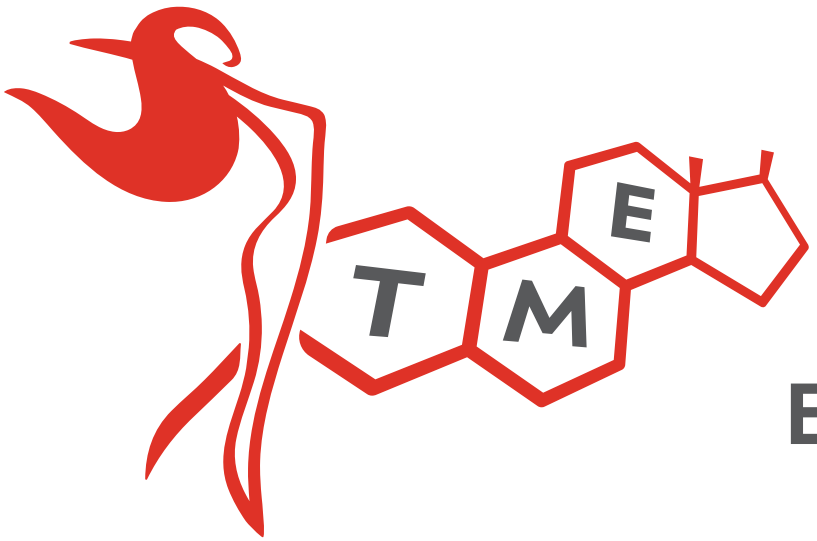

TRANSLATIONAL MOLECULAR ENDOCRINOLOGY

BOOK OF ABSTRACTS

Organized by the Program group P3-0449 of the Faculty of Medicine, University of Ljubljana.

Organizing committee

Tea Lanišnik Rižner, Maja Pušić Novak, Marija Gjorgoska

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Scientific meeting Translational Molecular Endocrinology

18th September 2024

Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

BOOK OF ABSTRACTS

Dear Colleagues,

In 2024, a new program group **P3-0449** ***Translational Molecular Endocrinology for Women's Health*** was established at the Faculty of Medicine of the University of Ljubljana, focusing on the models of hormone-dependent diseases, pathophysiology, discovery of new drug targets and diagnostic and prognostic biomarkers. This has prompted us to organize the first scientific meeting of the program group with invited speakers from abroad, which will take place on **18 September 2024** at the Faculty of Medicine.

The first scientific meeting "**Translational Molecular Endocrinology**" will include three sessions. **Models for hormone dependent diseases,** from primary cell cultures to cell lines, 3D cultures, organoids to patient derived explants will be presented in the first session by members of the program group and Dr. Daniela Annibali from KU Leuven and Dr. Andrea Romano from University of Maastricht.

The second session will focus on **molecular mechanisms of hormone dependent diseases**, in particular the role of estrogens and androgens presented by members of the program group, novel compounds targeting enzymes of local estrogen formation introduced by Dr. Erzsebet Mernyak from University of Szeged and finally, mathematical modeling of steroid metabolism explained by Dr. Karl Storbeck from Stellenbosch University.

Diagnostic and prognostic biomarkers of hormone dependent gynecological diseases will be the topic of the third session. The members of the program group will present their studies on biomarker discovery using metabolomics, steroidomics, proteomics and transcriptomics approaches as well as the construction of diagnostic and prognostic models for endometriosis, endometrial and ovarian cancer.

The aim of the first meeting is to present the current and new studies being conducted in collaboration with the University Medical Centre Ljubljana and colleagues from abroad. There will be plenty of time for networking and establishing new contacts and collaborations that will contribute to high-quality studies in the field of translational molecular endocrinology in the coming years.

We would like to thank the invited speakers, the members of the program group, the sponsors and all those involved in the organization of this event.

You are cordially invited to attend the 2nd meeting Translational Molecular Endocrinology in 2025.

