Cancer Cell International

RESEARCH

Open Access

A novel serum-based steroid-protein panels for differentiating ovarian cancer from non-malignant adnexal masses

Marija Gjorgoska[1], Boštjan Pirš[2,3], Špela Smrkolj[2,3] and Tea Lanišnik Rižner[1*]

Abstract

Background Ovarian cancer is the deadliest gynecological malignancy, largely due to the advanced stage at diagnosis in most patients. This study investigates whether systemic steroids can serve as biomarkers to distinguish malignant ovarian tumors from non-malignant adnexal masses.

Methods This prospective, single-center observational study included 99 women with adnexal masses who underwent surgery between December 2021 and February 2025. Preoperative serum levels of 17 steroid hormones, including androgens, 11-oxyandrogens, glucocorticoids, and mineralocorticoids, were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Machine learning was employed to assess the diagnostic potential of these steroids in distinguishing ovarian cancer ($n=43$) from non-malignant adnexal masses ($n=56$).

Results Patients with ovarian cancer had lower levels of 11β-hydroxy-testosterone (11OHT), 11-keto-testosterone (11KT), and testosterone compared to controls. Using stepwise feature selection, we developed two diagnostic models incorporating three 11-oxyandrogens (11KT, 11OHT, and 11β-hydroxy-androstenedione), patient age, and either cancer antigen 125 (CA-125) or human epididymis protein 4 (HE4) for distinguishing malignant from non-malignant adnexal masses. The model including CA-125 achieved AUC of 0.907, 88.9% sensitivity and 82.0% specificity, while the model including HE4 achieved AUC of 0.911, 94.4% sensitivity and 77.3% specificity as evaluated by cross-validation. Both models significantly outperformed CA-125, HE4, and the Risk of Ovarian Malignancy Algorithm (ROMA) index alone.

Conclusion Patients with ovarian cancer exhibit distinct steroid profiles compared to those with non-malignant adnexal masses. If validated, the models could enhance diagnosis, reducing unnecessary surgeries for benign conditions while ensuring timely treatment for ovarian cancer, particularly when conventional biomarkers are inconclusive.

Keywords Ovarian cancer, Adnexal masses, Steroids, Machine learning, Diagnostic models

*Correspondence:
Tea Lanišnik Rižner
tea.lanisnik-rizner@mf.uni-lj.si
[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, Ljubljana 1000, Slovenia
[2]Department of Gynecology, University Medical Centre, Šlajmerjeva 3, Ljubljana 1000, Slovenia
[3]Department of Gynecology and Obstetrics, Faculty of Medicine, University of Ljubljana, Šlajmerjeva 3, Ljubljana 1000, Slovenia

Background

Ovarian cancer (OC) is the eighth most diagnosed cancer in women and most common cause of gynecologic cancer death in developed countries. In 2022, 324,603 new cases and 206,956 deaths were reported globally [1]. Epithelial ovarian cancer is the most common histological type and includes four main subtypes: serous, clear cell, mucinous, and endometrioid carcinoma. The serous subtype is further classified into low-grade and high-grade, with high-grade serous ovarian carcinoma (HGSOC) being both the most prevalent and the most lethal form [2]. Prognosis varies significantly by stage, with 5-year survival rates of 93% for cancer confined to the ovaries (localized), 75% for cancer that has spread nearby (regional), and only 31% for cancer that has spread to distant organs (distant) [3, 4]. Unfortunately, over a third of cases are diagnosed at an advanced stage, largely due to nonspecific symptoms.

Most symptomatic patients or those with abnormal clinical findings, such as suspicious ultrasound results or elevated cancer antigen 125 (CA-125) levels, do not have ovarian cancer. A study found that only 3% of premenopausal and 18% of postmenopausal patients referred through the UK's National Health Services (NHS) expedited pathway were actually diagnosed with ovarian cancer [5]. Clearly, accurate, accessible, and cost-effective diagnostic methods are needed to distinguish malignant tumors from non-malignant adnexal masses and reduce unnecessary procedures.

Currently, several risk-prediction models, based on either ultrasound features or blood biomarker levels exist that can be used to triage patients with an adnexal mass. These include CA-125, HE4, the Risk of Malignancy Index (RMI1), the Risk of Ovarian Malignancy Algorithm (ROMA), the International Ovarian Tumor Analysis (IOTA) Simple Rules, the IOTA Assessment of Different Neoplasias in the Adnexa (ADNEX) model, and the Ovarian-Adnexal Reporting and Data System (ORADS) [6–10]. These diagnostic tools differ in performance. CA-125 at the 35 kU/L threshold has high sensitivity (>80%) but low specificity (< 80%); RMI1, ROMA and IOTA Simple Rules achieve a better balance, exceeding 80% sensitivity and specificity for diagnosing ovarian cancer in symptomatic postmenopausal women; ADNEX has very high sensitivity (>95%) but low specificity (< 60%), leading to more false positives [11].

CA-125, used alone or as part of ROMA, lacks specificity due to its elevation in benign conditions common in premenopausal patients such as menstruation, endometriosis, and peritoneal inflammation [12], as well as conditions common in postmenopausal and elderly patients such as heart failure, cirrhosis, chronic kidney disease, and intraabdominal infections [13, 14]. Other models relying on ultrasound findings require validation in routine practice, as scans are often performed by non-specialists. Emerging molecular biomarkers, including circulating tumor DNA, tumor cells, exosomes, and metabolites, show promise for ovarian cancer diagnosis but remain unadopted due to limited validation and high costs [15–17].

This study investigates serum steroids as biomarkers for diagnosing ovarian cancer in patients with adnexal masses, using machine learning. We measured preoperative serum levels of classic androgens, 11-oxyandrogens, glucocorticoids, and mineralocorticoids in 43 patients with primary/recurrent ovarian cancer and 56 with non-neoplastic adnexal masses, benign or borderline ovarian tumors, using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Logistic regression was used to assess the diagnostic potential of these hormones, alone and in combination with CA-125, human epididymis protein 4 (HE4), and clinical parameters.

Materials and methods

Study population and design

Adult women (≥ 18 years) who were scheduled for surgery for an adnexal mass or presumed/histologically proven ovarian cancer at the Department of Gynecology, University Medical Center Ljubljana, were enrolled. Exclusion criteria were age < 18, active non-ovarian malignancy, previous ovarian malignancy, polycystic ovary syndrome (PCOS) and non-ovarian endometriosis.

Blood samples were collected between December 2021 to February 2025, 1–7 days before surgery, alongside lifestyle, medication, and clinical data. Written informed consent was obtained from all participants. Morning samples were collected and processed following a standardized procedure specifically adapted for metabolomics studies [18]; a detailed description of the collection and processing steps is provided in Sect. 1.1. in Supplementary File 1. Tissue specimens were examined by a certified pathologist according to WHO Classification of tumors, 5th edition and ICCR dataset [19].

