis (MS) remains elusive, with a potential interplay between environmental factors and genetic variants influencing disease susceptibility.

Objective and Design: This study, comprising 200 patients diagnosed with MS and 207 non-patients volunteered from Greece, explores the frequency of two inflammation-related genetic polymorphisms (rs1800795-IL6, rs1800629-TNFA). These polymorphisms have been associated with MS in other populations. Whole blood samples were collected from participants, followed by DNA isolation. Genotype analysis for the investigated polymorphisms was performed using KASP PCR, and the results underwent thorough statistical analysis.

Results and Conclusion: The statistical analysis of genotype distribution for the studied polymorphisms between patient and control populations yielded no statistically significant results (p -value > 0.05). Based on the data from this study, no correlation appears to exist between the investigated polymorphisms and the occurrence of MS in Greek patients. However, further studies involving a larger population sample are warranted to validate these findings and provide additional insights into the role of these polymorphisms in the occurrence of MS.

24. Gait Alterations in the Prediction of Metabolic Syndrome in Patients With Schizophrenia: A Pilot Study With PODOSmart[®] Insoles

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Background: Second-generation antipsychotics (APs) are associated with metabolic syndrome (MetS), characterized by abnormal pro-inflammatory cytokine production and oxidative stress due to the reduced antioxidant systems, and neurological effects, including mobility impairment. This pilot study investigated relationships between inflammatory-metabolic biomarkers, MetS and gait alterations in patients with psychosis treated with APs.

Methods: Patients with psychosis treated with APs, 20 with MetS (MPS group) and 20 without MetS (PS group) were studied, using anthropometric data, blood measurements and gait analysis performed with the PODOSmart[®] gait analysis device.

Results and Discussion: The MPS group had significantly higher mean body mass index (BMI) and arterial blood pressure (BP) than the PS group. PODOSmart[®] gait analysis recorded significant differences between groups in pronation-supination at Heel Off (HO), gaitline HO and gaitline Toe Off (TO). Multifactorial elastic net regression models demonstrated significant association with MetS of inflammatory markers, specific AP2 treatment, gender, age; BMI; BP and smoking (accuracy λ = 0.08), and in relation to gait parameters (accuracy λ = 0.750), the three pronation-supination variables, i.e., at HO, flat foot in (AP2 related) and TO, and propulsion speed. The gait parameters were at the edges of the model, thus indicating a more significant role of these parameters compared to the other clinical variables. Early diagnosis of MetS in patients with schizophrenia via identification of gait alterations can be a screening measure for serious cardiovascular complications related to psychosis and APs, to enable timely dietary intervention that can control the pro-inflammatory state and reduce oxidative stress.

25. Diagnostic accuracy of platelet count, mean platelet volume and platelet to lymphocyte ratio in colorectal carcinoma

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Background: Clinical values of platelet count (PC) and mean platelet volume (MPV), as the parameters of whole blood count, as well as MPV/PC ratio and platelet to lymphocyte ratio (PLR), have been studied in different types of malignant tumours. In our recent study, we found that patients with colorectal carcinoma (CRC) have significantly higher PC and PLR and significantly lower MPV and MPV/PC compared to patients with adenoma. Our research aimed to study diagnostic accuracy of PC, MPV, MPV/PC ratio and PLR in distinguishing patients with CRC and patients with adenomas.

Objective and Design: The study included 155 patients who were divided into two groups according to pathohistological results; 74 patients with adenomas and 81 patients with confirmed CRC. A blood sample was collected in a K₃EDTA tube after overnight fasting. A routine examination of the complete blood count was conducted on Sysmex XN1000 (Sysmex Inc, Kobe, Japan). MPV/PC values were calculated from the MPV and PC, while PLR was calculated from the platelet count and the absolute lymphocyte count. Statistical analysis was performed using ROC curve analysis on MedCalc® Statistical Software version 22.014 (MedCalc Software Ltd, Ostend, Belgium).

Results: ROC curve analysis showed good diagnostic accuracy for PC (AUC = 0.72, 95% CI 0.65 – 0.79, P < 0.001, Se = 69.41%, Sp = 70.27%, cut-off value >238), MPV/PC (AUC = 0.72, 95% CI 0.64 – 0.79, P < 0.001, Se = 81.48%, Sp = 55.41%, cut-off value ≤ 0.04) and PLR (AUC = 0.71, 95% CI 0.64 – 0.78, P < 0.001, Se = 62.96%, Sp = 77.03%, cut-off value > 150.88). MPV exhibited the lowest diagnostic accuracy (AUC = 0.63, 95% CI 0.55 – 0.71, P = 0.004, Se = 58.02%, Sp = 66.22%, cut-off value ≤ 9.3).

Conclusion: PC, MPV/PC and PLR showed good diagnostic accuracy in differentiating between patients with CRC and patients with adenomas.

Acknowledgment: The Croatian Science Foundation funded this research, grant number IP-2019-04-4624 (Project “Genetic, protein and RNA profiling of colorectal cancer using liquid biopsy”).

26. Inflammation-related biomarkers from complete blood count in patients with colorectal carcinoma

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Background: Inflammation has a very important role in the process of carcinogenesis, and chronic inflammation affects all stages of tumor development. The clinical usefulness of inflammation-related biomarkers available from routine complete blood (CBC) examination such as LWR (lymphocyte-to-leukocyte ratio), LMR (lymphocyte-to-monocyte ratio), NMR (neutrophil-to-monocyte ratio) and LCR (lymphocyte-to-CRP ratio), has been studied in many types of cancer. The aim of this study was to research whether there are differences in leukocyte count, neutrophil, lymphocyte and monocyte count, as well as in calculated values from those parameters LWR, LMR, NMR and LCR between patients with colorectal carcinoma (CRC) and patients with adenomas.