On behalf of the organizing committee

Head of the program group P3-0449

Prof. dr. Tea Lanišnik Rižner

Program

10:00-10:05	**Welcome**
	Vita Dolžan, Head of the Institute of Biochemistry and Molecular Genetics, UL, Faculty of Medicine
10:05-10:25	**Tea Lanišnik Rižner**, UL, Faculty of Medicine, Ljubljana, Slovenia *Presentation of the program group Translational molecular endocrinology for women's health*
10:30-11:50	**Models of hormone dependent gynecological diseases**
	Chair: Maja Pušić Novak
10:30-10:50	**Maja Pušić Novak,** UL Faculty of Medicine, Ljubljana, Slovenia *From primary cells to immortalized endometriotic cell lines*
10:50-11:10	**Ivana Jovčevska**, UL Faculty of Medicine, Ljubljana, Slovenia *3D models of high grade serous ovarian cancer*
11:10-11:30	**Daniela Annibali**, KU Leuven, Leuven, Belgium *Ovarian cancer patient derived explants*
11:30-11.50	**Andrea Romano**, University of Maastricht, Maastricht, The Netherlands *Organoids as models of endometrial cancer*
11:50-13:00	**Lunch break**
13:00-14:20	**Molecular mechanisms of hormone dependent diseases**
	Chair: Marija Gjorgoska
13:00-13:20	**Marija Gjorgoska**, UL, Faculty of Medicine, Ljubljana, Slovenia *Androgens in endometrial and ovarian cancers*
13:20-13:40	**Nika Marolt**, UL, Faculty of Medicine, Ljubljana, Slovenia *Estrogens in high grade serous ovarian cancer*
13:40-14:00	**Erzsebet Mernyak**, University of Szeged, Szeged, Hungary *Targeting enzymes of local estrogen formation*
14.00-14:20	**Karl Storbeck**, Stellenbosch University, Stellenbosch, South Africa *Mathematical modeling of steroid metabolism*
14:20- 14:50	**Coffee break**
14:50 - 16:50	**Diagnosis and prognosis of hormone dependent gynecological diseases**
	Chair: Tea Lanišnik Rižner
14:50 - 15:10	**Jerzy Adamski**, UL Faculty of Medicine, Ljubljana, Slovenia; National University of Singapore *Search for diagnostic biomarkers with metabolomics: endometriosis case*
15:10 – 15:30	**Vid Janša**, University Medical Centre Ljubljana, Ljubljana, Slovenia *Biomarkers of endometriosis discovered by proteomics*
15:30 - 15:50	**Maja Pušić Novak**, UL, Faculty of Medicine, Ljubljana, Slovenia *Transcriptomic approach for discovery of biomarkers for peritoneal endometriosis*
15:50 – 16:10	**Luka Roškar**, Hospital Murska Sobota, Murska Sobota, UL Faculty of Medicine, Ljubljana, Slovenia *Angiogenesis factors as candidate biomarkers for EC*
16:10 – 16:30	**Tea Lanišnik Rižner**, UL, Faculty of Medicine, Ljubljana, Slovenia *Proteomics/metabolomics models for diagnosis and prognosis of EC*
16:30 – 16:50	**Marija Gjorgoska**, UL, Faculty of Medicine, Ljubljana, Slovenia *Steroidomics for discovery of biomarkers*
16:50- 16:55	**Concluding remarks**

Presentation of the program group Translational molecular endocrinology for women's health

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Program group P3-0449 *Translational Molecular Endocrinology for Women's Health* was established at the Faculty of Medicine of the University of Ljubljana in January 2024. The new program addresses unmet clinical need for early diagnosis, reliable prognosis allowing individualized therapy, and new treatment options for hormone-dependent gynecological diseases that affect women throughout their lives and are associated with tremendous health burden and economic loss. Endometriosis, characterized by the growth of endometrial-like tissue outside the uterine cavity, affects up to 10% of reproductive age women and 190 million women worldwide. Hormone-dependent cancers occur most frequently after menopause and account for more than 35% of all cancers in women, with endometrial cancer (EC) being the most common and ovarian cancer (OC) the deadliest gynecologic cancer. Currently, there is a lack of appropriate models for endometriosis EC and OC, therapies to reverse chemoresistance, and clinically relevant diagnostic and prognostic biomarkers for endometriosis, EC and OC.