Outcomes

The primary outcome was diagnostic accuracy of steroids for detecting ovarian cancer (binary outcome), defined as primary or recurrent malignant ovarian neoplasms ($n = 43$) versus non-malignant adnexal masses (defined as non-neoplastic adnexal masses or benign or borderline ovarian tumors) ($n = 56$), confirmed by histological examination obtained by surgery (reference standard). Five additional sub-analyses were performed to further evaluate the models: (1) upon excluding borderline ovarian tumors from the control group; (2) upon excluding premenopausal patients from both groups; (3) upon excluding patients with advanced-stage disease (stage III-IV

according to International Federation of Gynecologic Oncology (FIGO)) from the case group; (4) upon excluding patients with non-epithelial ovarian tumors from the case group; and (5) upon excluding patients with non-HGSOC from the case group.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Unconjugated steroids (n = 17) were quantified using a previously validated LC-MS/MS method described in Gjorgoska et al. [20]. Schematic representation of the position of these steroids in steroid biosynthesis is given in Supplementary Fig. 1. Briefly, 180 µL of thawed serum, calibrators, and QC samples were mixed with stable isotope-labeled internal standards (see Sect. 1.2. in Supplementary File 1) and incubated for 15 min. After protein precipitation with 180 µL 3 M Na_2SO_4, samples were extracted using methyl-*tert*-butyl ether (MTBE). The organic layer was evaporated and the extracted analytes were reconstituted in LC-MS grade methanol-water (50:50) (v/v). Steroids were chromatographically separated using a Phenomenex Kinetex XB-C18 column on a Shimadzu Nexera LC system, with mass spectrometry detection on a Sciex 3500 triple quadrupole mass spectrometer using electrospray ionization. Data acquisition was performed using Analyst 1.6.2.

Steroid quantification was based on peak area ratios of analytes to internal standards. All samples were anonymized prior to analysis. Steroid concentrations below the lower limit of quantification (LLOQ) were replaced by 0.5 x LLOQ for statistical analysis. Aldosterone, 5α-dihydrotestosterone (DHT), and progesterone were below the lower limit of quantification (LLOQ) in 69.2%, 58.6%, and 39.4%, respectively, and were excluded from further analysis.

Proteins

Serum CA-125 (kU/L) and HE4 (pmol/L) levels were routinely measured at the Clinical Institute for Clinical Biochemistry, University Medical Center, Ljubljana, using clinically validated electroluminescent immunoassays (ECLIAs), for CA-125, REF: 11,776,223,190, for HE4, REF: 05950929190, on a Cobas e411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The ROMA index score was calculated based on the formula established by Moore and collaborators [21].

Statistical analysis

Data were anonymized and analyzed using R Studio (version 4.3.0 or higher). Statistical significance was defined as $p < 0.05$. Continuous variables were expressed as median values with interquartile range (IQR) and compared using the Mann-Whitney U test. Robust ANCOVA (Analysis of Covariance) was used to adjust for age and menopause status. Categorical variables were expressed as frequencies with percentages and compared using Fisher exact test or Chi square test of independence.

Machine learning was performed using the caret library [22], with a 5 × 5-fold cross-validation protocol (1000 iterations). We tested 20 variables, including 12 steroid hormones, 3 steroid pools (androgen, 11-oxy-androgen and glucocorticoid pool; see Sect. 1.3. in Supplementary File 1 for their definitions), two proteins (CA-125, HE4), ROMA index and 2 clinical parameters (age, menopause status). Continuous variables were ln-transformed and standardized. Feature selection was performed via stepAIC (MASS library). Multicollinearity was assessed using the Variance Inflation Factor (VIF) in a logistic regression model, with acceptable values < 5. Diagnostic accuracy was assessed using the area under the receiver operating curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), F1 score and Akaike information criteria (AIC). DeLong's test was used to compare AUCs between different models.

Results

Description of the cohort

Between December 2021 and February 2025, a total of 103 participants were recruited, and preoperative morning serum samples were collected (Fig. 1). Among them, 47 (45.6%) had ovarian cancer, including 42 (89.4%) with primary ovarian cancer, one (2.1%) with recurrent disease, and four (8.5%) with metastatic disease from other primary sites. Participants with metastatic disease were excluded from the primary outcome analysis. Of the remaining 43 participants with ovarian cancer, 39 (90.7%) had epithelial tumors, with 32 (74.4%) classified as HGSOC, 2 (4.7%) as clear-cell, 2 (4.7%) as endometrioid, 2 (4.7%) as carcinosarcoma, and 1 (2.3%) as low-grade serous carcinoma. The remaining 4 cases (9.3%) had sex-cord stromal malignant tumors, including 3 (75%) with adult granulosa cell tumors and 1 (25%) with high-grade Sertoli-Leydig tumor. Regarding cancer staging, 7 (16.3%) cases were diagnosed at FIGO stage I, 2 (4.7%) at FIGO stage II, 26 (60.5%) at FIGO stage III, and 6 (14.0%) at FIGO stage IV; data was missing for two patients (4.7%). In terms of treatment status, 39 patients (90.7%) were treatment-naïve, four (9.3%) had been admitted after receiving neoadjuvant chemotherapy.

The control group consisted of 56 participants with non-malignant adnexal masses, including 8 (14.3%) with non-neoplastic adnexal masses, 33 (58.9%) with benign ovarian tumors, and 15 (26.8%) with borderline ovarian tumors. Among borderline ovarian tumors, 10 (66.7%) were FIGO stage I, one (6.7%) was FIGO stage II, and 3 (20%) were FIGO stage III. Table 1 provides an overview of the basic demographics and clinical characteristics of

