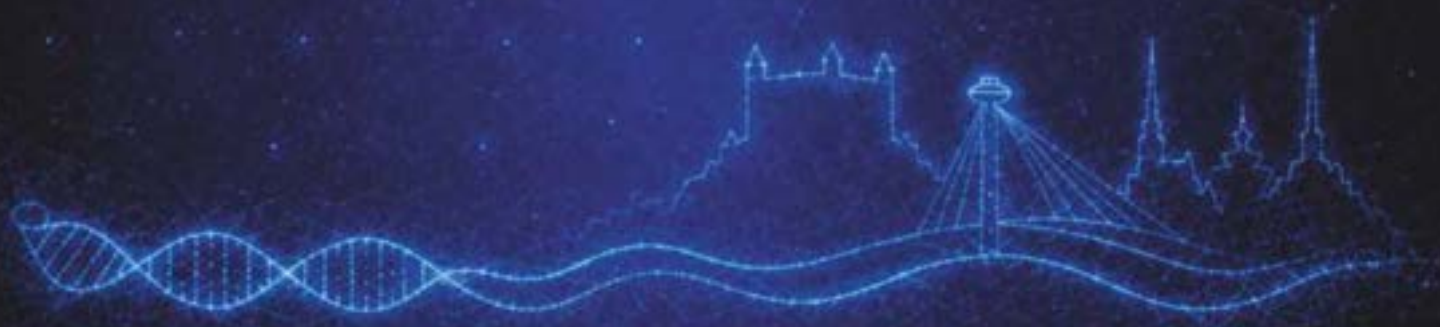


**7th Central & Eastern
European Congress
on Free Nucleic Acids**



ABSTRACT BOOK

**September 10-11, 2026
Bratislava, Slovakia**

Keynote & Invited Lectures (I01 - I14)

Young Scientific Poster Session (Y01 - Y15)

Regular Scientific Poster Session (R01 - R11)

I01.

Detection of Occult Malignancies in Pregnant Women Using Cell-Free DNA

Diana W. Bianchi

National Institutes of Health, Bethesda, MD, USA

The development of massively parallel sequencing techniques has made it possible to efficiently and cost-effectively determine the sequence of cell-free (cf) DNA fragments circulating in the blood of pregnant women. In a little over a decade cfDNA sequencing has translated to routine clinical care as a non-invasive screen for fetal chromosome aneuploidies. In approximately 1 in 8,000 tests, however, the laboratory's algorithm is unable to interpret the test results. Occult maternal malignancy is one potential explanation for these so-called nonreportable results. In my presentation I will discuss the prospective IDENTIFY study (NCT04049604) performed at the NIH Clinical Center in Maryland, USA, that aims to identify biological causes underlying nonreportable cfDNA sequencing results and to generate evidence to inform reporting guidelines and the subsequent diagnostic workup and treatment of pregnant women with cancer. At enrollment, study participants may be pregnant or up to two years postpartum and perceive themselves to be asymptomatic. Enrollees undergo a standardized cancer screening evaluation, including history and physical examination, whole-body magnetic resonance imaging (MRI), repeat research cfDNA sequencing using a common genome-wide platform, fecal occult blood test, pap/HPV test, and hematological and biochemical laboratory tests. When available, placentas are analyzed for confined placental mosaicism. As of July 2026, we have enrolled over 320 participants and ~ 52% of them have a solid tumor that is shedding cfDNA into their circulation. MRI was critical for detecting malignancy in this asymptomatic cohort. We are exploring additional noninvasive biomarkers. Our results highlight the importance of a timely and thorough clinical evaluation of pregnant women to permit treatment as soon as possible during pregnancy. We hypothesize that detecting and treating maternal malignancies before development of symptoms will improve health and extend life.

102.

Leveraging the Biology of Cell-Free DNA for Enhancing the Performance of Liquid Biopsy Approaches

Irena Hudecova

Astellas Pharma, Addlestone, UK

While circulating tumour DNA (ctDNA) has transformed precision oncology, applications such as minimal residual disease (MRD) monitoring remain challenging due to the low abundance of tumour-derived signals. Tumour-informed ultrasensitive approaches have been developed to address these challenges, and are expected to play an increasingly important role in clinical care. In contrast, tumour-naïve methods, which do not have access to tumour tissue, can improve sensitivity by integrating features that reflect the underlying biology of cell-free DNA (cfDNA). This presentation will explore how the biological characteristics of cfDNA, particularly its fragmentation patterns, can be leveraged to enrich tumour-derived signals. We will discuss how integrating these distinct biological features into a multi-modal framework can enhance assay sensitivity. The presentation will highlight technical innovations designed to capture previously overlooked aspects of cfDNA biology, including single-stranded cfDNA, as well as novel sampling approaches such as dried blood spots, to enable point-of-care testing and longitudinal cancer monitoring. Finally, we will discuss ongoing clinical trials evaluating some of these approaches and their utility in real-world settings.

103.

Fragmentomics analysis of cell-free DNA as a new dimension for liquid biopsy

Wai Kei Jacky Lam

The Chinese University of Hong Kong, Hong Kong SAR

Fragmentomics analysis of cell-free DNA (cfDNA) has emerged as a rapidly advancing area in liquid biopsy, leveraging the principle that cfDNA fragmentation in blood is non-random and reflects underlying biological processes in health and disease. Building on our earlier work on preferred end motifs on the 5' end, we revisited the role of cfDNA termini in light of limitations in conventional sequencing workflows that introduce artificial end repair and obscure native 3' end information. Using single-stranded DNA sequencing, we captured the true 5' and 3' end sequence features (EM5 and EM3), together with the nucleotide sequence characteristics immediately upstream and downstream of these ends (PREM and POEM), to construct a holistic end-motif profile of plasma cfDNA. In a hepatocellular carcinoma (HCC) model, comprehensive analysis integrating EM5, EM3, PREM, and POEM outperformed traditional approaches that relied solely on 5' end features. Beyond diagnosis, we further explored whether fragmentomics could inform future cancer risk, using nasopharyngeal carcinoma (NPC) as a model. Applying fragmentomics to plasma Epstein–Barr virus (EBV) DNA, we identified NPC-associated molecular features that conferred an 87-fold increased risk of developing NPC over four years compared with individuals negative for plasma EBV DNA. This risk-informed fragmentomics framework enables more accurate stratification of individuals into distinct risk tiers, thereby optimising surveillance intensity and intervals according to underlying molecular risk, with important implications for healthcare resource allocation in endemic settings.

104.

Unraveling the genetics of cell-free DNA using GWAS

Jasper Linthorst

Amsterdam UMC, Department of Human Genetics, Amsterdam, The Netherlands

Plasma cell-free DNA (cfDNA) is a biomarker of increasing importance. It enables non-invasive screening applications, such as Non-invasive Prenatal Testing (NIPT) and Cancer Liquid Biopsies, in which differences in fragmentation patterns of cfDNA are extensively used in complex predictive models. However, the biology behind these patterns is not completely understood. Additionally, the biological mechanisms behind cfDNA are linked to (auto)immunity, inflammation, and coagulation.

To explore the effect of common genetic variants on concentration and fragmentation properties of cfDNA, we performed multiple genome-wide association studies (GWAS) on imputed genotypes obtained from low-coverage whole genome sequencing data from over 100.000 Dutch NIPT screens.

We found that all cfDNA properties studied have significant, partially distinct, heritable components. We detected hundreds of genome-wide significant loci. A common missense variant in DNASE1L3 (p.Arg206Cys) has the strongest effect on most cfDNA traits. Especially in samples which are imputed to be homozygous for this variant, sequenced fragments were longer, less frequently ended in CC/GG and plasma cfDNA concentrations were lower. For the clinical application of NIPT, we show that the altered fragmentation patterns cause predictors to overestimate fetal DNA fractions. Despite this apparent increase in fetal DNA, the reduced cfDNA concentration causes a 14x increased odds of NIPT failure. In addition, we found significant genetic correlations between our cfDNA traits and GWAS of blood traits, systemic autoimmune diseases and thrombosis.

In conclusion, we find that a considerable fraction of the variation in cfDNA measurements is attributable to common genetic factors which should be addressed in future clinical applications of cfDNA.

105.

Advancing Early Cancer Detection: Translational Liquid Biopsy Studies at MMCI

Roman Hrstka, Kateřina Ondrášová, Vít Weinberger, Milan Anton, Matouš Cwik,
Petr Karásek, Ondřej Horký

Masaryk Memorial Cancer Institute, Brno, Czech Republic

Research Centre for Applied Molecular Oncology (RECAMO)

Department of Gynecology and Obstetrics, University Hospital Brno and Faculty of Medicine, Masaryk University

Early cancer detection remains one of the major challenges in modern oncology, particularly for malignancies lacking effective screening strategies or presenting with limited symptoms at early stages. We have been developing several translational liquid biopsy approaches across multiple cancer types, integrating molecular diagnostics with prospective clinical studies.

We investigated molecular analysis of uterine lavage fluid for detecting endometrial precancerous lesions and early-stage endometrial cancer. In a prospective case-control study of 257 women with endometrial intraepithelial neoplasia (EIN), early-stage endometrial carcinoma, and healthy controls, we analyzed 22 genes associated with endometrial carcinogenesis. The study demonstrated the feasibility of mutation profiling from uterine lavage samples but also revealed limitations related to specificity and background mutation burden in controls.

We further analyzed circulating tumor DNA in serum from 110 colorectal cancer patients using ddPCR-based detection of KRAS, NRAS, and BRAF mutations and compared the results with matched tumor tissue. The study demonstrated the applicability of ctDNA analysis as a complementary tool for molecular diagnostics and disease monitoring while illustrating current limitations in sensitivity and concordance.

Building on previous lipidomic studies showing promising diagnostic performance in pancreatic cancer, the SCREPAN academic study was launched, incorporating KRAS mutation testing as a complementary biomarker strategy for high-risk individuals.

Together, these studies illustrate both the opportunities and limitations of translational liquid biopsy approaches for early cancer detection and highlight the importance of integrating molecular technologies with clinically well-characterized patient cohorts.

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106.

Improving early detection of cancer in Lynch Syndrome through liquid biopsy innovations

Mev Dominguez-Valentin

Department of Tumor Biology, Oslo University Hospital, Oslo, Norway

Lynch syndrome is one of the most common hereditary cancer syndromes and is caused by pathogenic variants in mismatch repair genes, conferring a high lifetime risk of colorectal, endometrial, and several other cancers across multiple organs. Although current surveillance strategies have improved outcomes, they still rely largely on invasive procedures and do not adequately address the need for tolerable, scalable, and multi-organ early detection approaches for the growing population of individuals at risk. This presentation highlights emerging liquid biopsy strategies as a promising frontier in precision oncology for Lynch syndrome, with the potential to enable earlier, less invasive, and more personalized cancer detection. Particular emphasis is placed on the Horizon Europe–unded PREDI-LYNCH project, a six-year Pan-European initiative designed to validate non-invasive liquid biopsy-based tests in 2,000 Lynch syndrome carriers recruited across 27 clinical centers in 9 countries, supported by a consortium of 28 partners across 16 European countries and a €3.6 million investment. The project integrates clinical surveillance, multi-omic technologies, artificial intelligence–riven risk prediction, and biobanking to improve detection of colorectal, endometrial, and urothelial cancers while also evaluating cost-effectiveness, health impact, and ethical, legal, and social acceptability. By moving beyond painful and repetitive invasive surveillance toward risk-adapted, patient-centered, and clinically actionable testing, this work aims to advance precision prevention and support future policy integration and adoption of non-invasive screening strategies for individuals with Lynch syndrome.