In endometriosis there is a clear need for a better understanding of the pathophysiology, early non-invasive diagnosis in combination with new treatment options for patients. There is also a need to improve the treatment of OC and EC patients through the development of non-/minimally invasive tools for early diagnosis, prognostic tools that allow individualized treatment and prevent over- or under-treatment, and treatment strategies to combat chemoresistance. The new program aims to construct models, elucidate the pathophysiological mechanisms at the molecular level, identify new drug targets, especially in the context of chemoresistance, discover biomarkers and develop multi-omics diagnostic and prognostic algorithms for these hormone-dependent diseases. These goals will be achieved through state-of-the-art methodological approaches and global collaboration.

Models of hormone dependent gynecological diseases

From primary cells to immortalized endometriotic cell lines

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Endometriosis is a chronic gynecological disorder characterized by the presence of endometrial-like tissue outside the uterus, affecting approximately 190 million women globally. Despite its prevalence, the etiology and pathophysiology of endometriosis remain unclear, leading to problems with delayed diagnosis and inadequate treatment. Most of the currently established endometriotic cell lines lack thorough phenotypic characterization. This study aims to obtain and thoroughly characterize endometriotic primary cell cultures and to establish novel immortalized cell lines for endometriosis research.

Endometriotic tissue samples (n=7) were collected at the Department of Gynecology at UMC Ljubljana following a strict standard operating procedure. The collected samples were used to establish a protocol for isolation and cultivation of endometriotic primary cells. Different digestion solutions and cell separation methods were tested. Two cell fractions were obtained and their viability, proliferation rate and population doubling time were determined. All isolated primary cells were screened for presence of mycoplasma.

A protocol for the isolation of endometriotic primary cells was successfully established, with all cells testing negative for mycoplasma. The first cell fraction required an average of 16.2 ± 7.3 days to reach confluence, with a population doubling time of 2.7 ± 0.7 days, while the second fraction reached confluence in 13.6 ± 4.3 days with a population doubling time of 2.5 ± 0.9 days. Both cell fractions maintained high viability (>90%) through passage 4.

Ongoing experiments are focused on analyzing the expression of estrogen-associated genes and proteins in the primary cells obtained. Future experiments will include a complete characterization of the cells by assessing their migratory capacity, cell senescence and identifying morphological markers to detect contaminating cells. After confirmation of cell purity, the cells will be immortalized using the human telomerase reverse transcriptase (hTERT) plasmid.

This research was supported by ARIS grants Z3-4522 to M.P.N. and P3-0499 to T.L.R, and the EU H2020-MSCA-RISE project TRENDO.

3D tumor modeling reveals key insights into EMT, gene expression, and treatment development

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Traditional research on high grade serous ovarian cancer (HGSOC) uses 2D monolayer cellular models that only partially resemble the spatial characteristics of the tumor. Current drug screening assays use 2D models, which do not correlate with results obtained in clinical trials. Lately, cancer research has shown that 3D spheroid models better mimic the *in vivo* tumor and its microenvironment than 2D monolayer models. Their use will result in improved tumor phenotyping, proliferation dynamics and better understanding of ovarian cancer biology. At last, cost-effective 3D cellular models with consistent properties can be used as high-throughput drug screening platforms or for modeling tumor microenvironment.

Here, we examine differences between 2D and 3D models of OVCAR-4, OVSAHO, COV362, and Kuramochi cell lines, which best match the genomic picture of HGSOC. **Aim:** to develop reproducible protocols for generating 3D cellular models of HGSOC and characterizing them using various methods, and to develop and optimize a protocol for spheroid dissociation into single cells for downstream analysis. **Morphology:** 3D spheroid models show dynamic structural changes and cell migration patterns like *in vivo* tumors after characterization. Key cancer-progression genes (*WNT11B*, *BRCA1*, *VIM*, *TP53*, *MMP2*, *VEGFA*, *Ki67*) are expressed differently in 3D than 2D models. **EMT and Signaling:** *WNT11B* and *VIM* upregulation in 3D models suggests enhanced EMT which makes cancer cells more invasive. **Proliferation and Microenvironment:** *BRCA1* and *Ki67* downregulation in 3D suggests slower cell division due to the spheroid environment. **Treatment development:** We will perform drug screening assays with selected chemotherapeutics (eg. carboplatin, olaparib) to determine differences in IC_{50} values between 2D and 3D models. **Conclusion:** 3D spheroids better model the complex biology of HGSOC. The choice of *in vitro* models can influence key processes like EMT and cell proliferation which can lead to inaccurate prediction of drug responses *in vivo*.