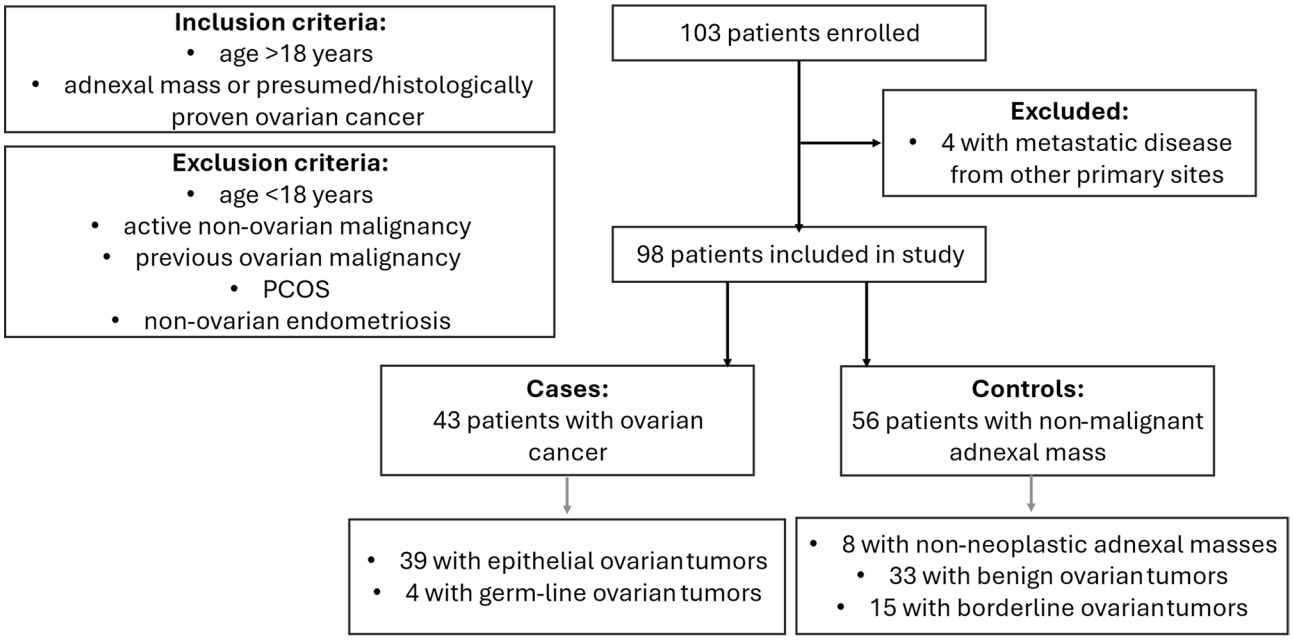

Fig. 1 Study flow diagram. PCOS, polycystic ovary syndrome

the cohort. Cases and controls differed significantly in age at operation, height, and history of oral contraceptive use. No significant differences were found for oral contraceptive use in the past 3 months, weight, BMI, menopausal status, smoking status, use of hormone replacement therapy (past or in the previous 3 months), or parity. A detailed description of the clinical and histopathological characteristics of the study participants is provided in Supplementary Table 1.

Preoperative serum steroid levels differ between patients with ovarian cancer and control women with non-malignant adnexal masses

Preoperative serum steroid levels differed between patients with ovarian cancer and patients with non-malignant adnexal masses (Table 2). Univariate analysis showed significantly lower levels of testosterone (T), 11β-hydroxy-testosterone (11OHT), and 11-keto-testosterone (11KT) in cases compared to controls ($p = 0.012$, < 0.001, < 0.001, respectively). After adjusting for age and menopause, 11OHT and 11KT remained significantly lower ($p_{adj}<0.001$, 0.003, respectively), while T showed borderline significance ($p_{adj}=0.08$). In contrast, cortisol levels were higher in cases vs. controls, after adjusting for age and menopause (p_{adj}: 0.027). No other significant hormone differences were found between the two groups. As expected, patients with ovarian cancer had higher CA-125 and HE4 levels as well as higher ROMA index score compared to controls. Steroid hormones showed generally poor correlations with CA-125 and HE4 levels, with 11KT exhibiting the strongest negative association with CA-125 (Spearman's $\rho = -0.34$, $p = 0.003$) and DHEA showing the strongest positive association with HE4 (Spearman's $\rho = 0.33$, $p = 0.001$) (Supplementary Table 2).

These trends of lower T, 11OHT, and 11KT and higher cortisol levels in cases compared to controls were consistent when patients with borderline ovarian tumors were excluded from the control group (Supplementary Table 3).

In a separate sub-analysis comparing patients with HGSOC to controls with non-malignant adnexal masses, similar trends were observed. Patients with HGSOC had significantly lower levels of T ($p_{adj}=0.012$), 11OHT ($p_{adj}=0.001$), and 11KT ($p_{adj}=0.003$), as well as higher cortisol levels ($p_{adj}=0.039$), compared to controls (Supplementary Table 4). Cases with HGSOC had also significantly higher levels of CA-125, HE4 levels and ROMA index score compared to controls.

Development of machine learning diagnostic models for ovarian cancer

In univariate logistic regression, HE4 was the most effective biomarker for distinguishing primary/recurrent ovarian cancer from non-malignant adnexal masses, with an AUC of 0.873. Among steroids, 11KT performed best (AUC: 0.709) (Table 3). Performance metrics for other steroid hormones are presented in Supplementary Table 5.

For multivariate models, stepwise feature selection identified 11KT, 11β-hydroxy-androstenedione (11OHA4), 11OHT, and age at operation as the top predictors for distinguishing cases from controls. The two-variable model incorporating 11KT and 11OHA4,

Table 1 Demographics and clinical characteristics of participants, by primary outcome definition of ovarian cancer

	Diagnosis based on reference standard		All participants (*n* = 99)
	Primary/recurrent ovarian cancer (*n* = 43)	Non-malignant adnexal masses (*n* = 56)	
Age (years); p = 0.006			
Median (IQR)	63.00 (53.00–68.00)	54.50 (47.00–64.00)	57.00 (50.00–65.50.00.50)
Height (cm); p = 0.041			
Median (IQR)	164.00 (159.20–167.00)	165.00 (162.00–170.00.00.00)	165.00 (160.00–169.00.00.00)
Missing	5 (11.6%)	7 (12.5%)	12 (12.1%)
Weight (kg)			
Median (IQR)	66.00 (59.00–75.00)	65.00 (60.00–80.25.00.25)	65.00 (59.00–75.75.00.75)
Missing	5 (11.6%)	6 (10.7%)	11 (11.1%)
Body mass index (kg/m^2)			
Median (IQR)	23.00 (22.00–27.90.00.90)	23.80 (22.00–28.10.00.10)	23.75 (22.00–28.00)
Missing	6 (14.0%)	7 (12.5%)	13 (13.1%)
Menopausal status			
Premenopausal	9 (20.9%)	21 (37.5%)	30 (30.3%)
Postmenopausal	34 (79.1%)	35 (62.5%)	69 (69.7%)
Race or ethnicity			
White	39 (90.7%)	52 (92.9%)	91 (91.9%)
Missing	4 (9.3%)	4 (7.1%)	8 (8.1%)
Smoking status			
Never	24 (55.8%)	32 (57.1%)	56 (56.6%)
Current	13 (30.2%)	12 (21.4%)	25 (25.3%)
Ex-smoker	3 (7.0%)	8 (14.3%)	11 (11.1%)
Missing	3 (7.0%)	4 (7.1%)	7 (7.1%)
History of hormone replacement therapy use			
No	34 (79.1%)	48 (85.7%)	82 (82.8%)
Yes	5 (8.9%)	3 (5.4%)	8 (8.1%)
Missing	4 (7.1%)	5 (8.9%)	9 (9.1%)
Hormonal replacement therapy in the past 3 months			
No	37 (86.0%)	49 (87.5%)	86 (86.9%)
Yes	2 (4.7%)	1 (1.8%)	3 (3.0%)
Missing	4 (9.3%)	6 (10.7%)	10 (10.1%)
History of oral contraceptive use; p = 0.018			
No	25 (58.1%)	21 (37.5%)	46 (46.5%)
Yes	12 (27.9%)	31 (55.4%)	43 (43.4%)
Missing	6 (14.0%)	4 (7.1%)	10 (10.1%)
Oral contraceptive use in the past 3 months			
No	40 (93.0%)	51 (91.1%)	91 (91.9%)
Yes	0	1 (1.8%)	1 (10%)
Missing	3 (7.0%)	4 (7.1%)	7 (7.1%)
Parity			
Median (IQR)	2.00 (1.00–2.00)	2.00 (1.50–2.00.50.00)	2.00 (1.00–2.00)
Missing	21 (48.8%)	21 (37.5%)	42 (42.4%)
Tumor stage according to FIGO			
I	7 (16.3%)	10 (BOTs, 66.7%)	17 (17.2%)
II	2 (4.7%)	1 (BOTs, 6.7%)	3 (3.0%)
III	26 (60.5%)	3 (BOTs, 20%)	29 (29.3%)
IV	6 (14.0%)	–	6 (6.1%)
Unknown	2 (4.7%)	–	2 (2.0%)