107.

Clinical utility of cfDNA and ctDNA in testicular germ cell tumors

Michal Mego

Faculty of Medicine, Comenius University & National Cancer Institute, Bratislava, Slovakia

Testicular germ cell tumors (TGCTs) are among the most curable solid malignancies; however, clinically relevant challenges remain in risk stratification, early relapse detection, assessment of post-chemotherapy residual disease, and management of relapsed or refractory tumors. Conventional serum tumor markers and imaging are essential components of current care, but their limitations are well recognized, particularly in marker-negative disease, stage I surveillance, viable residual disease, and teratoma.

Liquid biopsy represents a promising complementary strategy in TGCTs. While circulating miR-371a-3p has emerged as the most extensively validated marker of active germ cell tumor burden, cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) may provide additional and biologically distinct information. Total cfDNA may reflect not only tumor burden, but also systemic inflammation, treatment-related tissue injury, and overall disease activity. In contrast, ctDNA enables tumor-specific molecular characterization, including estimation of tumor fraction, genomic instability, and copy-number alteration burden.

Our previous work has shown that circulating biomarkers are clinically informative in TGCTs. Pretreatment miR-371a-3p positivity was associated with metastatic burden, IGCCCG risk group, treatment response, and survival outcomes, supporting the value of liquid biopsy as a dynamic marker of disease biology. Emerging data further suggest that cfDNA and ctDNA may be particularly useful in advanced and relapsed/refractory TGCTs, where conventional prognostic tools remain imperfect and early identification of treatment failure is clinically important. The clinical utility of cfDNA and ctDNA is likely to be context-dependent. Potential applications include refinement of minimal residual disease assessment, monitoring of treatment response, early relapse detection, and prognostic stratification before salvage treatment. Key limitations include low ctDNA fraction in selected patients, assay standardization, uncertain performance in teratoma, and the need for prospective validation. Ultimately, cfDNA and ctDNA should be integrated with miRNA, imaging, pathology, and clinical parameters to personalize the management of patients with TGCTs.

108.

The role of whole genome sequencing in the diagnosis of hereditary colorectal cancer predisposition syndromes

Vince Kornél Grolmusz

*Department of Clinical and Molecular Genetics, National Institute of Oncology, Budapest, Hungary
Lendület "Momentum" Hereditary Cancers Systems Biology Research Group, Hungarian Academy of Sciences -
National Institute of Oncology
HUN-REN-NIO-TTK-HCEMM Oncogenomics Research Group*

Hereditary colorectal cancer (CRC) predisposition syndromes encompass hereditary polyposis and nonpolyposis syndromes. Germline pathogenic variants in APC result in familial adenomatous polyposis, while germline pathogenic variants in genes contributing to the mismatch repair machinery are associated with the most frequent hereditary CRC predisposition, Lynch syndrome (LS). Although most pathogenic variants are located within coding exons, noncoding variants can also contribute to disease. For example, variants that activate deep intronic cryptic splice donor or acceptor sites may induce the exonization of noncoding sequences, thereby conferring pathogenicity.

Our research group is interested in the comprehensive germline and somatic analyses in rare and complex hereditary CRC predisposition. The first part of the presentation will introduce our laboratory-developed workflow on including whole genome sequencing (WGS) in the diagnostic workup of LS. Following the diagnostic multigene panel test, multiple tissue- and RNA-based assays are used to prioritize samples for WGS. In the second part of the talk an integrative framework will be presented to decipher carcinogenic mechanisms in complex CRC predisposition syndromes. In cases where multiple germline pathogenic variants can individually increase CRC risk, somatic molecular testing including immunohistochemistry, specified MSI testing and comprehensive genomic profiling are applied to detect relevant mechanisms.

In summary, comprehensive germline and somatic molecular analyses can optimize the diagnostic workflow of hereditary CRC syndromes and can identify key molecular mechanisms driving carcinogenesis in complex CRC predisposition.

109.

Analysis of longitudinal cancer data in circulating DNA

Simone Ciccolella, Tomáš Szemes, Marcel Kucharík, Juraj Gazdarica

Department of Informatics, Systems and Communication, University of Milano-Bicocca, Milan, Italy

Geneton Ltd. Bratislava, Slovakia

Cell-free DNA (cfDNA) sequencing has emerged as a powerful, minimally invasive tool for liquid biopsy in oncology. By analyzing cfDNA reads, we can reconstruct copy number variation (CNV) profiles of cancer patients before, during, and after treatment. However, the clinical utility of longitudinal cfDNA analysis is frequently hampered by the extremely low sequencing coverage typical of these samples, which limits the resolution and reliability of CNV calls and complicates direct comparison across timepoints and patients.

To address this, we present novel clustering methodologies designed to associate CNVs across multiple timepoints despite sparse, low-coverage data. Our computational framework serves a dual objective. First, it effectively tracks intra-patient tumor evolution and genomic changes over time. Tracking these temporal shifts enable us to monitor tumor evolution under therapeutic pressure and to evaluate treatment efficacy and adapt personalized therapeutic interventions for specific individuals.

Second, our method identifies recurrent subsets of hallmark CNVs that are shared across a diverse population of patients with the same tumor type. Isolating these consensus genomic signatures reveals candidate biomarkers for more accurate, early-stage cancer diagnosis.

Together, these two directions, individual-level tracking and population-level hallmark discovery, demonstrate how low-coverage cfDNA CNV clustering can support both personalized treatment monitoring and broader diagnostic applications, despite the technical constraints inherent to liquid biopsy data.

I10.

Translational Significance of microRNAs as Diagnostic and Prognostic Biomarkers in Colorectal Cancer

Jasenska Wagner Kostadinović, Andreja Bartulić, Dominik Seletković, Luka Klobučarić

Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

Colorectal cancer (CRC) remains one of the most common malignancies worldwide and a major cause of cancer-related mortality. Since prognosis strongly depends on the stage at diagnosis, there is a growing need for reliable, minimally invasive biomarkers that could complement current screening and diagnostic approaches. Circulating microRNAs (miRNAs), small non-coding RNA molecules involved in post-transcriptional regulation of gene expression, have emerged as promising candidates for liquid biopsy due to their remarkable stability in serum, plasma, and extracellular vesicles.

Dysregulated miRNA expression contributes to colorectal carcinogenesis by affecting key biological processes, including cell proliferation and apoptosis, inflammation, angiogenesis, epithelial–mesenchymal transition, invasion, and metastasis. These effects are mediated through major signalling pathways involved in CRC development and progression, particularly Wnt/ β catenin, TGF- β PI3K/AKT/mTOR, and MAPK/ERK. Depending on their molecular targets, miRNAs may function as oncogenic miRNAs or tumour suppressors. Among the most extensively investigated candidates, miR-21 and miR-92a show particularly strong potential as diagnostic and prognostic biomarkers, while miR-122 has also demonstrated high diagnostic accuracy. Tumour-suppressive miRNAs, including miR-30a-5p and exosomal miR-150-5p, may be particularly valuable for postoperative monitoring.

Current evidence suggests that panels combining several miRNAs may provide greater diagnostic accuracy than individual biomarkers and could facilitate discrimination between healthy individuals, premalignant adenomas, and CRC. Circulating and exosomal miRNAs therefore represent a promising non-invasive approach for early detection, monitoring of treatment response, assessment of disease progression, and prediction of patient outcome. However, methodological heterogeneity, pre-analytical variability, and insufficient disease specificity currently limit their clinical implementation. Standardisation of analytical procedures and validation of optimized miRNA panels in large prospective cohorts will be essential for translating these biomarkers into personalized CRC management.

I11.

**Plasma Exosomal microRNA Profiles in Preterm Children:
Associations with Bronchopulmonary Dysplasia History and Current
Echocardiographic Findings**

Ilona Hromadníková, Katerina Kotlabova, Ivan Berka, Jan Sirc

Institute for the Care of Mother and Child, Third Faculty of Medicine, Charles University, Prague, Czech Republic

Department of Molecular Biology and Cell Pathology, Gynaecology and Obstetrics Clinic

Neonatology Clinic, Institute for the Care of Mother and Child, Third Faculty of Medicine, Charles University, Prague, Czech Republic

We aimed to investigate the associations between plasma exosomal microRNA profiles, a history of bronchopulmonary dysplasia (BPD), and concurrent echocardiographic (ECHO) abnormalities - including elevated pulmonary vascular resistance (PVR) - in preschool- and school-aged children born extremely or very preterm. A total of 125 preterm children (median age: 5.42 years) were included, of whom 37 had a history of BPD and 88 did not. An additional 39 age-matched term-born children served as controls. Expression levels of 40 microRNAs, selected based on prior studies in animal models and human cohorts, were analyzed using real-time PCR. Echocardiographic assessments evaluated both structural and functional cardiac parameters. Compared to term controls, preterm children exhibited widespread alterations in plasma exosomal microRNA profiles, with 30 microRNAs significantly upregulated regardless of BPD history. Two microRNAs were uniquely dysregulated in children without BPD, and one in those with BPD. No significant differences in microRNA expression were observed among preterm children based on current ECHO findings. Furthermore, no correlations were found between microRNA levels and Doppler-derived indicators of pulmonary hypertension (PH) risk. These findings suggest that prematurity alone exerts a lasting influence on plasma exosomal microRNA profiles, independent of BPD history or current echocardiographic abnormalities. Although ECHO abnormalities are more prevalent in preschool- and school-aged children born extremely or very preterm compared to normal term children, they do not appear to influence circulating exosomal microRNA expression profiles.

I12.

Intra-amniotic inflammatory complication in pregnancy

Marian Kacerovský

Faculty of Medicine and Dentistry, Palacký University & University Hospital Olomouc, Czech Republic

The intra-amniotic environment plays a central role in the maintenance of pregnancy and the initiation of parturition. Intra-amniotic inflammation may develop in the presence of microbial invasion of the amniotic cavity, representing intra-amniotic infection, or in the absence of detectable microorganisms and/or their nucleic acids, referred to as sterile intra-amniotic inflammation. The lecture will review the principal mechanisms underlying these conditions, including ascending microbial invasion from the lower genital tract, activation of pattern-recognition receptors, tissue stress, release of alarmins, and the potential contribution of nuclear and mitochondrial cell-free DNA. Current diagnostic approaches based on amniotic fluid analysis will also be discussed, with particular emphasis on interleukin-6 measurement and microbiological methods, including culture and molecular techniques.