This research was supported by ARIS grant L4-4565 to I.J. and P3-0449 to T.L.R.

Ovarian cancer patient-derived explants

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Rapid and effective clinical implementation of preclinical findings often remains a significant hurdle, mainly because of scarcity of suitable preclinical models capable of accurately predicting responses to both traditional and innovative therapies. This is particularly evident within the realm of gynecological cancers, where the effectiveness of emerging targeted therapies and combination treatments has been limited, compared to other cancer types, and the standard of care is still represented by platinum-based chemotherapy. To improve this, we started the PETITE, a collection of patient-derived explant (PDE) models established from different gynecological cancers. PDEs can be established//biobanked directly from surgical specimens – also from rare and difficult-to treat subtypes - and cultured ex vivo as tumor-derived fragments or precision-cut slices, allowing functional testing of live tumor tissues without previous major manipulation or dissociation.

The initial characterization of the ovarian cancer PETITE models revealed that they maintain a marked intra- and inter-tumor heterogeneity in terms of morphology and metabolic activity. Moreover, our findings confirm that PDEs in culture preserve original tumor characteristics and retain their value in predicting responses to chemotherapy. Encompassing most common but also rarer subtypes, such as mucinous ovarian carcinomas or granulosa cell tumors, the PETITE collection represents a robust platform for exploring translational research avenues and tailoring personalized treatment approaches for patients with ovarian cancers.

This research was supported by https://giving.kuleuven.be/projects/research-into-mucinous-ovarian-cancer and UZ Leuven Fund for Academic Studies (KOOR).

Organoids as models of endometrial cancer

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Organoid technology was developed a few decades ago as a model of intestinal crypts, and since then, it has been applied to a large variety of tissues.

Organoids are organized as three-dimensional cultures where cells are dispersed and suspended in an extra cellular matrix milieu and thus maintain cell-cell interactions, rather than establishing strong - unphysiological - binding with the coating of a culture plate as in the case of monolayer cell cultures. To establish organoids, epithelial primary cells are cultured in medium containing a cocktail of growth factors and intracellular signaling activators or inhibitors aimed at maintaining a pool of stem cells and at the same time inhibit terminal differentiation that allow maintaining and passaging these cultures for extremely long time. Through passaging, organoids maintain the main features of the tissue of origin and are generally genetically stable. Finally, since organoids are derived from primary patient material, these models are suitable to study personalized approaches and medicine.

Endometrial organoids were described first in 2017. The lecture aims to give an overview of the current state in the development and use of endometrial organoids, with special focus on endometrial cancer organoids and the experiences at the Maastricht University. Opportunities and challenges will be overviewed.

This research was supported by Dutch Cancer Society, project number 2022-4 EXPL/14795.

Molecular mechanisms of hormone dependent diseases

Androgens in endometrial and ovarian cancers

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Endometrial cancer (EC) is the most common gynecological cancer in high-income countries, with a rising global incidence. High-grade serous ovarian cancer (HGSOC) is the most lethal ovarian cancer subtype, mainly due to its diagnosis at advanced stages. Understanding the molecular basis of EC and HGSOC can inform prevention, detection, and treatment strategies. In our study, we focused on androgens' intracrinology in these cancers. More specifically, we conducted *in vitro* metabolism studies using a panel of four well-characterized EC and six HGSOC cell lines to examine the intra-tumoral formation of bioactive androgens and 11-oxyandrogen metabolites from steroid precursors. Additionally, we analyzed publicly available datasets from the Cancer Genome Atlas Network to explore the association between androgen receptor (AR) expression and clinical outcomes in both cancers.