Table 1 (continued)

	Diagnosis based on reference standard		All participants (*n* = 99)
	Primary/recurrent ovarian cancer (*n* = 43)	Non-malignant adnexal masses (*n* = 56)	
Histopathological type			
Epithelial	39 (90.7%)	–	39 (39.4%)
High-grade serous	32 (74.4%)	–	32 (32.3%)
Low-grade serous	1 (2.3%)	–	1 (1.0%)
Clear cell	2 (4.6%)	–	2 (2.0%)
Endometrioid	2 (4.6%)	–	2 (2.0%)
Carcinosarcoma	2 (4.6%)	–	2 (2.0%)
Sex-cord stromal	4 (9.3%)	–	4 (4.0%)
Primary/recurrent disease			
Primary disease	42 (97.7%)	–	42 (42.4%)
Recurrent disease	1 (2.3%)	–	1 (1.0%)
Chemotherapy status			
Treatment-naïve	39 (90.7%)	–	39 (39.4%)
Post NACT	4 (9.3%)	–	4 (4.0%)
Non-malignant adnexal masses			
Non-neoplastic adnexal masses			
Normal adnexa with other pelvic pathology	–	5 (0.9%)	5 (5.1%)
Sactosalpinx	–	1 (1.8%)	1 (1.0%)
Paraovarian cyst	–	1 (1.8%)	1 (1.0%)
Ovarian torsion with necrosis	–	1 (1.8%)	1 (1.0%)
Benign ovarian tumors			
Simple cyst	–	9 (16.1%)	9 (9.1%)
Mature teratoma	–	6 (10.7%)	6 (6.1%)
Fibroma	–	3 (5.4%)	3 (3.0%)
Endometrioma	–	4 (7.1%)	4 (4.0%)
Serous cystadenoma or cystadenofibroma	–	7 (12.5%)	7 (7.1%)
Mucinous cystadenoma or cystadenofibroma	–	4 (7.1%)	4 (4.0%)
Borderline ovarian tumors			
Serous	–	7 (12.5%)	7 (7.1%)
Mucinous	–	6 (10.7%)	6 (6.1%)
Sero-mucinous	–	2 (3.6%)	2 (2.0%)

Data are n (%) unless otherwise specified. Statistical significance was determined by Mann-Whitney U test for continuous variables and Fisher exact test or Chi square test of independence for categorical variables. Two-sided p values below the significance threshold 0.05 are reported. All participants were female. BOT, borderline ovarian tumor; FIGO, the International Federation of Gynecology and Obstetrics; NACT, neoadjuvant chemotherapy.

achieved an AUC of 0.770. Adding 11OHT and age at operation improved the AUC to 0.813. Incorporating CA-125 into this four-parameter model (11KT, 11OHA4, 11OHT, and age at operation) further increased the AUC to 0.907, significantly outperforming CA-125 alone (AUC: 0.868; $p = 0.0003$) and the ROMA index (AUC: 0.884; $p = 0.039$) (Table 3; Fig. 2A, C). Similarly, adding HE4 instead of CA-125 to the four-parameter model improved the AUC to 0.911, significantly outperforming HE4 alone ($p = 0.0001$) and the ROMA index ($p = 0.016$) (Table 3; Fig. 2B, C).

In the subset analysis excluding borderline ovarian tumors from the control group, HE4 remained the best-performing single biomarker (AUC: 0.898), whereas 11OHT performed best among steroids (AUC: 0.701). The four-parameter model achieved an AUC of 0.823. The four-parameter model combined with CA-125 achieved an AUC of 0.922, significantly outperforming CA-125 alone (AUC: 0.872; $p < 0.0001$) and the ROMA index (AUC: 0.901; $p = 0.03$). Similarly, the four-parameter model combined with HE4 achieved an AUC of 0.932, significantly outperforming HE4 alone ($p = 0.0003$) and the ROMA index ($p = 0.004$) (Supplementary Table 6).

In the second sub-analysis (postmenopausal patients only), the CA-125 was the best performing biomarker (AUC: 0.837), while 11OHT was the top steroid predictor (AUC: 0.705). The four-parameter model achieved an AUC of 0.777. The four-parameter model plus CA-124 achieved an AUC of 0.873, significantly outperforming CA-125 alone (AUC: 0.837; $p = 0.002$) as well as ROMA (AUC: 0.846; $p = 0.041$). The four-parameter model plus HE4 achieved an AUC of 0.872, performing better than HE4 alone (AUC: 0.832; $p = 0.005$), and ROMA alone ($p = 0.065$) (Supplementary Table 7).

Table 2 Preoperative serum concentrations of steroid hormones and CA-125 in patients with ovarian cancer ($n=43$) and controls ($n=56$), based on the primary outcome definition of ovarian cancer

Analyte	Primary/recurrent OC ($n=43$)		Non-malignant adnexal masses ($n=56$)		*P* value	
	Median	IQR	Median	IQR	Unadjusted	Adjusted
Classic androgens (nM)						
DHEA	7.04	3.82–13.47	9.44	4.95–17.36	0.061	0.453
A4	2.21	1.51–3.63	2.82	1.89–3.93	0.151	0.467
T	0.55	0.36–1.06	0.80	0.58–1.18	**0.012**	0.080
11-oxyandrogens (nM)						
11OHA4	5.26	3.67–7.40	4.94	3.17–7.59	0.518	0.659
11KA4	0.53	0.25–0.70	0.57	0.39–0.89	0.249	0.295
11OHT	0.34	0.16–0.56	0.68	0.41–0.97	**< 0.0001**	**< 0.0001**
11KT	0.49	0.17–0.62	0.68	0.51–0.95	**< 0.0001**	0.003
Glucocorticoids (nM)						
17α-hydroxy-progesterone	0.81	0.56–1.63	1.13	0.65–1.87	0.105	0.286
11-deoxycortisol	0.50	0.14–0.99	0.63	0.36–1.02	0.344	0.351
Cortisol	542.10	459.30–648.60.30.60	482.20	350.80–581.00	0.075	**0.027**
Cortisone	43.09	31.69–58.10	51.15	35.51–63.73	0.108	0.230
Mineralocorticoids (nM)						
Corticosterone	14.07	9.18–20.81	12.62	7.75–23.41	0.646	0.357
Clinical biomarkers						
CA-125 (kU/L)	325.00	55.00–779.5.00.5	21.67	14.70–34.25.70.25	**< 0.0001**	0.081
HE4 (pmol/L)	245.00	99.25–848.25.25.25	57.50	51.00–70.50.00.50	**< 0.0001**	0.431
ROMA index score	84.39	57.57–96.02	14.24	9.40–21.41.40.41	**< 0.0001**	**< 0.0001**