The prevalence and clinical significance of intra-amniotic inflammatory complications will be illustrated across several obstetric conditions, including preterm prelabor rupture of membranes, preterm labor with intact membranes, early recurrent pregnancy bleeding, and prolapsed fetal membranes. Evidence supporting the use of amniocentesis to distinguish intra-amniotic infection, sterile intra-amniotic inflammation, microbial invasion without inflammation, and the absence of both microorganisms and inflammation will be presented. Finally, the potential of this classification to guide individualized clinical management will be explored, together with current evidence regarding the role and optimal timing of antimicrobial treatment in reducing or resolving intra-amniotic inflammation.

I13.

Noninvasive fetal blood group genotyping

Frederik Banch Clausen

Department of Clinical Immunology, Copenhagen University Hospital, Copenhagen, Denmark

Noninvasive fetal blood group antigen genotyping serves as a diagnostic tool to predict the fetal antigen status which then allows assessment of the risk of hemolytic disease of the fetus and newborn in pregnancies of immunized women. In addition, fetal RHD genotyping is used as an antenatal screening to guide targeted use of anti-D immuno-globulin prophylaxis in non-immunized RhD negative, pregnant women. Based on testing of cell-free DNA extracted from maternal plasma, these noninvasive assays demonstrate high diagnostic accuracy. Consequently, noninvasive fetal blood group antigen genotyping has become standard care in transfusion medicine.

Noninvasive fetal blood group antigen genotyping was among the first clinical applications of cell-free DNA. In Denmark, we have offered fetal RHD genotyping as a nationwide screening test since 2010, and KEL testing since 2013.

I will present an overview of clinical experience with fetal RHD genotyping and other blood group antigens, such as KEL and RHCE. I will discuss challenges and solutions related to the implementation of these assays, including different testing platforms such as next-generation sequencing (NGS) and droplet digital PCR (ddPCR).

I14.

Future use of cfDNA in prenatal testing

Erik Sistermans

Amsterdam UMC, Department of Human Genetics, Amsterdam, The Netherlands

The use of extracellular, or cell-free, DNA (cfDNA) has found wide application in clinical practice. The most widespread example is the use of fetal cfDNA for the detection of fetal aneuploidies, often referred to as non invasive prenatal testing or screening (NIPT/NIPS). However, several developments support the feasibility of a much broader use of cfDNA in future prenatal testing.

First, the entire fetal genome is present in maternal plasma, allowing, for instance, non invasive prenatal fetal whole exome and whole genome sequencing (WES/WGS). Second, maternal plasma contains cfDNA and cfRNA derived from microorganisms such as viruses and bacteria. NIPT platforms based on WGS will sequence these as well, but they are currently filtered out. Specific analysis of these existing sequencing data focused on microbial sequences has the potential to detect infections that may threaten a healthy pregnancy at a stage when early treatment is still possible. Promising results have already been obtained for Cytomegalovirus (CMV).

Third, cfDNA contains much more information than the nucleotide sequence alone. Biological properties including fragment length, end motifs, and genomic distribution are collectively referred to as “fragmentomics.” Fragmentomic and epigenetic data can be used alone or in combination to predict other threats to a healthy pregnancy, such as pre eclampsia, intrauterine growth restriction, and preterm birth. Finally, it is possible to impute common maternal and fetal variants from NIPT data. This not only enables genome wide association studies (GWAS) on these data, but also the calculation of polygenic risk scores and the assessment of carriership for common pathogenic variants.

During the presentation, these opportunities will be discussed and placed in the context of future cfDNA based prenatal testing. Although the main focus will be on technology, potential ethical and practical implications will also be addressed.

Y01.

NIPT in Pregnancy Loss: Experience in Centre of Medical Genetics and Reproductive Medicine Gennet.

Ludvík T.¹, Bittóová M.¹, Koudová M.¹, Stejskal D.¹, Čadová P.¹, Sheardová J.¹, Šimáčková Š.¹

¹ GNTlabs by GENNET, Centre for Medical Genetics and Reproductive Medicine, Prague, Czech Republic

Introduction: cffDNA-based testing in pregnancy loss (niPOC) is an alternative method for aneuploidy detection in product of conception in specific circumstances with proved advantages and known limitations.

Methods: Maternal blood samples were collected within the optimal sampling window following pregnancy loss between January 2024 and May 2026. NIPT was performed using the VeriSeq™NIPT Solution v2 assay, supplemented by WisecondorX and BinDel. Furthermore, real-time PCR (qPCR) was utilized to estimate viral loads (IU/ml) for cytomegalovirus (CMV) and parvovirus B19.

Results: We present results together with our niPOC workflow and follow-up examination in 30 niPOC cases tested in our laboratory.

Conclusions: With two years of clinical experience with more than 10,000 routine NIPT cases, this study demonstrates that niPOC serves as a valuable and feasible non-invasive alternative for identifying chromosomal cause of pregnancy loss, particularly when traditional tissue sampling is compromised or unavailable. In addition, our vision regarding testing for CMV (cytomegalovirus) and parvovirus B19.

Y02.

Extracellular DNA Dynamics in Rheumatoid Arthritis Patients and Experimental Arthritis Models

Kristína Macáková¹, Veronika Borbélyová¹, Emőke Šteňová², Peter Celec^{1,3}, Barbora Vlková¹

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, 81108 Bratislava, Slovakia

² 1st Department of Internal Medicine, Faculty of Medicine, University Hospital, Comenius University, Mickiewiczova 13, 82101 Bratislava, Slovakia

³ Institute of Pathophysiology, Faculty of Medicine, Comenius University, 81108 Bratislava, Slovakia

Introduction: Extracellular DNA (ecDNA) is increasingly recognized as a potential mediator and biomarker of inflammation in autoimmune diseases. In rheumatoid arthritis (RA), ecDNA may originate from several cellular sources, including neutrophil extracellular traps (NETs), and could reflect ongoing inflammatory processes. The aim of this study was to evaluate ecDNA dynamics in RA patients in relation to disease activity and to explore the effects of ecDNA-related pathways in experimental arthritis.

Methods: Plasma ecDNA concentrations were analyzed in RA patients before and after treatment with biological disease-modifying antirheumatic drugs. Clinical disease activity was assessed using standard indices and inflammatory markers. In parallel, experimental arthritis models were used to evaluate the effects of modulating ecDNA pathways through enzymatic degradation of extracellular DNA and inhibition of NETs formation.

Results: Anti-inflammatory treatment was associated with significant improvement in clinical disease activity and inflammatory markers, accompanied by changes in circulating ecDNA concentrations. In experimental models, modulation of ecDNA-related pathways showed differential effects on disease severity, with inhibition of NETs formation demonstrating a more consistent reduction in arthritis severity compared to direct extracellular DNA degradation.

Conclusions: The observed changes in circulating ecDNA following treatment, together with the findings from experimental arthritis models, indicate a possible association between ecDNA-related pathways and RA. However, the precise role of ecDNA in disease pathogenesis remains to be determined.

Y03.

Sex-Specific Effects of Peptidyl Arginine Deiminase 4 Deficiency in the Cafeteria Diet-Induced Obesity-Associated Metabolic Complications

Feješ A.^{1,2}, Bečka E.¹, Feješ Slivková I.¹, Macáková K.¹, Šteňová E.³, Pastorek M.¹, Celec P.^{1,4}, Šebeková K.¹, Gardlík R.^{1,4}, Borbélyová V.¹

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

² 5th Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

³ 1st Department of Internal Medicine, Faculty of Medicine, University Hospital, Comenius University, Bratislava, Slovakia

⁴ Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction: Obesity promotes low-grade inflammation partly through neutrophil extracellular trap (NET) formation, driven by peptidyl arginine deiminase 4 (PAD4). However, whether circulating NET-related markers, including extracellular DNA (ecDNA), myeloperoxidase (MPO)-DNA complexes, and DNase activity, reflect obesity status, PAD4 genotype, or sex remains unclear.

Methods: Male and female C57BL/6 wild-type (WT) and Pad4(-/-) mice (n=5/group) were fed a control or cafeteria (CAF) diet for 22 weeks. Plasma ecDNA, DNase activity, MPO-DNA complexes, and NETotic polymorphonuclear neutrophils (PMNs) were quantified, alongside body composition, glucose and lipid metabolism, and liver-damage markers.

Results: Pad4(-/-) mice of both sexes showed lower percentages of circulating NETotic PMNs than WT mice. In contrast, plasma ecDNA concentrations showed no robust effect of genotype or sex: a weak diet effect ($p < 0.05$) was observed in males without significant pairwise differences, while no effect was detected in females. MPO-DNA complexes were similarly unchanged across all groups. In males, DNase activity was unaffected by the CAF diet, but differed by genotype ($p < 0.05$), with Pad4(-/-)/CAF males displaying lower DNase activity than WT/CAF males. No differences in DNase activity appeared in females. Metabolically, PAD4 deficiency delayed obesity onset and improved dyslipidemia in CAF-diet-fed males but not females. Pad4(-/-)/CAF females exhibited impaired glucose tolerance, worsened lipid profile, and higher hepatic triglyceride accumulation.

Conclusions: Total circulating ecDNA and MPO-DNA complexes do not reliably reflect PAD4-derived NET formation in this obesity model, likely because ecDNA has multiple cellular origins and is rapidly cleared. Lower DNase activity in Pad4(-/-) males suggests sex-dependent changes in neutrophil/DNA-clearance biology. Overall, PAD4 deficiency has sex-specific metabolic consequences, supporting sex as a critical biological variable in NET-targeted obesity research.

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Y04.

Deoxyribonuclease-Resistant Extracellular DNA

L'ubica Janovičová¹, Nikola Pribulová¹, Katarína Kmet'ová¹, Peter Celec¹

¹ *Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Sasinkova 4, Bratislava, Slovakia*

Introduction: Extracellular DNA (ecDNA) is an important source of disease markers, but also an inducer of sterile inflammation. A fraction of ecDNA, termed protected ecDNA, resists degradation by deoxyribonuclease I (DNase 1) and is likely responsible for immunogenicity of ecDNA. However, standard sample processing steps may substantially decrease the measurable concentration of protected ecDNA, confounding its use as a biomarker. This study aimed to determine how centrifugation and freeze-thawing affect the recoverable fraction of protected ecDNA in plasma.

Methods: Heparinized blood was collected from eighteen healthy probands. Blood was centrifuged at $1600 \times g$ to remove cells, and plasma was centrifuged a second time at $16,000 \times g$. Aliquots of once- and twice-centrifuged plasma were either frozen or treated with DNase 1 to isolate the protected ecDNA fraction.