In EC, we found that the ability of *in vitro* models to metabolize classic androgen precursors into bioactive androgens varied but was not linked to tumor grade or molecular phenotype. Low-grade EC models showed a higher capacity to produce bioactive 11-oxyandrogens from 11-oxyandrogen precursors compared to normal endometrium and high-grade EC models. Furthermore, *AR* expression was highest in tumor-adjacent endometrium and lowest in high-grade endometrial tumors, and it was associated with improved disease-specific survival (HR: 0.41, 95% CI, 0.18-0.95, p = 0.04). In HGSOC, we identified a subset of cell models with a strong ability to transform 11-oxyandrogen precursors into bioactive 11-oxyandrogens, characterized by high expression of cell differentiation markers. Furthermore, *AR* expression was significantly higher in primary versus metastatic HGSOC tumors and in chemo-sensitive versus refractory disease. Higher *AR* expression was also associated with a better disease-free interval (HR: 0.79, 95% CI, 0.61-1.00, p = 0.06). Altogether, our results support the further investigation of AR as prognostic biomarker in both EC and HGSOC.

This research was supported by ARIS grants J3-2535 and P3-0449 both to T.L.R.

Estrogens in high grade serous ovarian cancer

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High-grade serous ovarian carcinoma (HGSOC) is the most common ovarian cancer, often developing resistance to chemotherapy and involving disrupted estrogen action. The role of estrogens in HGSOC chemoresistance remains unclear. This study had two aims: i) to contribute to better understanding of estrogen metabolism and the role of estrogens in the proliferation, migration and sensitivity to carboplatin of HGSOC cell lines; ii) to identify potential chemoresistance biomarkers among genes involved in estrogen biosynthesis and action using transcriptomic data from platinum sensitive/resistant HGSOC tissues.

The formation of estrogens from estrone sulfate (E1S) was analyzed by LC-MS/MS. QPCR was used to analyze the expression of genes involved in estrogen biosynthesis, transport and action in six HGSOC cell lines with different sensitivities to carboplatin. The effects of estrogens and sulfatase inhibitor (STX64) on the proliferation and migration of and sensitivity to carboplatin were examined using the Alamar assay and wound healing test. RStudio was used for bioinformatic analysis of the selected transcripts from HGSOC tissues.

LC-MS/MS showed active estrogen formation in most carboplatin-sensitive cells, except COV362. E1S and estradiol influenced proliferation in OVSAHO, OVCAR-3 and Caov-3 cells. Ethinylestradiol (EE2) and equilin (EQ) reduced proliferation in OVCAR-4, Kuramochi, Caov-3, and COV362, while EQ increased proliferation of OVSAHO. STX64 decreased E1S-dependent proliferation in Caov-3 and OVSAHO, impacted carboplatin response in Caov-3, and reduced viability in OVSAHO and Kuramochi. E1S inhibited migration and enhanced carboplatin response in Kuramochi and Caov-3, while EQ improved migration inhibition with carboplatin in Kuramochi. EE2 combined with carboplatin was more effective in reducing migration in Kuramochi and COV362 compared to individual treatments. Transcriptomic analyses of HGSOC tissues and cell lines identified potential target genes affecting treatment response. This study elucidates the role of estrogens in proliferation and migration, identifies biomarker candidates for HGSOC chemoresistance and suggests new strategies for overcoming chemoresistance.

This research was supported by ARIS grant P3-0449 to T.L.R. and a Young Researcher grant to N.M.

Targeting enzymes of local estrogen formation

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The core-modified 13α-estrone (13αE1) is a promising starting material in the design of bioactive estrone-based agents lacking estrogenic side effects. We have recently described halogenations, Pd-catalyzed cross couplings (C–C, C–N or C–P) or directed C–H modifications at the A-ring of 13αE1. Depending on the reaction conditions and reagents, *ortho* or *meta* substituted 13αE1 derivatives were synthesized. Certain potent inhibitors of established anticancer drug targets, namely 17β-hydroxysteroid dehydrogenase 1 (17β-HSD1), steroid sulfatase (STS), organic anion transporting polypeptide 2B1 (OATP2B1) and AKR1C1–3 enzymes have been identified, and important structure-activity results appeared. Several halogenated 13αE1 derivatives exerted outstanding OATP2B1 inhibition with submicromolar IC_{50} values. Biphenyl derivatives proved to be antiproliferative against human reproductive cancer cell lines. 3-Hydroxy-2-phenylethynyl compounds exerted marked inhibitory action against 17β-HSD1 enzyme. C-2 phosphonated derivatives proved to be dual OATP2B1 and 17β-HSD1 inhibitors. We have identified several potent, selective AKR1C inhibitors, acting in the submicromolar range. It should be underlined that the biological activity of A-ring-modified 13αE1 compounds greatly depends on the substitution pattern of the aromatic ring. Overall, these results indicate that this compound group has the potential for development of new antitumor agents.