Unadjusted p values were calculated using the non-parametric Mann-Whitney *U* test. Adjusted p value were calculated with robust ANCOVA (Analysis of Covariance) test with age at operation and menopause status as confounders. P values below 0.05 were considered statistically significant. 11OHA4, 11β-hydroxy-androstenedione; 11OHT, 11β-hydroxy-testosterone; 11KA4, 11-keto-androstenedione; 11KT, 11-keto-testosterone; A4, androstenedione; BOT, borderline ovarian tumor; CA-125, cancer antigen 125; DHEA, dehydroepiandrosterone; IQR, interquartile range; OC, ovarian cancer; T, testosterone.

In the third sub-analysis (early-stage ovarian cancer vs. non-malignant adnexal masses), CA-125 was the best-performing individual biomarker (AUC: 0.710), while 11KT performed best among steroids (AUC: 0.679). The four-parameter model alone performed comparably to CA-125 (AUC: 0.706). The four-parameter model plus CA-125 achieved an AUC of 0.808 and significantly outperformed CA-125 alone ($p=0.001$) as well as the ROMA index (AUC: 0.714, $p=0.002$). The four-parameter model plus HE4 achieved an AUC of 0.770, significantly outperforming HE4 (AUC: 0.642, $p=0.001$) but was comparable to the ROMA index ($p=0.155$) (Supplementary Table 8).

In the fourth sub-analysis (epithelial ovarian cancer vs. non-malignant adnexal masses), HE4 was the best performing biomarker (AUC: 0.919), while 11KT performed best among steroids (AUC: 0.732). The four-parameter model achieved an AUC of 0.817. The four-parameter model plus CA-125 achieved an AUC of 0.923 and significantly outperformed CA-125 alone (AUC: 0.903, $p=0.011$). The four-parameter model plus HE4 achieved an AUC of 0.935 and performed better than HE4 alone ($p=0.054$). Both steroid-protein models performed comparably to the ROMA index (AUC: 0.924, $p>0.05$ in both cases) (Supplementary Table 9).

In the fifth sub-analysis (HGSOC vs. non-malignant adnexal masses), HE4 was the best-performing biomarker (AUC: 0.940), while 11KT was the top steroid predictor (AUC: 0.707). The four-parameter model achieved an AUC of 0.786. The four-parameter model plus CA-125 achieved an AUC to 0.933, significantly outperforming CA-125 alone (AUC: 0.907; $p=0.006$) but performing comparably to ROMA (AUC: 0.921; $p=0.576$). The four-parameter model plus HE4 achieved an AUC of 0.930, performing slightly worse than HE4 alone ($p=0.042$) but comparable to ROMA ($p=0.262$) (Supplementary Table 10).

Discussion

In this biomarker discovery study, we analyzed preoperative steroid hormone levels in 99 women with adnexal masses to evaluate their potential in distinguishing ovarian cancer from benign conditions. Incorporating 11-oxyandrogens, patient age, and either CA-125 or HE4, we developed two steroid-protein diagnostic models. Both models significantly outperformed CA-125 or HE4 alone ($p<0.001$ for both), as well as the ROMA index ($p<0.05$), highlighting the added diagnostic value of steroid hormone profiling. This advantage likely reflects the ability of 11-oxyandrogens to capture tumor-associated steroidogenic activity not detected by conventional markers, thereby providing a more comprehensive

Table 3 Diagnostic performance statistics of univariate and multivariate models by primary outcome definition of ovarian cancer (cases, *n* = 43; controls, *n* = 56)

Model	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	F1 score (%)	AIC
Univariate							
11OHT	0.705 (0.696–0.712)	79.5 (78.6–80.7)	49.3 (48.1–50.0)	67.6 (67.0–68.2.0.2)	64.3 (63.0–65.6.0.6)	73.1 (72.4–73.8)	122.741
11KT	0.709 (0.704–0.713)	82.7 (81.4–83.9)	45.9 (44.8–47.1)	67.1 (66.5–67.7)	66.6 (64.9–68.5)	74.1 (73.3–74.9)	119.641
CA-125	0.868 (0.862–0.873)	87.5 (87.1–87.5)	71.1 (70.0–71.9.0.9)	80.2 (79.5–80.6)	81.0 (80.5–81.2)	83.7 (83.3–83.9)	91.293
HE4	0.873 (0.865–0.878)	92.7 (921.–92.9)	70.4 (69.5–71.4)	80.7 (80.1–81.3)	87.9 (87.1–88.2)	86.3 (85.9–86.7)	88.717
Multivariate							
Best 2 parameters	0.770 (0.764–0.774)	88.0 (86.8–88.9)	51.5 (50.5–52.4)	70.7 (70.2–71.3)	76.3 (74.6–77.9)	78.4 (77.8–79.0)	112.820
Best 3 parameters	0.793 (0.787–0.797)	86.8 (85.7–87.5)	61.8 (60.0–63.3.0.3)	75.2 (74.3–76.0)	77.9 (76.4–78.9)	80.6 (79.9–81.3)	108.552
Best 4 parameters	0.813 (0.808–0.817)	85.2 (83.9–86.4)	64.5 (63.3–65.7)	76.2 (75.5–76.9)	76.6 (74.9–78.2)	80.5 (79.5–81.3)	104.380
ROMA index	0.884 (0.875–0.891)	91.1 (91.1-91.1-1)	78.5 (78.1–78.6)	85.0 (84.7–85.0)	86.8 (86.8–86.8)	87.9 (87.8–87.9)	79.334
Best 4 parameters + CA-125	0.907 (0.904–0.910)	88.9 (88.2–89.3)	82.0 (81.4–82.8)	86.4 (86.1–87.1)	84.9 (84.2–85.6)	87.6 (87.1–88.0)	79.293
Best 4 parameters + HE4	0.911 (0.908–0.913)	94.4 (94.0–94.6.0.6)	77. 3 (76.7–77.7)	84.4 (84.1–84.7)	91.3 (90.8–91.7)	89.1 (88.9–89.3)	80.428

11OHT, 11β-hydroxy-testosterone; 11KT, 11-keto-testosterone; AIC, akaike information criteria; AUC, area under the curve; CA-125, cancer antigen 125; NPV, negative predictive value; PPV, positive predictive value; Multivariate model with best 2 parameters = 11KT + 11OHA4; model with best 3 parameters = 11KT + 11OHA4 + age; model with best 4 parameters = 11KT + 11OHA4 + age + 11OHT.