Results: The second centrifugation step removed 95% of ecDNA from fresh plasma but only half of ecDNA from previously frozen plasma. This indicates that freezing shifts ecDNA into a more sedimentable form in plasma that likely contains microvesicles and apoptotic bodies. The freeze-thaw cycle reduced the protected ecDNA fraction: in once-centrifuged plasma, protected ecDNA fell from 84% to 15% of total ecDNA after freezing, and in twice-centrifuged plasma from 66% to 28%.

Conclusions: Since freezing before processing is common and often unavoidable, the loss of protection may lead to systematic underestimation of this biologically relevant ecDNA fraction. The disrupted protection structures of ecDNA may include histones, vesicles, neutrophil extracellular traps etc. These findings indicate that a fraction of protected ecDNA, potentially carrying valuable biological information, is lost during centrifugation, warranting further mechanistic investigation.

Y05.

The Role of Sterile Inflammation in Autoimmune Hepatitis

Gromová B¹, Gaál Kovalčíková A², Kosnáčová J², Lichá K³, Celec P^{1,4}, Gardlík R^{1,4}

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

² Department of Pediatrics, National Institute of Children's Diseases and Faculty of Medicine, Comenius University, Bratislava, Slovakia

³ Institute of Medical Physics and Biophysics, Faculty of Medicine, Comenius University, Bratislava, Slovakia

⁴ Institute of Pathological Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction: Autoimmune hepatitis (AIH) is a chronic, immune-mediated liver disease of largely unknown etiology. Cell death releases damage-associated molecular patterns (DAMPs), including extracellular DNA (ecDNA), which circulates either free or in complexes. Moreover, recruited neutrophils release DNA as neutrophil extracellular traps (NETs), fueling sterile inflammation. Deoxyribonuclease (DNase) is an enzyme that cleaves ecDNA and may counteract this accumulation. The aim of this study was to characterize the contribution of these circulating DAMPs in pediatric AIH patients and a Con A-induced mouse model.

Methods: Plasma samples from pediatric AIH patients (n=10) and age-matched healthy controls (n=6) were analyzed. Levels of circulating ecDNA were quantified fluorometrically, and DNase activity was measured by gel radial diffusion. Intact and nonintact mitochondria within microparticles as well as NET formations were characterized by flow cytometry. In parallel, we examined Con A-induced AIH in Aire-deficient mice (n=15) to characterize this disease model, with liver injury assessed biochemically.

Results: Total ecDNA and DNase activity were significantly higher in pediatric AIH patients compared with healthy controls. A corresponding trend toward higher ecDNA concentrations and DNase activity was observed in the Con A-induced mouse model. NET formations did not differ between AIH and healthy mice; however, upon stimulation with mitochondria or *Escherichia coli*, neutrophils from AIH mice tended to produce fewer NETs than those from healthy mice. Assessment of microparticles revealed reduced presence of intact/nonintact mitochondria in pediatric AIH patients compared with healthy controls.

Conclusion: Based on the preliminary results, the elevated ecDNA in animal model does not appear to derive primarily from NETs or from intact/nonintact mitochondria carried in microparticles. The detectable DNase response in AIH patients and Con A-induced model suggests an imbalance between release and clearance, which may influence disease severity. Further studies should identify the cellular source of ecDNA and evaluate pharmacological strategies to modulate its generation and recognition.

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Y06.

Analysis of cell-free DNA in Alzheimer's disease

**L. Lukyová^{1,2,3}, Z. Holešová¹, Z. Hanzlíková^{1,3,4}, N. Forgáčová^{2,4,5}, T. Sládeček^{1,2,3}, A. Zatlková^{1,4},
J. Gazdarica^{1,2}, J. Minárik⁴, J. Styk^{1,2}, T. Sedláčková^{1,2}, T. Szemes^{1,2,3}**

¹ Geneton Ltd., Bratislava, Slovakia

² Comenius University Science Park, Bratislava, Slovakia

³ Comenius University Faculty of Natural Sciences, Department of Molecular Biology, Bratislava, Slovakia

⁴ Institute of Clinical and Translational Research, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

⁵ National Institute of Children's Diseases, Bratislava, Slovakia

Introduction: High costs associated with advanced neuroimaging techniques and the invasive nature of cerebrospinal fluid biomarker analysis, both currently used in the diagnosis of Alzheimer's disease (AD), are driving the search for accurate, minimally invasive diagnostic approaches. Although most research has focused on blood-based protein biomarkers, growing interest has also been directed toward cell-free DNA (cfDNA). As a minimally invasive blood biomarker, cfDNA shows promise for the early detection of progressive neurodegenerative processes underlying AD.

Methods: Low-coverage whole-genome sequencing (~1×) was performed to analyze cfDNA from individuals in the early stages of AD and from age-matched cognitively healthy controls. Sequencing data were processed using a custom bioinformatics pipeline designed for cfDNA analysis, with an emphasis on comprehensive characterization of fragmentomic features.

Results: Analysis of cfDNA fragmentomic features identified end-motifs as the parameter with the highest discriminatory power between patients with AD and controls. Based on these findings, an experimental panel of end-motifs was constructed, reflecting underlying nuclease activity. This panel enabled reliable discrimination between AD patients and control individuals.

Conclusion: Our characterization of cfDNA in patients with AD identified a biological signal in the form of cfDNA end-motifs that enables discrimination between patients and controls. The resulting panel of end-motifs represents an experimental set of candidate biomarkers. The performance of this panel remains to be established in larger, independent cohorts.

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Y07.

Complementary cell-free DNA characteristics for discrimination of malignant and non-malignant endometrial pathology

**T. Ďuranová^{1,2}, J. Styk^{1,3}, Z. Hanzlíková^{3,4,5}, O. Pös^{1,3}, I. Lojová^{1,5}, S. Bokorová^{1,3}, T. Sládeček^{1,3},
M. Kubáňová^{1,2}, J. Sitarčík^{1,3,6}, W. Kramp^{1,3}, T. Sedláčková^{1,3}, P. Janega⁸, V. Repiská²,
J. Radvánszky^{1,4,5}, J. Budiš^{1,3,6}, T. Szemes^{1,3,4}**

¹ Comenius University Science Park, Bratislava, Slovakia

² Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University Bratislava, Slovakia

³ Geneton Ltd., Bratislava, Slovakia

⁴ Department of Molecular Biology, Faculty of Natural Sciences, Comenius University Bratislava, Slovakia

⁵ Institute of Clinical and Translational Research, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

⁶ Slovak Center of Scientific and Technical Information, Bratislava, Slovakia

⁷ Faculty of Informatics and Information Technologies, Slovak University of Technology, Bratislava, Slovakia ⁸ Institute of Pathological Anatomy, Faculty of Medicine, Comenius University Bratislava, Slovakia

Introduction: Endometrial cancer (EC) and non-malignant endometrial pathologies (EP) frequently present with overlapping clinical manifestations, making differential diagnosis challenging. Both conditions may also induce systemic biological changes reflected in plasma cell-free DNA (cfDNA), potentially generating partially overlapping cfDNA signatures. Consequently, distinguishing EC from EP represents a substantially greater challenge than the conventional cancer-versus-healthy comparison. As genome-wide copy number alterations and cfDNA fragmentomic features capture complementary aspects of disease biology, we investigated whether their integration improves discrimination between EC and EP using low-pass whole-genome sequencing (lpWGS).

Methods: Preoperative plasma cfDNA from 109 patients with EC and 77 patients with heterogeneous non-malignant EP, including benign and premalignant endometrial conditions, was profiled by lpWGS (~1× coverage). Genome-wide copy number profiles and fragmentomic features were derived from lpWGS data using established analytical tools and in-house bioinformatic approaches.

Results: EC and EP exhibited distinct genome-wide copy number and fragmentomic characteristics in plasma cfDNA. Combined analysis consistently outperformed individual feature classes in differentiating malignant from non-malignant endometrial pathology.

Conclusion: Combined analysis of genome-wide copy number alterations and fragmentomic characteristics derived from a single lpWGS assay captures complementary cfDNA information and provides a promising framework for plasma-based discrimination between EC and EP. This approach maximizes the biological information obtained from a single plasma sample while maintaining the simplicity of a single sequencing assay.

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Y08.

Pilot comparison of genomic and fragmentomic profiles in EBC- and plasma-derived cfDNA from patients with pulmonary diseases

M. Kubáňová^{1,6}, T. Forgáč³, O. Pös^{1,2}, Z. Hanzlíková^{2,3,4}, S. Bokorová^{1,2}, T. Sedláčková^{1,2}, A. Dzian⁷, V. Holubeková⁸, E. Halašová⁸, V. Repiská⁶, J. Budiš^{1,2,5}, T. Szemes^{1,2,3}

¹ Comenius University Science Park, Bratislava, Slovakia

² Geneton Ltd., Bratislava, Slovakia

³ Department of Molecular Biology, Faculty of Natural Sciences, Comenius University Bratislava, Slovakia

⁴ Institute of Clinical and Translational Research, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

⁵ Slovak Center of Scientific and Technical Information, Bratislava, Slovakia

⁶ Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University Bratislava, Slovakia

⁷ Department of Thoracic Surgery, Jessenius Faculty of Medicine in Martin, Comenius University Bratislava, Martin, Slovakia

⁸ Biomedical Centre Martin, Jessenius Faculty of Medicine in Martin, Comenius University Bratislava, Martin, Slovakia

Introduction: Exhaled breath condensate (EBC) is a non-invasive biofluid containing extracellular nucleic acids from the respiratory tract. Compared with blood-based liquid biopsies, EBC may provide lung-specific molecular information and represents a potential biomarker source for pulmonary diseases. While EBC-derived cell-free DNA (cfDNA) is technically sequenceable, its analytical performance and disease-discriminatory potential relative to plasma remain insufficiently characterized.

Methods: As part of an ongoing study, EBC and plasma samples were collected from 17 patients with malignant and benign pulmonary diseases. EBC was obtained using the RTube collection device under standardized conditions. cfDNA was analyzed using low-coverage whole-genome sequencing (lcWGS). Genomic and fragmentomic features, including copy number variations (CNVs) and insert-size metrics, were assessed between EBC and plasma samples.

Results: Despite the ultra-low DNA input of EBC, optimized workflows enabled lcWGS of sufficient quality for downstream analyses in most samples. Due to limited EBC yield and hemolysis in plasma, library preparation was successful in 13/17 EBC samples and 15/17 plasma samples. EBC-derived cfDNA showed lower median insert sizes (≈ 0 –5 bp) compared with plasma (~ 164 bp), along with broader fragment-size distributions and higher variability. CNV analysis revealed increased CNV counts and total CNV burden in EBC compared with plasma across both subgroups. Although the currently assessed genomic and fragmentomic features did not reveal statistically significant malignant–benign differences in this pilot cohort, the dataset supports further development of the analytical framework, including expansion toward additional fragmentomic features and validation in larger cohorts.