This research was supported by NKFIH SNN 139323 grant.

Mathematical modeling of steroid metabolism

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Hormone-dependent cancers are driven by sex steroids and rely on both peripheral and local activation of these hormones. Recently, a novel group of adrenal-derived androgens, known as 11-oxygenated androgens, has been identified in humans. The active forms, 11-ketotestosterone (11KT) and 11-keto-5α-dihydrotestosterone (11KDHT), are potent androgen receptor (AR) agonists with potencies and efficacies similar to testosterone and DHT, respectively. Our group has shown that, although there is overlap in the enzymes involved in the pre- and post-receptor metabolism of classic and 11-oxygenated androgens, there are significant differences in enzyme substrate preferences.

We are employing a bottom-up systems biology approach to build a comprehensive computational model of classic and 11-oxygenated androgen metabolism that can be adapted to enzyme expression levels in specific cancer settings and used to predict the effects of changes in enzyme expression or inhibition. This process involves kinetically characterizing individual enzymes expressed in non-steroidogenic HEK293 cells and using the resulting parameterized rate equations to construct a computational model comprising ordinary differential equations (ODEs).

We subsequently validate the model by its ability to independently predict the conversion of substrates by enzymes co-expressed in HEK293 cells at different ratios or to predict the metabolism in cell lines or human tissue explants endogenously expressing various combinations and ratios of enzymes. We have also demonstrated that the model can predict relative enzyme expression levels *in vivo* based on enzyme kinetics and the measured circulating concentrations of each steroid substrate and product.

Taken together, our data illustrate the power and utility of a bottom-up systems biology approach for investigating complex and overlapping metabolic pathways, such as those observed in steroid hormone biosynthesis and metabolism. This approach may be a valuable tool in predicting steroid metabolism within hormone-dependent cancers.

This research was supported by The National Research Foundation (NRF) of South Africa and International Centre for Genetic Engineering and Biotechnology (ICGEB).

Diagnosis and prognosis of hormone dependent gynecological diseases

Search for diagnostic biomarkers with metabolomics: endometriosis case

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The development of biomarkers for the diagnosis of human diseases is a challenging process. The biomarkers must be characteristic of a certain phenotype, quantifiable, actionable for intervention, safe to collect and sustainable to produce. The most common problem in the search for a new biomarker is its lack of specificity for a particular medical indication. Biomarkers can fail because they also change in other health conditions or diseases. In post-genomic diagnostics, the functional aspect of omics such as the quantification of miRNA profiles, protein/peptide or metabolite signatures is becoming increasingly important.

Endometriosis is a disease caused by ectopic growth of endometrial tissue that affects 190 million women worldwide and is characterized by pelvic pain, infertility and inflammatory complications. Treatment includes hormone therapy and surgery. Currently, only advanced stages of endometriosis can be clearly diagnosed, and the gold standard is invasive laparoscopy followed by histologic examination. In the search for non-invasive biomarkers for endometriosis, we investigated metabolomic signatures in venous blood. Concentrations of single metabolites were not facilitating distinction of healthy and affected individuals. However, using generalized linear model approach we were able to discover specific metabolite signatures that support the diagnosis of endometriosis in early stages. This approach has the great advantage of being insensitive to the variability of the human metabolome caused by confounding factors such as ethnicity, age, diet, lifestyle or medication. At present we prepare for clinical validation of approach.

This research was supported by a grant from Helmholtzgemeinschaft to J.A and ARIS grants J3-6799, J3-1755 and P3-0449 to T.L.R.

Biomarkers of endometriosis discovered by proteomics

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Endometriosis is a common benign gynecological disease, with a high incidence in patients experiencing fertility problems. The diagnosis of ovarian endometriosis or deep endometriosis can be made with transvaginal ultrasound, while peritoneal endometriosis needs to be proven through surgical procedure - laparoscopy. The desire for non-invasive diagnostics is the basis for our research.