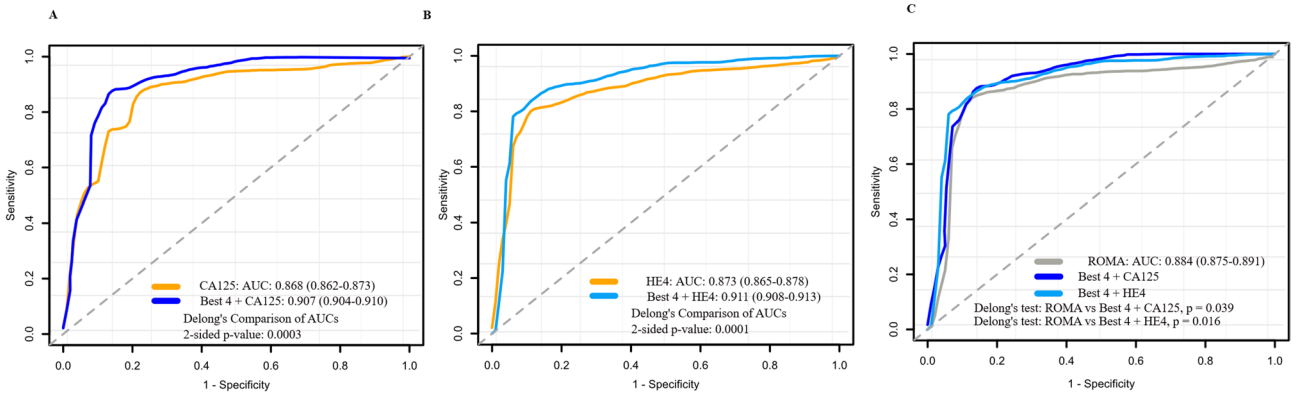

Fig. 2 Performance of logistic regression models in detecting ovarian cancer. (**A**) AUC curves comparing the Best 4 + CA-125 model to CA-125 alone for distinguishing ovarian cancer (*n* = 43) from non-malignant adnexal masses (*n* = 56). (**B**) AUC curves comparing the Best 4 + HE4 model to HE4 alone for distinguishing ovarian cancer (*n* = 43) from non-malignant adnexal masses (*n* = 56). (**C**) AUC curve comparison of the Best 4 + CA-125 model, the Best 4 + HE4 model, and the ROMA index for distinguishing ovarian cancer (*n* = 43) from non-malignant adnexal masses (*n* = 56). The Best 4 model includes 11KT, 11OHA4, age, and 11OHT as predictive parameters. 11OHA4, 11β-hydroxy-androstenedione; 11OHT, 11β-hydroxy-testosterone; 11KT, 11-keto-testosterone; AUC, area under the receiver operating characteristic curve; CA-125, cancer antigen 125; HE4, human epididymis protein 4; ROMA, risk of ovarian malignancy algorithm

biochemical profile than CA-125, HE4, or the ROMA index alone.

The superior performance of the models remained consistent after excluding borderline tumors from the control group and when restricted to postmenopausal patients. Both steroid-protein models also outperformed CA-125 and HE4 in detecting early-stage disease, with the steroids + CA-125 model further surpassing ROMA. However, in subgroup analyses restricted to epithelial ovarian cancer or HGSOC versus non-malignant adnexal masses, both steroid-protein models performed comparably to the ROMA index, although they were superior in the main malignant versus non-malignant comparison. This difference may reflect the smaller number of patients

in the sub-analyses or suggest that further refinement of the models is needed for histology-specific diagnosis. Overall, these subgroup findings require confirmation in larger cohorts.

To the best of our knowledge, this is the first study to characterize 11-oxyandrogen levels in women with adnexal masses. It is also the first study to apply machine learning to evaluate circulating steroids alone and in combination with proteins for distinguishing malignant from non-malignant cases. Given that 11-oxyandrogens are altered in hormone-sensitive cancers [23, 24], their integration into diagnostic models represents a novel and biologically relevant improvement.

Apart from hormone-sensitive cancers, 11-oxyandrogens have been linked to disorders such as congenital adrenal hyperplasia and PCOS [25]. Their elevated levels in PCOS suggest a role of adipose tissue in regulation. However, in our study, 11OHT, 11KT, and T were lower in cases than in controls, despite no significant differences in body weight. One possible explanation is intra-tumoral steroid metabolism. We recently showed that HGSOC tumors express key steroid-metabolizing enzymes [26], potentially accounting for systemic reductions in these steroids. If these metabolites are produced locally in ovarian tumors, they could impact key cellular processes, as 11KT and 11-keto-DHT activate the androgen receptor (AR) with a potency comparable to testosterone and DHT, respectively [27–29]. AR expression has been linked to improved survival and greater platinum sensitivity in HGSOC [30], though mechanistic studies on its signaling in ovarian tumors are lacking.

In contrast to 11-oxyandrogens, classic androgens have already been investigated as risk factors for ovarian cancer, however data are conflicting. Some studies suggest higher T levels may increase the risk of endometrioid and mucinous ovarian tumors [31], while others found no link [32–34]. Contrariwise, Mendelian randomization studies suggest higher T might lower the risk of ovarian cancer [35], including HGSOC [36]. Glucocorticoids have also been linked to ovarian cancer progression. Schrepf et al. reported that patients with ovarian cancer exhibited disrupted cortisol rhythms before surgery [37], which may explain the higher cortisol levels we observed in cases compared to controls in our study. Disrupted cortisol rhythms have also been associated with systemic inflammation and poorer survival [37], whereas chemotherapy was shown to normalize these rhythms [38].

Our study has several limitations. First, it is based on a small, single-center cohort. Second, technical constraints of the LC-MS/MS method prevented quantification of low-abundance steroids, such as DHT. Third, the small number of patients with early-stage ovarian cancer in our study limits the generalizability of the findings for this subgroup. As earlier detection is associated with substantially improved survival outcomes [3, 4], further validation of the models in larger early-stage cohorts is warranted. Fourth, the small number of premenopausal patients restricted assessment of model performance in this subgroup. While 11-oxyandrogens remain stable throughout life, one key steroid in our models, 11OHT, differs significantly between pre- and postmenopausal women [39]. This difference may introduce variability or limit the models' applicability in premenopausal patients, so this should be investigated further. Finally, despite internal cross-validation, the lack of external validation is a major limitation that may affect generalizability of our findings. Validation in larger, independent, ideally multicentric cohorts is essential to confirm robustness and support clinical translation.

Nonetheless, our study has several key strengths, including the collection of serum samples following a strict SOP adapted for metabolomics studies, the use of a validated LC-MS/MS method for multi-steroid profiling, and the incorporation of tumor biomarkers routinely employed in clinical practice. Moreover, the steroid-protein models developed in this study improved the distinction between malignant and benign adnexal masses. This has the potential to improve preoperative risk assessment, leading to more accurate triage and better clinical decision-making. Such improvements could reduce unnecessary surgeries for benign conditions and ensure timely treatment for ovarian cancer, particularly in cases where conventional biomarkers are inconclusive. With validation in larger, independent cohorts, these models have the potential to become valuable diagnostic tools, especially because mass spectrometry for steroid profiling is already well established in clinical practice. In addition, integrating further preoperative clinical data, such as ultrasound features based on IOTA criteria and computed tomography characteristics of adnexal masses, could further increase model accuracy. A further important direction for future studies is longitudinal sampling and clinical follow-up, which would allow assessment of whether circulating steroid levels are influenced by chemotherapy or tumor burden, and whether they could complement established biomarkers such as CA-125 in monitoring disease progression or treatment response.