Conclusions: Our findings demonstrate the feasibility of low-coverage whole-genome sequencing of extracellular DNA from EBC, with sufficient quality for genomic and fragmentomic analyses in most samples. Despite technical variability and limited statistical power, these results support further investigation of EBC as a complementary source of lung-associated genomic information in larger studies.

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Y09.

Stimulus Dictates NET Architecture and DNase I Clearance

Hudecová L.¹, Bečka E.¹, Bertovič P.¹, Strečanský T.¹, Pastorek M.¹

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava

Introduction: During sepsis, excessive production of neutrophil extracellular traps (NETs) can contribute to tissue damage and organ dysfunction. While the host relies on nuclease to digest DNA backbone of NETs, DNase I activity is significantly impaired during sepsis. NETs DNA is structurally stabilized and shielded from nucleases particularly by cationic peptides. Because distinct pathogen trigger divergent neutrophil signaling, we hypothesized that NETs induced by different stimuli would yield unique proteome, directly affecting their clearance from the circulation.

Methods: Blood and neutrophils isolated from healthy volunteers and C57/BL6J were stimulated by *E. coli*, *S. aureus*, and mitochondria to induce NETs. Following DNase I and DNase IL3 treatment, DNA was isolated using the DNA Mini Blood Kit, quantified using Qubit™1X dsDNA High Sensitivity, and the fragmentation pattern was observed using the QSep Bio-Fragment Analyzer.

Results: Human NETs induced by *S. aureus* exhibited the highest resistance to degradation by nucleases compared to other groups. At the same time, human NETs induced by *E. coli* have a high content of short fragments ranging from 100 to 200 bp, with a higher potential to activate a TLR9 receptor-mediated response. In contrast to human NETs, mouse NETs induced by mitochondria exhibited the highest resistance to nuclease degradation, and their fragmentation profile differs from that of human NETs.

Conclusion: The data demonstrate that the inciting stimulus dictates the structural architecture and clearance kinetics of NETs. Furthermore, differences between human and mouse NET degradation profiles warrants caution in using a mouse model of sepsis with NET degradation therapies.

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Y10.

DNase1L3 exhibits bacteriostatic properties in case of liquid cultures of *S. aureus*

Filip Baran¹, Patrik Hains¹, Emma Skybová¹, Ľubica Janovičová¹, Peter Celec^{1,2}

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

² Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction: During sepsis, a state of life-threatening organ dysfunction caused mostly by bacteria, dying cells release excessive numbers of damage-associated molecular patterns (DAMPs). One such DAMP is extracellular DNA (ecDNA), which worsens sepsis severity by triggering sterile inflammation. Exogenous supplementation with deoxyribonuclease I (DNase I), an enzyme able to cleave ecDNA, was shown to prevent this proinflammatory signaling and alleviate the severity of sepsis. In our current work, we investigated whether representatives of the DNase enzyme family, namely DNase I and DNase1L3, might also exhibit antibacterial properties, which would further mitigate the disease.

Methods: *E. coli* CFT073 (ATCC-700928) or *S. aureus* subspecies aureus Rosenbach (NCTC-8530) were cultivated in the presence of DNase I or DNase1L3 either in liquid media or on solid agar plates. Growth of bacteria in liquid media was recorded over a period of 48h through optical density measurement at 600nm every 30 minutes. In case of agar plates, bacteria were seeded using the drop-plate method and incubated for 24h, after which the size and number of bacterial colonies were analyzed.

Results: DNase I did not exhibit any kind of direct bactericidal or bacteriostatic effects at any used concentration. Dynamics of bacterial growth, the size and number of bacterial colonies remained unaffected, with no significant differences. Higher concentrations of DNase1L3 significantly influenced the dynamics of growth of *S. aureus* in liquid media, with potent bacteriostatic effects observed. However, treatment with this enzyme had no effect on the size or number of bacterial colonies.

Conclusion: DNase I does not possess any direct antibacterial properties, and its beneficial use in sepsis can be ascribed to its ability to degrade excessive concentrations of immunogenic ecDNA. Higher concentrations of DNase1L3 exhibit strain specific bacteriostatic properties not described in literature so far. The exact mechanism causing this inhibition will be examined in future experiments.

Y11.

Evaluation of a Custom Targeted cfDNA Sequencing Workflow in Colorectal Cancer and non-cancer Lynch Syndrome patients

Yuliia Fridland^{1,2}, Jakub Styk^{2,3}, Ondrej Pös^{2,3}, Zuzana Hanzlíková^{1,3,4}, Marcel Kucharík^{2,3,4}, Werner Krampfl^{2,3}, Jaroslav Budiš^{2,3,5}, Ján Radvánszky^{1,2,4}, Tatiana Sedláčková^{2,3}, Tomáš Szemes^{1,2,3}

¹ Department of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovak Republic;

² Comenius University in Bratislava Science Park, Bratislava, Slovak Republic; ³ Geneton Ltd., Bratislava, Slovak Republic;

⁴ Institute of Clinical and Translational Research, Biomedical Research Center of the Slovak Academy of Sciences, Bratislava, Slovak Republic;

⁵ Slovak Centre of Scientific and Technical Information, Bratislava, Slovak Republic.

Introduction: Plasma cell-free DNA (cfDNA) profiling offers minimally invasive access to molecular information and enables repeated assessment. Because tumor-derived fragments may be highly diluted in total cfDNA, targeted enrichment can achieve greater depth across selected regions than genome-wide approaches. This study evaluates targeted plasma cfDNA sequencing for molecular profiling of colorectal cancer (CRC) patients and Lynch syndrome carriers using an adapted custom Twist panel originally developed for tissue DNA.

Methods: The study included plasma cfDNA from 50 pre-operative CRC patients and 50 Lynch syndrome carriers who had no active malignancy at the time of blood collection. Libraries were prepared using an adapted Twist Library Preparation EF 2.0 workflow optimized for plasma cfDNA, covering microsatellite instability (MSI) markers, mismatch repair (MMR) genes, selected CRC-associated genes, and hotspot regions. Libraries were enriched and sequenced on an Illumina NextSeq 2000 platform. Available clinicopathologic and molecular data were used to contextualize plasma findings, including mismatch repair immunohistochemistry, microsatellite instability status, previous MMR-gene testing, and CRC molecular classification.

Results: Targeted sequencing successfully generated plasma cfDNA data suitable for MSI-marker analysis and targeted variant detection in both study cohorts. The adapted workflow supports evaluation of cfDNA-level molecular profiles in CRC patients and cancer-free Lynch syndrome carriers, including identification of potentially informative cases for comparison with available molecular and clinicopathologic findings.

Conclusions: Plasma cfDNA sequencing provides a practical framework for minimally invasive molecular profiling in CRC and hereditary colorectal cancer research. This approach supports further evaluation of plasma MSI markers and targeted genomic biomarkers, particularly in relation to Lynch syndrome-associated molecular features, while final analysis will define its analytical performance, limitations, and potential applications in CRC and Lynch syndrome research.

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Y12.

Impaired clearance of cell-free DNA and myeloid dysregulation in a murine model of sepsis

Bertovič P.¹, Bíla K.¹, Bečka E.¹, Hudecová L.¹, Pidlypska T.¹, Baláž M.², Szabó J.³, Pastorek M.¹

¹ *Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava*

² *Biomedical Research Center, Slovak Academy of Sciences, 845 05 Bratislava, Slovakia*

³ *Institute of Medical Physics and Biophysics, Faculty of Medicine, Comenius University, Bratislava*

Introduction: During sepsis, neutrophil extracellular traps (NETs) are released to capture pathogens, but their increased formation can further drive pathological inflammation. Physiologically, NETs are cleared via action of plasmatic nucleases and macrophage phagocytosis. We hypothesize that this clearance mechanism fails during sepsis, causing the accumulation of NETs, which drives persistent inflammation and can contribute to post-septic complications. We evaluated functional shifts in neutrophils and macrophages post-sepsis, focusing on NET clearance and sex-specific differences.

Methods: Sepsis was induced in C57BL/6 mice using a fecal slurry injection. One month post recovery, mice received a secondary *Staphylococcus aureus* challenge. Flow cytometry was used to characterize myeloid cell phenotype, including maturation, activation, degranulation, and NETosis. NET phagocytosis by macrophages was assessed using live-cell imaging. Neutrophil mitochondrial respiration was evaluated via Seahorse analysis.

Results: Acute sepsis caused a rapid disappearance of resident peritoneal macrophages that were replaced by infiltrating monocytes. One month post-sepsis, plasma NET levels remained elevated, reflecting a systemic failure in their clearance. In vivo, post-septic monocyte-derived macrophages exhibited a mixed M1, and M2 phenotype characterized by CD86 and CD206 expression. Concurrently, neutrophil counts remained elevated, displaying a mature phenotype. Despite retaining their degranulation capacity, post-septic neutrophils suffered from impaired capacity for early chromatin decondensation and NETosis. Notably, males demonstrated higher neutrophil burden, elevated mitochondrial respiration, and persistently altered macrophage subsets compared to females.

Conclusion: Surviving sepsis induces profound myeloid alterations characterized by an intrinsic defect in neutrophil NET formation and impaired macrophage-mediated extracellular DNA clearance. The sexual dimorphism observed during late-stage resolution in neutrophil dynamics suggests that the female survival advantage is probably driven by superior myeloid cell functionality.

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Y13.

From global instability metrics to regional genomic architecture: resolving CNV patterns in ovarian cancer cfDNA

Z. Hanzlíková^{1,2,3}, J. Styk^{3,4}, O. Pös^{3,4}, O. Biró^{5,6}, L. Lukyová^{1,3,4}, J. Sitarčík^{3,4}, T. Sládeček^{1,3,4}, W. Krampfl^{3,4}, A. Mészáros^{5,6}, Sz. Máté⁷, J. Rigó⁸, J. Radvánszky^{1,2,4}, J. Budiš^{3,4,9}, T. Szemes^{1,3,4}

¹ Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

² Institute of Clinical and Translational Research, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

³ Geneton Ltd., Bratislava, Slovakia

⁴ Comenius University Science Park, Bratislava, Slovakia

⁵ Clinomics Europe Ltd., Budapest, Hungary

⁶ Semmelweis University Doctoral School, Surgical Medicine Division, Budapest, Hungary

⁷ First Department of Obstetrics and Gynaecology, Semmelweis University, Budapest, Hungary

⁸ Department of Clinical Studies in Obstetrics and Gynecology, Faculty of Health Sciences, Semmelweis University, Budapest, Hungary

⁹ Slovak Center of Scientific and Technical Information, Bratislava, Slovakia

Introduction: Plasma cell-free DNA (cfDNA) analysis using low-coverage whole-genome sequencing (lcWGS) enables detection of ovarian cancer (OC)-associated genomic instability through genome-wide copy number variants (CNV). While global instability metrics summarize genomic disruption, they provide limited insight into the regional genomic structures underlying these signals. We hypothesized that CNV profiles contain recurrent regional alteration patterns that improve biological interpretation and refine predictive models.