Objective and Design: The study included 74 patients with pathohistologically confirmed adenomas and 81 patients with confirmed CRC. A blood sample was collected in a K₃EDTA tube after overnight fasting. CRP was measured on Alinity ci-series

(Abbot Diagnostics, Abbot Park, IL, USA). CBC was analysed on Sysmex XN1000 (Sysmex Inc, Kobe, Japan). LWR, LMR, NMR and LCR values were calculated. Statistical examinations were performed using the Mann-Whitney-test.

Results: Leukocytes [8.3 (6.9-11.1) vs 7.20 (6.2-9.3) $\times 10^9/L$; $P=0.006$], neutrophils [5.70 (4.49-8.02) vs 4.82 (3.88-6.05) $\times 10^9/L$; $P=0.005$], monocytes [0.70 (0.60-0.89) vs 0.63 (0.50-0.78) $\times 10^9/L$; $P=0.007$] were significantly lower in CRC patients. Lymphocytes [1.81 (1.50-2.30) vs 1.58 (1.29-2.10) $\times 10^9/L$; $P=0.059$] were similar between CRC and adenoma groups. All calculated values, LWR [0.21 (0.18-0.25) vs 0.22 (0.18-0.26); $P=0.432$], LMR [2.50 (1.94-3.29) vs 2.60 (2.10-3.27); $P=0.492$], NMR [8.04 (6.33-10.05) vs 8.00 (6.35-9.85); $P=0.983$], LCR [0.27 (0.09-0.56) vs 0.36 (0.16-0.68); $P=0.153$] were similar in both groups.

Conclusion: CRC patients have lower leukocytes, neutrophils and monocytes count, however our study did not show differences in the LCR, LMR, LWR and NMR between patients with CRC and adenoma.

Acknowledgment: This research was funded by the Croatian Science Foundation, grant number IP-2019-04-4624 (Project: Genetic, protein and RNA profiling of colorectal cancer using liquid biopsy).

27. Interventions to monitor and tackle obesity at European level: lessons learned to support BETTER4U project

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Background: Obesity is an ongoing public health emergency. Literature highlights the importance of assessing and implementing comprehensive interventions. Previous interventions (i.e. the European Childhood Obesity Surveillance Initiative (COSI), the CO-CREATE project (COC) and the BigO project) have set the basis for the identification of obesogenic determinants.

Objective: To describe previous and ongoing projects for monitoring and tackling obesity at European level to further support the BETTER4U project.

Design: BETTER4U includes contributions from previous and ongoing projects regarding, among others, the analysis of determinants and monitoring of childhood overweight and obesity rates across Europe (COSI), and policy changes for healthy eating and physical activity targeting adolescent obesity (COC). COSI is a system measuring trends in childhood overweight and obesity for over 15 years and providing nationally representative analysis of the associated determinants from over 500 000 children. COC aimed to co-create policy ideas for obesity prevention, where adolescents from 5 European countries actively participated. The multicenter trial conducted during the BigO project, developed a decision support system for measuring and tracking obesogenic behaviors in free living children and adolescents in relation to their local environment. Similarly, BETTER4U will implement a lifestyle intervention in 7 European countries, using artificial intelligence, and monitoring tools to implement personalised recommendations for obesity prevention.

Results: Collection of the 2007–2017 COSI data in 11 European countries showed that the prevalence decreased in countries with high prevalence and remained stable or slightly increased in the other countries. In the COC project, 60 adolescents developed system maps to identify the factors driving obesity. Three novel policies were co-created along with researchers and were further discussed with relevant stakeholders at national and European level.

Conclusions: Successful community-based approaches from previous European projects provide valuable insight for obesity prevention. BETTER4U will integrate all described results to develop and successfully deliver the proposed lifestyle intervention.

28. Saliva Proteome of Primary Sclerosing Cholangitis: A Pilot Study to demonstrate its clinical utility

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Background: Primary sclerosing cholangitis (PSC) is a rare chronic inflammatory liver disease characterized by biliary strictures and cholestasis. The aetiology and pathogenesis of PSC remain very poorly understood, making difficult the development of effective pharmacological therapies, which are actually aimed at treating symptoms and managing complications. In addition, the lack of serological markers for a certain diagnosis remains a challenge.

Objective: In this project, we analysed the saliva proteome to discover proteins that could be used as novel markers for a non-invasive screening and diagnosis of PSC.

Design: EVs-enriched saliva from 10 PSC patients and 10 healthy controls were analysed using a mass spectrometry technique, and a bioinformatic approach was applied to detect the differentially expressed proteins, their related biological functions and pathways, and the correlation with the clinical evidence in order to identify possible markers for the PSC group.

Results: We identified 25 differentially expressed proteins in PSC patients when compared to the healthy control group. Among them, disulfide-isomerase A3 and peroxiredoxin-5 exhibited an area under the curve values of 0.900 and 0.865, respectively, suggesting these saliva proteins as good discriminators between the two groups. The point-biserial analysis highlighted significant positive correlations between the increased serum alkaline phosphatase levels and the salivary amounts of core histone macro-H2A.1 and immunoglobulin lambda variable 3-19. Moreover, significant positive correlations were also identified between the presence of ulcerative colitis and the salivary levels of nicotinamide phosphoribosyl-transferase and hyaluronan synthase 1.

Conclusion: To the best of our knowledge, this is the first salivary proteomics study based on PSC patients and healthy subjects in which we succeeded in demonstrating the potentiality of saliva as a useful biofluid in biomarker discovery for non-invasive screening and diagnosis of PSC.

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