Conclusions

In conclusion, circulating steroid hormones, particularly 11-oxyandrogens, offer a valuable and noninvasive biomarker class for improving the preoperative differentiation of malignant and benign adnexal masses. When combined with CA-125 or HE4, these hormones significantly enhance diagnostic accuracy beyond conventional markers and the ROMA index. The steroid-protein models remain robust across clinical subgroups and provide insights into tumor-specific steroidogenic activity,

supporting their potential as a clinically meaningful tool for risk stratification and improved surgical decision-making in women with adnexal masses.

Abbreviations

11KA4	11-keto-androstenedione
11KT	11-keto-testosterone
11OHA4	11β-hydroxy-androstenedione
11OHT	11β-hydroxy-testosterone
17OHP4	17α-hydroxy-progesterone
A4	Androstenedione
ADNEX	Assessment of different neoplasias in the adnexa
AIC	Akaike information criteria
ANCOVA	Analysis of covariance
AUC	Area under the receiver operating curve
BOT	Borderline Ovarian Tumor
CA125	Cancer antigen 125
DHEA	Dehydroepiandrosterone
DHT	5α-dihydrotestosterone
ECLIA	Electroluminescent immunoassays
FIGO	International Federation of Gynecologic Oncology
HE4	Human epididymis protein 4
HGSOC	High-grade serous ovarian cancer
ICCR	International Collaboration on Cancer Reporting
IOTA	International Ovarian Tumor Analysis
IQR	Interquartile range
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LLOQ	Lower limit of quantification
NPV	Negative predictive value
OC	Ovarian cancer
ORADS	Ovarian-Adnexal Reporting and Data System
PCOS	Polycystic ovary syndrome
PPV	Positive predictive value
RMI1	Risk of Malignancy Index 1
ROMA	Risk of Ovarian Malignancy Algorithm
T	Testosterone
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12935-025-04047-8.

Supplementary Material 1

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

We thank Ms. Vesna Sekelj Rangus from the Department of Gynecology and Obstetrics and Ms. Vera Troha Poljančič from the Clinical Institute of Chemistry and Clinical Biochemistry, University Medical Center, Ljubljana for their help in sample collection and processing. We also thank Ms. Vera Troha Poljančič and Prof. Joško Osredkar from the Clinical Institute of Chemistry and Clinical Biochemistry, University Medical Center, Ljubljana for additional CA-125 and HE4 measurements in a subset of patients.

Author contributions

Marija Gjorgoska: Conceptualization, Data curation; Formal analysis, Investigation; Methodology, Visualization, Writing - original draft, Writing - review & editing. Boštjan Pirš, Methodology, Writing - review and editing. Špela Smrkolj: Writing - review and editing. Tea Lanišnik Rižner: Conceptualization, Funding acquisition, Supervision, Writing - review & editing. All authors reviewed the manuscript.

Funding

This study was funded by the Slovenian Research Agency, grant number J3-2535 and J3-60065 and program funding P3-0449, to T.L.R.

Data availability

All data related to this article can be found in supplementary materials.

Declarations

Ethics approval and consent to participate

The study was approved by the Slovenian Ethics Committee ((ID: 0120–487/2020/3) and carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from each patient before sample collection.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 5 May 2025 / Accepted: 21 October 2025
Published online: 17 November 2025

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021(3). https://doi.org/10.3322/caac.21660.
2. Kim J, Park EY, Kim O, et al. Cell origins of high-grade serous ovarian cancer. Cancers. 2018. https://doi.org/10.3390/cancers10110433.
3. Ruhl JL, Callaghan C, Schussler N (eds.) Summary Stage 2018: Codes and Coding Instructions, National Cancer Institute, Bethesda, MD, 2024. https://seer.cancer.gov/tools/ssm/
4. American Cancer Society. Cancer Society; 2025. SEER*Explorer: An interactive website for SEER cancer statistics [Internet]. Surveillance Research Program, National Cancer Institute. Accessed 25.02.2025. https://seer.cancer.gov/explorer/
5. Kwong FL, Kristunas C, Davenport C, et al. Investigating harms of testing for ovarian cancer – psychological outcomes and cancer conversion rates in women with symptoms of ovarian cancer: a cohort study embedded in the multicentre rockets prospective diagnostic study. BJOG: An International Journal of Obstetrics & Gynaecology. 2024;131(10):1400–10. https://doi.org/10.1111/1471-0528.17813.
6. Ovarian masses in. premenopausal women, management of suspected (Green-top Guideline no. 62). Royal College of Obstetricians and Gynaecologists. Accessed 25.02.2025. https://www.rcog.org.uk/guidance/browse-all-guidance/green-top-guidelines/ovarian-masses-in-premenopausal-women-management-of-suspected-green-top-guideline-no-62/
7. Van Calster B, Van Hoorde K, Valentin L, et al. Evaluating the risk of ovarian cancer before surgery using the ADNEX model to differentiate between benign, borderline, early and advanced stage invasive, and secondary metastatic tumours: prospective multicentre diagnostic study. BMJ: Br Med J. 2014;349:g5920. https://doi.org/10.1136/bmj.g5920.
8. Andreotti RF, Timmerman D, Strachowski LM, et al. O-RADS US Risk Stratification and Management System: A Consensus Guideline from the ACR Ovarian-Adnexal Reporting and Data System Committee. Radiology. 2019;294(1):168–85. https://doi.org/10.1148/radiol.2019191150. 2020/01/01.
9. Jacobs I, Oram D, Fairbanks J, et al. A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer. BJOG: Int J Obstet Gynecol. 1990;10(10):922–9. https://doi.org/10.1111/j.1471-0528.1990.tb02448.x. /01 1990.
10. Kaijser J, Bourne T, Valentin L, et al. Improving strategies for diagnosing ovarian cancer: a summary of the international ovarian tumor analysis (IOTA) studies. Ultrasound Obstet Gynecol. 2013;41(1):9–20. https://doi.org/10.1002/uog.12323.
11. Sundar S, Agarwal R, Davenport C, et al. Risk-prediction models in post-menopausal patients with symptoms of suspected ovarian cancer in the UK (ROCkeTS): a multicentre, prospective diagnostic accuracy study. Lancet Oncol. 2024;25(10):1371–86. https://doi.org/10.1016/S1470-2045(24)00406-6.