Methods: Plasma cfDNA from 82 OC patients before surgery and 41 age-matched healthy controls was profiled using lcWGS (~1× coverage). Genome-wide CNV profiles were summarized using global instability measures and used to train machine learning (ML) models for cancer classification. Recurrent CNV regions were further characterized through regional similarity analysis and gene-level annotation to identify shared functional contexts and disease-associated loci.

Results: ML models based on global CNV-derived metrics achieved a sensitivity of 85%, specificity of 50%, and AUC of 0.855. Individual CNV-derived features showed variable predictive performance (AUC range: 0.623–.946), suggesting that global metrics capture informative signals but may obscure underlying regional patterns. Regional analysis revealed heterogeneous but recurrent chromosomal alteration patterns, with selected CNV regions showing distinct frequencies between OC patients and controls. Functional mapping identified 20 genes affected in 30% of OC samples with limited or absent representation in controls. Regional similarity analysis revealed partially shared CNV architectures among patient subsets, suggesting convergent chromosomal remodeling patterns within a heterogeneous disease landscape.

Conclusions: ML model development and biological interpretation represent an iterative process, where predictive performance guides refinement of genomic feature representation. Regional and functional characterization of CNV events improves interpretability of cfDNA-based genomic instability profiling beyond global metrics.

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Y14.

TLR9 and DNase1L3 Deficiency Impair Memory Formation in a Sex-Specific Manner

Emma Skybová¹, Jakub Szabó², Filip Baran¹, Ľubica Janovičová¹, Barbara Wegiel³, Peter Celec^{1,4}

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

² Institute of Medical Physics and Biophysics, Faculty of Medicine, Comenius University, Bratislava, Slovakia

³ Department of Surgery, Division of Surgical Sciences, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA

⁴ Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction: Extracellular DNA (ecDNA) is found outside of cells and can be recognized by Toll-like receptor 9 (TLR9) which was implicated in DNA damage-repair-dependent memory formation. EcDNA can be cleaved by specialized enzymes, Deoxyribonucleases (DNases). However, TLR9 involvement beyond fear-related memory, and the cognitive phenotype of DNase1L3-deficient mice, remain uncharacterized. We hypothesized that TLR9 and DNase1L3 deficiency would affect memory formation accompanied by altered neuroinflammation.

Methods: Eighty adult C57BL/6 mice: wild-type (13 male, 8 female), DNase1L3-deficient (22 male, 12 female), and Tlr9-deficient (14 male, 11 female) were included in this study. Locomotor activity and anxiety-like behavior were assessed with the Open Field test, recognition memory with the Novel Object Recognition test, and spatial learning, memory, and cognitive flexibility with the Morris Water Maze and Reversal Water Maze.

Results: DNase1L3^{-/-} and Tlr9^{-/-} females showed impaired spatial learning, and DNase1L3^{-/-} females showed approximately 2-fold reduction in spatial memory compared to WT females. Tlr9^{-/-} males showed markedly worse short-term recognition memory (memory index approximately 0.25 vs. 0.85 in WT) while DNase1L3^{-/-} males showed higher locomotor activity than WT and Tlr9^{-/-} males.

Conclusions: TLR9 and DNase1L3 deficiency affect memory formation and locomotor activity in sex-specific manner. Ongoing analyses of hippocampal neuroinflammatory markers, along with expanded measurements, will further clarify the impact of impaired ecDNA clearance on brain. These preliminary findings will be researched further to investigate the role for ecDNA-sensing in cognition.

Y15.

Absolute Quantification of Extracellular RNA from Plasma-Derived Extracellular Vesicles

Anna Sekyrova¹, Pavel Stejskal¹, Josef Srovnal¹

¹ *Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc, Czech Republic*

Introduction: Extracellular vesicles (EVs) represent an important source of biologically relevant information, and the RNA (exRNA) they carry has emerged as a promising biomarker reservoir for a range of pathological conditions. At the same time, reliable quantification of EV-associated RNA remains technically demanding, mainly because plasma-derived RNA is present in very low amounts and standard fluorometric or electrophoretic approaches often lack sufficient sensitivity. A further challenge lies in normalization, as commonly used housekeeping genes are not always suitable for exRNA analysis. The aim of this study was to develop and validate a protocol for exRNA quantification by RT-qPCR using appropriate reference genes.

Methods: EVs were isolated from plasma of healthy donors using the Norgen ExtraClean Plasma/Serum Exosome Purification and RNA Isolation Kit. The extracted exRNA was reverse-transcribed into cDNA using the SuperScript IV First-Strand Synthesis System. RT-qPCR quantification was performed with PrimePCR SYBR Green Assay. Four EV-associated genes, SNRPG, TOMM7, NOP10, and OST4, were evaluated as candidate reference genes. Standard curves were generated from DNA templates corresponding to these genes, and all RT-qPCR analyses were carried out on the CFX96 Real-Time PCR Detection System.

Results: RT-qPCR enabled reliable detection and quantification of exRNA even at very low input concentrations. For all tested reference genes, the standard curves were sufficiently linear and amplification efficiency was within the optimal range, confirming the suitability of the experimental design for absolute quantification. The tested exRNA samples isolated from plasma, as well as the reference cDNA, fell within the concentration range of the standard curves for all four genes, allowing calculation of transcript copy numbers per microliter of sample. The detected concentrations of the reference genes ranged approximately from 160 to 1600 copies/ μ L, confirming that the method is suitable for low-input exRNA quantification.

Conclusion: Absolute RT-qPCR based on standard curves provides a robust and sensitive strategy for quantifying plasma exRNA, even when RNA yield is extremely limited. In addition, the use of EV-enriched reference genes improves normalization and may enhance the reproducibility of exRNA-based biomarker studies.

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R01.

Differentially expressed plasma miRNAs associated with invasion markers in brain tumors

Beáta Soltész¹, Nikolett Németh¹, Álmos Klekner², Andor Karácsony², Péter Pozsgai³, Csilla Kompár¹, Gergely Buglyó¹, Bálint Nagy¹, István Balogh^{1,4}, József Virga³

¹ Department of Medical Genetics, Faculty of Medicine, University of Debrecen

² Department of Neurosurgery, Faculty of Medicine, University of Debrecen

³ Department of Oncology, Faculty of Medicine, University of Debrecen

⁴ Center for Clinical Genetics, Faculty of Medicine, University of Debrecen

Introduction: Primary brain tumors comprise a heterogeneous group of neoplasms, among which glioblastoma represents the most aggressive malignant group with an unfavorable prognosis. Although microRNAs (miRNAs) have been extensively investigated as diagnostic and prognostic biomarkers, their relationship with extracellular matrix (ECM)-associated invasion markers in biological fluids remains insufficiently explored.

Materials and methods: The expression profile of ECM-associated invasion markers was analyzed using TaqMan Low-Density Array in tumor tissues obtained from 40 patients representing four brain tumor groups (glioblastoma, astrocytoma, oligodendroglioma, and meningioma). Based on functional network analysis (NetworkAnalyst) and published literature, seven candidate miRNAs associated with the differentially expressed invasion markers were selected for validation in plasma samples by RT-qPCR. Statistical analyses were performed using the Mann–hitney U test.

Results: Comparative analysis of tumor tissues identified several ECM-associated genes showing significant differential expression among the investigated tumor entities. ERBB2, COL1A1, and LAMA4 exhibited significantly altered expression in glioblastoma, astrocytoma, and oligodendroglioma compared with benign meningiomas (all $p < 0.0001$). Furthermore, MMP2 and MMP9 discriminated predominantly among the malignant glioma groups ($p < 0.05$). Based on the predictions and literature, seven miRNAs potentially regulating these invasion-related genes were selected for plasma validation. Among them, miR-93-5p showed significantly altered expression in astrocytoma ($p < 0.05$), while miR-125b-5p was significantly dysregulated in both glioblastoma and oligodendroglioma compared with benign meningiomas ($p < 0.01$).

Conclusion: Our findings indicate that ECM-associated invasion markers exhibit tumor type specific expression patterns in diffuse gliomas and meningiomas, and that selected circulating miRNAs reflect these molecular alterations. In particular, miR-93-5p and miR-125b-5p emerge as promising minimally invasive biomarker candidates associated with tumor invasion. Further validation in larger patient cohorts is warranted to clarify their diagnostic and biological significance.

R02.

Neutrophil Extracellular Traps in Post-Sepsis Complications

Karolína Bíla¹, Emil Bečka¹, Letícia Hudecová¹, Patrik Bertovič¹, Jakub Szabó¹, Alexandra Gaál Kovalčíková², Ľudmila Podracká², Marcela Hortová-Kohoutková³, Jan Frič³, Michal Pastorek¹

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia.

² Department of Pediatrics, Faculty of Medicine, Comenius University and National Institute of Children's Diseases, Bratislava, Slovakia.

³ International Clinical Research Center, St. Anne's University Hospital, Brno, Czechia.

Introduction: Sepsis survivors experience substantial morbidity, with 75% developing Persistent Inflammation, Immunosuppression, and Catabolism Syndrome (PICS). Currently, no validated predictive markers for PICS exist. The BEATsep consortium aims to improve patient stratification by integrating multimodal longitudinal data in post-septic children and adults. As a part of the study, we investigated the persistence of circulating neutrophil extracellular traps (NETs), hypothesizing that their increased formation or impaired clearance drives chronic inflammation.

Methods: Circulating NETs (MPO-DNA complexes), soluble NET markers (MPO, NE, LL37, nucleosomes), and DNase I activity were analyzed in pediatric (n=32) and adult (n=43) sepsis survivors across three timepoints (3 days, 2 weeks, 6–2 months). Murine PICS model established via cecal slurry injection was used to investigate NET dynamics, myelopoiesis, macrophage phenotypes, behavior and response to a secondary *S. aureus* pneumonia challenge in sepsis survivors.

Results: Elevated NETs and soluble markers sustained for ≥4 days in ~46% of children and ~54% of adults. DNase I activity increased on day 3 and remained elevated through two weeks only in adults, positively correlating with soluble NET markers concentration. Circulating soluble NET markers concentration correlated with mature neutrophils, whereas NET-forming capacity and NETs associated with immature neutrophils (CD66blow, CD49d+). In mice, NETs persisted for ≥4 days, paralleling human kinetics. Post-septic mice exhibited dysregulated myelopoiesis, peritoneal macrophage phenotype shifts, and neutrophil metabolic reprogramming. These changes, alongside long-term mortality, delayed recovery, and behavioral deficits, were more pronounced in males. Secondary challenge revealed impaired pathogen clearance in ~50% of males, correlating with NET persistence after initial septic episode.

Conclusions: NETs persist in sepsis survivors, suggesting therapeutic opportunities targeting NETs even post-sepsis. Sex-stratified interventions may be warranted given male-specific immune hyperactivation and impaired pathogen responses.