The study was designed as a clinical prospective investigation. In the first part, peritoneal fluid (PF) samples were analyzed. A proteomic approach with antibody arrays was used in the discovery phase, while ELISA was used in the validation phase. In the second part of the study, proteins identified as potential biomarkers in PF were analyzed and validated in blood samples from 293 patients. We identified 16 proteins whose concentrations differed significantly between patients with endometriosis and those without endometriosis. Angiotensinogen (AGT), transforming growth factor-β-induced protein ig-h3 (TGFBI), cartilage oligomeric matrix protein (COMP), and angiopoietin-4 (ANGP4) have not previously been described in association with endometriosis. In the validation phase, we confirmed elevated concentrations of TGFBI and COMP in PF from patients with endometriosis. Discovery and validation studies in patients' plasma revealed higher TGFBI concentrations in patients with endometriosis. Importantly, in validation phase TGFBI showed good diagnostic potential for early-stage endometriosis with an area under the curve (AUC) of 0.74, sensitivity of 61%, and specificity of 83 %, compared to CA125, which had an AUC value of 0.62, sensitivity of 60% and specificity of 67%. TGFBI also showed good potential for the diagnosis of patients with peritoneal endometriosis, with an AUC of 0.76, sensitivity of 58%, and specificity of 89%. Finally, an SVM model combining TGFBI and CA-125 showed high AUC value of 0.94 and sensitivity of 95% for diagnosing moderate-to-severe endometriosis. TGFBI has good characteristics in the diagnosis of patients with early-stage endometriosis and might be considered as a candidate for Rule IN triage test after clinical validation.

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Transcriptomic approach for discovery of biomarkers for peritoneal endometriosis

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Endometriosis is a chronic gynecological condition affecting up to 10 % of all women in reproductive age. Diagnosing peritoneal endometriosis (PE) is particularly challenging, as it cannot be detected with standard imaging techniques. While numerous biomarkers of endometriosis have been identified to date, none have been clinically validated. However, recent advances in transcriptomics have created new opportunities for biomarker discovery. This study aimed to identify novel blood biomarkers for PE using whole blood transcriptomics combined with machine learning approaches.

Patients with symptoms of endometriosis who underwent laparoscopic surgery were enrolled in the study. They were categorized with presence of PE (n=20), peritoneal and ovarian endometriosis (PE+OE) (n=8), or absence of endometriosis (controls, n=20). Patient's blood samples were collected and whole genome RNA sequencing was performed. Differentially expressed genes (DEGs) and transcripts (DETs) were identified (FDR<0.05). Principal component analysis (PCA) and feature selection were conducted using transcripts per million data. The most important genes and transcripts were identified using mutual information, random forest, and support vector machine (SVM) feature selection methods. These selected features were then used to train an SVM classifier, which was subsequently tested on a separate set of unseen data to evaluate its performance.

In the proliferative phase, no DEGs and only 2 DETs were identified between the controls and the PE group, while in the secretory phase, 1,035 DEGs and 922 DETs were detected. There was no common DEGs and DETs in both menstrual phases. PCA clustered samples only based on their menstrual phase. A set of six transcripts identified by feature selection achieved the highest performance in SVM models, with an ROC AUC of 0.92, sensitivity of 0.75, and specificity of 1.0.

This is the first study to use whole genome RNA sequencing to discover blood biomarkers for PE. Validation of the selected DETs in a larger patient cohort is currently underway.

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Angiogenic factors as candidate biomarkers for endometrial cancer

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Endometrial cancer (EC) is a common gynecological cancer, with its growth and spread facilitated by an angiogenic switch involving the release of pro-angiogenic and suppression of anti-angiogenic factors (AFs). Studying these factors as biomarkers in plasma and tumor tissues could improve EC diagnosis and treatment.

Our research on angiogenesis in EC consisted of three phases. Initially, a prospective case-control study with 76 postmenopausal women examined 37 AFs as potential EC biomarkers. We found that plasma levels of sTie-2 and G-CSF were lower in EC patients, while leptin levels were higher.

Next, in a validation study with 202 patients (91 with EC and 111 with benign conditions), we measured pre-operative plasma concentrations of six angiogenic factors: leptin, IL-8, sTie-2, follistatin, neuropilin-1, and G-CSF. Using machine learning, we developed a diagnostic model based on these AFs. The leptin-based univariate model performed best, and the multivariate model, which included all six AFs, BMI, and age, achieved a ROC AUC of 0.89, suggesting strong predictive capability for EC risk.