12. Dochez V, Caillon H, Vaucel E, et al. Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. J Ovarian Res. 2019;27(1):28. https://doi.org/10.1186/s13048-019-0503-7.
13. Sevinc A, Adli M, Kalender ME, et al. Benign causes of increased serum CA-125 concentration. Lancet Oncol. 2007;8(12):1054–5. https://doi.org/10.1016/S1470-2045(07)70357-1.
14. Sikaris KA. CA125—a test with a change of heart. Heart Lung Circ. 2011;20(10):634–40. https://doi.org/10.1016/j.hlc.2010.08.001.
15. Zhu JW, Charkhchi P, Akbari MR. Potential clinical utility of liquid biopsies in ovarian cancer. Mol Cancer. 2022;21(1):114. https://doi.org/10.1186/s12943-022-01588-8.
16. Medina JE, Annapragada AV, Lof P, et al. Early detection of ovarian cancer using cell-free DNA fragmentomes and protein biomarkers. Cancer Discov. 2025;15(1):105–18. https://doi.org/10.1158/2159-8290.CD-24-0393.
17. Fahrmann JF, Ghasemi SM, Han CY, et al. A metabolite-based liquid biopsy for detection of ovarian cancer. Biomark Res. 2024;12(1):91. https://doi.org/10.1186/s40364-024-00629-2.
18. Rizner TL, Adamski J. Paramount importance of sample quality in pre-clinical and clinical research—need for standard operating procedures (SOPs). J Steroid Biochem Mol Biol. 2019;186:1–3. https://doi.org/10.1016/j.jsbmb.2018.09.017.
19. Gilks CB, Selinger CI, Davidson B, et al. Data set for the reporting of Ovarian, fallopian tube and primary peritoneal carcinoma: recommendations from the international collaboration on cancer reporting (ICCR). Int J Gynecol Pathol Nov. 2022;1(Suppl 1):S119–42. https://doi.org/10.1097/pgp.0000000000000908.
20. Gjorgoska M, Rižner TL. Simultaneous measurement of 17 endogenous steroid hormones in human serum by liquid chromatography-tandem mass spectrometry without derivatization. J Steroid Biochem Mol Biol. 2024;243:106578. https://doi.org/10.1016/j.jsbmb.2024.106578.
21. Moore RG, McMeekin DS, Brown AK, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. Gynecol Oncol. 2009;112(1):40–6. https://doi.org/10.1016/j.ygyno.2008.08.031.
22. Kuhn M. Building Predictive Models inRUsing thecaretPackage. J Stat Softw. 2008;28(5):1–26. https://doi.org/10.18637/jss.v028.i05.
23. Dahmani C, Caron P, Simonyan D, et al. Circulating adrenal 11-oxygenated androgens are associated with clinical outcome in endometrial cancer. Original Research. *Frontiers in endocrinology*. 2023-May-23. 2023;14. https://doi.org/10.3389/fendo.2023.1156680.
24. Dahmani C, Caron P, Simonyan D, et al. Preoperative circulating 11-oxygenated androgens are associated with metastasis-free survival in localized prostate cancer. J Urol. 2023;209(2):337–46. https://doi.org/10.1097/ju.0000000000003049.
25. Turcu AF, Rege J, Auchus RJ, et al. 11-Oxygenated androgens in health and disease. Nat Reviews Endocrinol. 2020;05(5):284–96. https://doi.org/10.1038/s41574-020-0336-x. /01 2020.
26. Gjorgoska M, Rižner TL. From fallopian tube epithelium to high-grade serous ovarian cancer: a single-cell resolution review of sex steroid hormone signaling. Prog Lipid Res. 2024;96:101302. https://doi.org/10.1016/j.plipres.2024.101302.
27. Pretorius E, Africander DJ, Vlok M, et al. 11-ketotestosterone and 11-ketodihydrotestosterone in castration resistant prostate cancer: potent androgens which can no longer be ignored. PLoS ONE. 2016;11(7):e0159867. https://doi.org/10.1371/journal.pone.0159867.
28. Storbeck K-H, Bloem LM, Africander D, et al. 11β-hydroxydihydrotestosterone and 11-ketodihydrotestosterone, novel C19 steroids with androgenic activity: a putative role in castration resistant prostate cancer? Mol Cell Endocrinol. 2013;377(1):135–46. https://doi.org/10.1016/j.mce.2013.07.006.
29. Handelsman DJ, Cooper ER, Heather AK. Bioactivity of 11 keto and hydroxy androgens in yeast and mammalian host cells. J Steroid Biochem Mol Biol. 2022;218:106049. https://doi.org/10.1016/j.jsbmb.2021.106049.
30. Tan J, Song C, Wang D, et al. Expression of hormone receptors predicts survival and platinum sensitivity of high-grade serous ovarian cancer. Biosci Rep. 2021. https://doi.org/10.1042/bsr20210478.
31. Ose J, Poole EM, Schock H, et al. Androgens are differentially associated with ovarian cancer subtypes in the ovarian cancer cohort consortium. Cancer Res. 2017;15(14):3951–60. https://doi.org/10.1158/0008-5472.Can-16-3322.
32. Tworoger SS, Lee IM, Buring JE, et al. Plasma androgen concentrations and risk of incident ovarian cancer. American J Epidemiology 2008/01//. 2008;167(2):211–8. https://doi.org/10.1093/aje/kwm278.
33. Trabert B, Michels KA, Anderson GL, et al. Circulating androgens and postmenopausal ovarian cancer risk in the women's health initiative observational study. Int J Cancer. 2019;15(8):2051–60. https://doi.org/10.1002/ijc.32157.
34. Ose J, Fortner RT, Rinaldi S, et al. Endogenous androgens and risk of epithelial invasive ovarian cancer by tumor characteristics in the European prospective investigation into cancer and nutrition. Int J Cancer. 2015;136(2):399–410. https://doi.org/10.1002/ijc.29000.
35. Ruth KS, Day FR, Tyrrell J. Using human genetics to understand the disease impacts of testosterone in men and women. Nat Med. 2020;26(2):252–8. https://doi.org/10.1038/s41591-020-0751-5.
36. Gjorgoska M, Rizner TL. The effect of androgens on the risk of endometriosis sub-phenotypes and ovarian neoplasms: a Mendelian randomization study. J Steroid Biochem Mol Biol. 2024;239:106482. https://doi.org/10.1016/j.jsbmb.2024.106482.
37. Schrepf A, Thaker PH, Goodheart MJ, et al. Diurnal cortisol and survival in epithelial ovarian cancer. Psychoneuroendocrinology. 2015;53:256–67. https://doi.org/10.1016/j.psyneuen.2015.01.010.
38. Schrepf A, Clevenger L, Christensen D, et al. Cortisol and inflammatory processes in ovarian cancer patients following primary treatment: relationships with depression, fatigue, and disability. Brain Behav Immun Mar. 2013;30(Suppl0):S126–34. https://doi.org/10.1016/j.bbi.2012.07.022.
39. Nanba AT, Rege J, Ren J, et al. 11-Oxygenated C19 steroids do not decline with age in women. The J Clin Endocrinol & Metabolism Jul. 2019;1(7):2615–22. https://doi.org/10.1210/jc.2018-02527.

Publisher's note