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R03.

Deoxyribonuclease Activity Assessment –the technical aspects

Lucia Krajčík Lauková¹, Barbora Vlková¹, Erik Vitáliš¹, Peter Celec¹

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction: Accurate measurement of deoxyribonuclease (DNase) activity is essential for understanding extracellular DNA (ecDNA) homeostasis. DNase 1 and DNase 1-like 3 (DNase1L3) play key roles in ecDNA degradation; however, currently available activity assays lack sufficient specificity to reliably distinguish between these enzymes. Several methods have been developed to assess DNase 1 activity, each with distinct methodological limitations. Therefore, we compared two commonly used functional assays for measuring DNase activity in different human blood sample types.

Methods: Human blood samples were collected in EDTA, heparin, or serum collection tubes. DNase activity was determined using the single radial enzyme diffusion (SRED) assay, based on clearance zone formation in DNA-containing agarose gels, and using a labeled DNA oligonucleotide assay, where probe degradation was quantified in time. Concentrations of DNase 1 and DNase1L3 were determined using commercially available enzyme-linked immunosorbent assay kits.

Results: DNase activity measured using SRED was ~30-fold higher than using the DNA probe degradation assay. The SRED assay revealed significantly lower DNase activity in EDTA plasma than in heparin plasma (by 39%) and serum (by 50%), whereas the probe-based assay detected no significant differences between the sample types. No significant correlation was observed between DNase activity obtained using the two methods. Concentrations of both, DNase 1 and DNase1L3 were higher in serum than in both plasma types.

Conclusions: The choice of analytical method and blood sample type substantially influences measured DNase activity. The lack of correlation between the assays described and used in the literature suggests they capture distinct aspects of DNase activity and are not interchangeable. Differences between plasma and serum highlight the impact of preanalytical conditions. These findings underscore the need for careful assay selection and standardization in studies of ecDNA degradation. Further research is needed to establish robust methods for specific DNase1 and DNase1L3 activity approaches.

R04.

Protein packaging protects mtDNA from DNase I and preserves its immunogenicity

Emil Bečka¹, Miroslava Baricová¹, Leticia Hudecová¹, Michal Pastorek¹

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction: Trauma-related tissue damage releases damage-associated molecular patterns, including mitochondrial proteins and DNA (mtDNA). Due to its hypomethylated CpG motifs, extracellular mtDNA can activate neutrophils via Toll-like receptor 9 (TLR9), triggering the neutrophil extracellular traps (NETs) formation. It remains unclear what affects the stability and immunogenicity of mtDNA in trauma patients. We hypothesize that mitochondrial proteins physically shield mtDNA from enzymatic degradation, thereby preserving its capacity to induce NET formation via TLR9.

Methods: An animal model of peritoneal surgery and administration of isolated mitochondria were used to analyze the dynamics of extracellular mitochondria and mtDNA. To assess degradation, isolated mitochondria and mtDNA were incubated in healthy donor plasma and measured via qPCR and spectrophotometry. Isolated mitochondria were pretreated with DNase I or proteinase K to deplete specific components before co-culture with neutrophils or HEK TLR9 reporter cells. NETs formation was quantified using real-time live-cell imaging.

Results: Mitochondria particles number increased one-hour post-surgery ($6.59 \times 10^6 \rightarrow 5.5 \times 10^7$; $p < 0.05$), whereas concentration of mitochondria dropped after one hour after administration ($7.58 \times 10^7 \rightarrow 3.55 \times 10^7$; $p > 0.05$). In human plasma, qPCR analysis showed naked mtDNA abundance decreased significantly (100% $\rightarrow$ 9%, $p < 0.01$), while mtDNA within intact mitochondria remained stable (100% $\rightarrow$ 120%, $p > 0.05$). This was supported by spectrophotometry, where mtDNA abundance in protein-depleted mitochondria and isolated mtDNA rapidly decreased (75,2% vs 44%, 40,7%; $p < 0.01$). Notably, intact mitochondria induced a significantly stronger TLR9 response compared to naked mtDNA (0.49 A.U. vs. 0.14 A.U., $p < 0.05$). Depletion of mitochondrial proteins significantly reduced NETs formation (1922.43 A.U. vs. 499.69, $p < 0.001$), with naked mtDNA being a poor inducer of NETs formation.

Conclusion: These findings demonstrate that mitochondrial proteins physically shield mtDNA from enzymatic degradation, preserving its immunogenicity and promoting TLR9-mediated NET formation during trauma.

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R05.

Extracellular DNA and DNases in Anorexia Nervosa

**Alexandra Gaál Kovalčíková¹, Peter Bielik², Katarína Šebeková², Ľubica Tichá¹,
Ľudmila Podracká¹, Peter Celec²**

¹ Department of Paediatrics, National Institute of Children's Diseases and Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovakia

² Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction: Anorexia nervosa (AN) is a severe psychosomatic eating disorder with complex systemic effects. Its pathophysiology is not fully understood, and robust biomarkers reflecting underlying molecular alterations remain limited. Extracellular DNA (ecDNA) has emerged as a potential biomarker reflecting tissue damage and inflammation, but also its cause. This study aimed to assess total ecDNA, its nuclear and mitochondrial fractions, ecDNA fragmentation patterns, and DNase activity in plasma from patients with AN and healthy controls.

Methods: The study included 96 female patients with restrictive-type AN and 29 age-matched healthy controls. Total ecDNA concentration was measured fluorometrically, nuclear and mitochondrial DNA fractions and fragmentation were quantified using qPCR. Nucleosomes were quantified using a Cell Death Detection ELISA. DNase was assessed using the single radial enzyme diffusion and ELISA.

Results: Total ecDNA, ncDNA, and mtDNA concentrations did not differ significantly between groups. In contrast, nucleosome concentrations were significantly higher in patients with AN compared to controls (by 58%, $p=0.01$). DNase activity showed no significant differences between groups. Correlation analysis revealed a weak negative association between body weight and ecDNA ($r=-0.20$, $p=0.02$), while no significant associations were observed for BMI or other evaluated parameters.

Conclusion: In conclusion, ecDNA quantity was not altered in AN; however, increased nucleosome concentrations indicate differences in cellular degradation processes. In line with recent fragmentomic studies on ecDNA in eating disorders, these findings suggest that qualitative features of ecDNA may be more informative than absolute concentration in AN. Whether these differences are involved in the pathogenesis of AN and its complications remain to be studied.

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R06.

Peripartum Changes in Plasma Extracellular DNA and DNase Activity After Vaginal Delivery and Cesarean Section

Erik Vitáliš¹, Peter Bielik¹, Lucia Krajčková¹, Petra Pšenková², Jozef Záhumenský², Peter Celec^{1,3}, Barbora Vlková¹

¹ Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, 811 08 Bratislava, Slovakia

² 2nd Department of Obstetrics and Gynecology, Faculty of Medicine, Comenius University, University Hospital Bratislava, Ruzinovska 6, 826 06, Bratislava, Slovakia

³ Institute of Pathophysiology, Faculty of Medicine, Comenius University, 811 08 Bratislava, Slovakia

Introduction: Childbirth involves tissue injury that can trigger the release of mitochondrial DNA (mtDNA) and pro-inflammatory proteins, contributing to sterile inflammation. We investigated peripartum changes in extracellular DNA (ecDNA) and DNase-related markers according to the delivery mode.

Methods: A total of 13 women undergoing vaginal delivery (VD, n = 3) or Cesarean section (CS, n = 10) were sampled at baseline, 2 hours, and 24–8 hours postpartum. Total ecDNA was quantified fluorometrically; nuclear DNA (ncDNA) and mtDNA were measured by qPCR; DNase 1 and DNase 1L3 were quantified by ELISA; and total DNase activity was assessed using a single radial enzyme diffusion (SRED) assay. The DNA integrity index (DII) was calculated from qPCR amplicon ratios for mtDNA and ALU. Repeated-measures two-way ANOVA or mixed-effects models were applied for statistical analysis.

Results: From baseline to 2 hours postpartum, total ecDNA increased 1.36-fold (VD) and 1.23-fold (CS); mtDNA increased 11.05-fold (VD) and 1.2-fold (CS); and ncDNA increased 1.4-fold (VD) and 1.37-fold (CS), all returning toward baseline levels by 24–8 hours. Baseline mtDNA was 2.45-fold higher in the CS group, but was 4.11-fold lower at 2 hours and 1.07-fold lower at 24–8 hours compared to VD. In the VD group, DNase 1 increased 1.33-fold at 2 hours but remained stable in the CS group; DNase 1L3 increased 1.29-fold in VD, whereas it decreased 1.52-fold in CS; and total DNase activity decreased 1.3-fold postpartum in VD while remaining unchanged in CS. DII showed no consistent pattern: ALU DII was 1.72-fold and 2.46-fold higher in CS at baseline and 2 hours, respectively, but was 1.55-fold lower at 24–8 hours; mtDNA DII was 1.39-fold lower in CS at baseline, comparable at 2 hours, and 4.03-fold higher in CS at 24–8 hours. None of the observed changes reached statistical significance ($p > 0.05$).

Conclusion: This preliminary study observed no sustained postpartum elevation in mtDNA or DNase activity. The transient early rise in ecDNA, mtDNA, and ncDNA likely reflects acute, delivery-associated DNA release. The pattern of DNA fragmentation suggests more pronounced DNA degradation following surgical delivery. Larger, well-balanced cohorts with earlier and more frequent sampling are warranted to elucidate the role of mtDNA in childbirth-induced sterile inflammation.

R07.

Cyclic electro-hydrodynamic stacking enables fg/ μ L-level DNA sizing for ultra-sensitive cell-free nucleosome analysis after plasma fractionation in liquid biopsy

Michaël Toublanc¹, Audrey Boutonnet², Marion Mano², Frédéric Ginot², Aurélien Bancaud¹

¹ CNRS, LAAS, 7 Avenue Du Colonel Roche, F-31400, Toulouse, France

² R&D Department, ADELIS, 31 670 Labège, France

Introduction: Circulating cell-free DNA (cfDNA) analysis in blood plasma provides valuable diagnostic information for cancer detection, transplant rejection monitoring, and prenatal screening. However, the low abundance of cfDNA remains a major analytical challenge that can limit sensitivity and reproducibility across studies. Here, we present an improved BIABooster DNA sizing method which allows to study size profile of cfDNA in various plasma compartments.

Methods: Cyclic electro-hydrodynamic stacking was performed by reversing the direction of the BIABooster concentration step. After such a reverse concentration, the concentrated DNA is located at the entry of the injection chamber, instead of being located at its exit. This allows to inject again a sample aliquot without losing the first one. Immunocaptured DNA was obtained by using antibodies binding to histone H3, double-stranded DNA, 5-methylCytosine DNA, or Histone H3 lysine 9 trimethylation (H3K9me3). Plasma fractionation was performed by density gradient ultracentrifugation and by size exclusion chromatography using qEV columns from Izon.