Finally, we used qPCR on 36 EC patients to test the expression of 15 angiogenesis-associated genes in tumor versus tumor-adjacent tissue. The results indicated that angiogenesis in EC is primarily driven by reduced expression of anti-angiogenic factors, with altered regulation in adjacent tissues linked to poorer prognosis.

Our findings suggest that plasma concentrations of AFs could serve as a supplementary diagnostic tool for early EC detection and prognosis, potentially guiding treatment decisions.

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Proteomics/metabolomics models for diagnosis and prognosis of EC

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Endometrial carcinoma (EC) is the most common gynaecological malignancy in the developed world. Currently, no valid non-invasive diagnostic or prognostic methods exist, making diagnosis and treatment rely on histopathological and surgical findings. ERA-NET Transcan2 project "Biomarkers for Diagnosis and Prognosis of Endometrial Carcinoma" (BioEndoCar; NCT03553589) coordinated by University of Ljubljana addresses this issue. A prospective observational case-control study was conducted at six medical centres across Europe. Plasma samples from women with diagnosed EC and controls were examined using non-targeted/targeted metabolomic and semi-quantitative immune-based proteomic approaches. The blood metabolomics (900 metabolites) and proteomics (>1000 proteins) data together with clinical and epidemiological data, were analysed using advanced artificial intelligence (AI) and machine learning (ML) methods to develop new diagnostic/prognostic models for early EC diagnosis and identifying patients with low/high risk for cancer progression and recurrence.

BioEndoCar has recruited over 440 patients, with strict standard operating procedures for sample collection, processing, and storage. The diagnostic/prognostic models based on all data developed using AI/ML methods showed promising characteristics with a repeated k-fold cross-validation with AUC > 0.8. The diagnostic models based on the top 25 combined features showed AUC of 0.94 in the test datasets. The best prognostic models in the test dataset included the top 25 proteins and top 25 combined features with AUC of 0.92 and 0.93, respectively. The developed models were then further refined using expert-led selection to reduce the number of features, with the number of metabolites in the combined model being reduced to 9, while maintaining high test AUC.

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Steroidomics for discovery of biomarkers

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Steroid hormones are crucial chemical messengers in human physiology, regulating a broad spectrum of essential functions. Systemic steroids are metabolized in peripheral tissues into bioactive and/or inactive metabolites, creating a complex circulating "steroidome", which can be altered in pathological conditions, potentially offering valuable diagnostic and prognostic insights. Endometrial cancer (EC) is the most common gynecological malignancy in high-income countries, with a rising incidence. Current diagnostic tests are invasive, and only 5-10% of symptomatic women are ultimately diagnosed with EC, emphasizing the need for noninvasive biomarkers. High-grade serous ovarian cancer (HGSOC) is the most lethal subtype of ovarian cancer, often diagnosed at advanced stages, highlighting the critical need for early detection biomarkers.

In our study, we analyzed a suite of androgens, 11-oxyandrogens, glucocorticoids and mineralocorticoids in serum using liquid chromatography-tandem mass spectrometry in two cohorts: EC cohort, including 62 patients with EC and 70 women with benign uterine conditions, and HGSOC cohort, including 21 patients with HGSOC and 28 women with benign ovarian conditions. We observed distinct alterations in the steroidome of patients with EC and HGSOC compared to their respective controls. Specifically, EC patients exhibited elevated levels of androgens, 11β-hydroxylated androgens, and glucocorticoids. Conversely, HGSOC patients had reduced levels of testosterone (T), 11-keto-T, and elevated cortisol compared to their controls. While the diagnostic potential of steroid hormones alone was modest for both EC and HGSOC, incorporating T and 11β-hydroxy-T into a multivariate logistic regression model alongside BMI, CA-125, and HE4 slightly improved diagnostic accuracy for EC (AUC: 0.83, sensitivity: 72%, specificity: 73%) compared to BMI, CA125 and HE4 model (AUC: 0.79, sensitivity: 70%, specificity: 70%). In HGSOC, steroids did not improve diagnostic models based on CA-125 and HE4. The inclusion of T and 11β-hydroxy-T into more complex diagnostic algorithms for EC could improve diagnostic performance.

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