Results: The Limit of Detection (LOD) of the 10 cycles injection method is 3 fg/ μ L for a given DNA fragment. This is more than 1000 fold more sensitive than “high sensitive” methods available with popular DNA analyzers like the BioAnalyzer or the Fragment Analyzer. Immunocapture of 5-methylCytosine cfDNA enriches significantly cfDNA in long fragments. As most of the cfDNA is smaller than 250 bp, it must be taken into account for interpretation of results obtained after such an immunocapture. In healthy donors, cfDNA circulates mainly as free nucleosomes, not associated to extracellular vesicles. This may be different in cancer patients.

Conclusions: We show that ultra-high sensitivity cfDNA size profiling brings new insights about cfDNA structure and biogenesis. This knowledge will contribute to improve liquid biopsy workflows.

R08.

Extracellular DNA composition, not concentration, reflects neutrophil extracellular trap formation in MASLD: evidence from patients and a cafeteria-diet mouse model

**Roman Gardlík¹, Andrej Feješ^{1,2}, Barbora Gromová¹, Emil Bečka¹, Regina Varga¹, Michal Pastorek¹,
Veronika Borbélyová¹, Mária Szántová³, Michal Hanák³, Viera Kupčová³,
Pavel Babál⁴, Katarína Šebeková¹**

¹ Institute of Molecular Biomedicine, Comenius University Faculty of Medicine, Bratislava, Slovakia

² 5th Department of Internal Medicine, Comenius University Faculty of Medicine, Bratislava, Slovakia

³ 3rd Department of Internal Medicine, Comenius University Faculty of Medicine, Bratislava, Slovakia

⁴ Institute of Pathological Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterised by chronic low-grade inflammation, in which activated neutrophils release neutrophil extracellular traps (NETs), chromatin fibres decorated with myeloperoxidase (MPO) and neutrophil elastase (NE). NETosis liberates DNA into the circulation, and total extracellular DNA (ecDNA) has therefore been proposed as an accessible marker of this response. Whether total ecDNA reflects NET formation in MASLD, or whether resolution of its nuclear (ncDNA) and mitochondrial (mtDNA) subfractions is required, remains undefined.

Methods and results: We first studied 51 MASLD patients (31 women, 20 men) and 16 controls (10 women, 6 men). Plasma was processed at 16,000g, and total ecDNA was quantified together with its nuclear and mitochondrial subfractions, MPO, NE, and soluble RAGE. Patients exhibited higher BMI, insulin resistance, and transaminases. Neutrophil activation was evident, with significant elevation of both MPO and NE. Total ecDNA, in contrast, did not differ between groups and therefore failed to detect this response. Subfraction analysis was discriminative: ncDNA increased approximately threefold, mtDNA decreased, and the mtDNA/ncDNA ratio declined severalfold. The concentration of circulating DNA was thus preserved, whereas its compartmental origin shifted toward nuclear DNA, consistent with a NET-derived source.

We next determined whether diet alone reproduces this phenotype, using thirteen female mice fed standard chow (CTRL, n = 7) or cafeteria diet (CAF, n = 6), an established model of obesity and MASLD. CAF mice developed obesity, insulin resistance and hepatic injury, with elevated MPO and NE and increased spontaneous and E. coli-induced ex vivo NET formation. Total serum ecDNA again remained unchanged.

Conclusions: Across both species, MASLD was accompanied by neutrophil activation and enhanced NET formation that total ecDNA failed to detect. Fraction-specific ecDNA —nuclear-dominant DNA and a reduced mtDNA/ncDNA ratio, captures this response, indicating that ecDNA composition, rather than concentration, constitutes the informative readout in metabolic liver disease.

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R09.

Non-invasive Fetal Antigen Genotyping Beyond RhD: Interim Results from the GeKon Study

Glodkowska-Mrowka E.¹, Kozioł K, Łpieńka H., Grażlis W, Laskowska P., Guz K, Mrówka P., Pelc-Kłopotowska M., Orzińska A.

¹ Institute of Hematology and Transfusion Medicine, Medical University of Warsaw, Warsaw, Poland

Introduction: Following the widespread implementation of anti-RhD prophylaxis, alloimmunization involving non-D antigens (anti-RhE, anti-Rhc, anti-RhC, anti-K) has become a significant contributor to HDFN. cfDNA-based non-invasive prenatal testing (NIPT) enables accurate fetal antigen prediction; however, unlike RhD testing, its application to other antigens remains limited to specialized centers. Prospective evidence on clinical outcomes, quality of life, and economic impact is needed to inform broader implementation.

Aim: To assess the routine application of NIPT for fetal non-D Rh (C, c, E) and K antigens in alloimmunized pregnancies and to examine its role in improving risk stratification.

Methods: This 14-month interim analysis includes 53 pregnant women enrolled in the prospective GeKon study with clinically relevant non-D alloantibodies: anti-c (n=3), anti-E (n=18), anti-K (n=15), and multispecific antibodies (n=17). Maternal plasma cfDNA was isolated and analyzed for RHCEc, RHCEE, RHCEC, RHD exons 5 and 7, CCR5, SRY, or biallelic polymorphisms using real-time PCR (LC480II, Roche) or for KEL01.01/*02 and SRY using digital PCR (Naica 6-color dPCR, Stilla). Clinical follow-up included antibody titers, paternal serology, cost analysis, and quality-of-life measures.

Results: NIPT identified 21/53 pregnancies as high risk due to detection of at least one incompatible fetal antigen. In 2 cases, risk remained unresolved because of additional antibodies. Among 17 pregnancies with anti- and Kk partners, 3/15 fetuses were predicted K-positive, below the expected 50%. For RhC/c/E, observed fetal antigen distribution followed Mendelian expectations. To date, 24 deliveries have occurred; among 18 with neonatal genotyping, concordance with NIPT was 100%. No moderate or severe HDFN was observed low-risk pregnancies. Quality of life analysis is ongoing.

Conclusions: NIPT demonstrated complete agreement with neonatal genotypes and enables reclassification of many pregnancies from antibody-defined high risk to truly low risk. These findings support a targeted monitoring approach in non- alloimmunization and contribute evidence toward broader clinical integration.

R10.

Quantification of Feto-Maternal ABO*A1 Chimerism by Digital PCR for HDFN Risk: A preliminary study

Agnieszka Orzińska¹, Sylwia Purchla-Szepiół, Katarzyna Guz, Eliza Głdkowska-Mrówka

¹ Institute of Hematology and Blood Transfusion, Warsaw, Medical University of Warsaw, Warsaw, Poland

Introduction: Hemolytic disease of the fetus and newborn (HDFN) due to ABO incompatibility is rare and typically occurs in mothers with high titers of antibodies, such as IgG anti-A, carrying a fetus expressing A antigens. Non-invasive determination of fetal ABO blood group from maternal plasma may support risk assessment and guide postnatal management. This preliminary study aimed to quantify feto-maternal ABO*A1 chimerism using digital PCR.

Methods: DNA from a mother and fetus (using a Fisher) on the Naica platform (Stilla/Bio-Rad). ABO*A chimerism was calculated as the number of ABO*A-positive events divided by all positive events $\times 100\%$.

Results: In the 0.5% ABO*A spike sample ABO*A chimerism was 0.37% (99 ABO*A-positive events). In the case with an O blood group partner, fetal ABO*A chimerism was 0.002% (one false ABO*A-positive event in one replicate). Among four cases with A blood group partners, two showed chimerism of 0.05% and 0.03% (mean ABO*A-positive events: 12 and 8, respectively), and fetuses were predicted to be blood group A. In the remaining two cases, chimerism was 0.004% (one false ABO*A-positive event in one replicate in both), and fetuses were predicted to be blood group O.

Conclusion: Digital PCR using the ABO*A1/non-A1 assay may enable precise detection of low-level feto-maternal ABO*A1 chimerism and could support risk assessment of ABO-related HDFN, particularly in pregnancies with a history of severe disease.

R11.

In Apparently Healthy Adolescents, Extracellular DNA Metabolism is Linked to Cardiometabolic Risk in a Sex-dependent Manner

Katarína Šebeková¹, Ľubica Janovičová¹, Radana Gurecká², Peter Celec¹

¹ Institute of Molecular Biomedicine, Medical Faculty, Comenius University in Bratislava, Bratislava, Slovakia

² Institute of Medical Physics and Biophysics, Medical Faculty, Comenius University in Bratislava, Bratislava, Slovakia

Introduction: In adults, obesity and metabolic syndrome (MetS) are associated with higher extracellular DNA (ecDNA) levels and lower DNase activity. However, in apparently healthy adolescents, ecDNA levels are associated with increased cardiometabolic risk (estimated as continuous MetS score [cMSS = $FPG/5.6 + SBP/130 + WHtR/0.5 + TAG/1.7 - HDL-C/1.02$ (males) 1.28 (females)]) rather than obesity. Associations between DNase activity, ecDNA, its nuclear (nc) and mitochondrial (mt) fractions, and cMSS in healthy adolescents remain unclear. We hypothesized that ecDNA levels and DNase activity are associated with cardiometabolic risk.

Methods: In 572 healthy adolescents (43% male) aged 16.8 ± 1.3 years, anthropometrics, blood pressure, blood counts, and standard blood chemistry were assessed. Plasma ecDNA was isolated and measured by fluorometry, ncDNA and mtDNA were quantified by real-time PCR, DNase activity by a SRED method.

Results: Males showed higher cMSS (2.2 ± 0.4 vs. 1.9 ± 0.4), higher total plasma ecDNA [14 (10–1) vs. 11 (8–6) ng/mL; median (interquartile range)], nuclear [1,743 (973–3,281) vs. 1,008 (570–1,171) genome equivalents (GE)/mL], and mitochondrial [36,814 (13,398–95,066) vs. 29,646 (10,626–2,286) GE/mL] DNA, with effect sizes of 0.8, 0.8, and 0.6, respectively. DNase activity was similar (7.1 ± 2.8 vs. 7.0 ± 2.6 K.U./mL). EcDNA, ncDNA, and mtDNA showed strong interrelations in both males ($r=0.38$ to 0.72) and females ($r=0.43$ to 0.60), but they did not correlate with DNase activity in either sex. CMSS correlated with DNase activity in males ($r=0.282$, $p<0.001$) and with ecDNA ($r=0.116$, $p=0.035$) and ncDNA ($r=0.114$, $p=0.046$) in females. The multivariate OPLS regression indicated that total body fat, DNase activity, and ecDNA levels are predictors of cMSS in males, while metabolic and inflammatory parameters predict cMSS in females.

Conclusion: In apparently healthy adolescents, ecDNA metabolism is linked to cardiometabolic risk in a sex-dependent manner. Our findings identify ecDNA turnover as a potential biomarker of cardiometabolic risk and highlight the importance of considering sex differences in future studies.