**Endometrial cancer diagnostic and prognostic algorithms based on proteomics, metabolomics and clinical data:**

**A systematic review**

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**Supplementary Materials:**

**Supplementary Table S1: Search strategy for Pubmed and OVID Embase.**

**Supplementary Table S2:** Selected signaling questions to assess the quality of the selected manuscripts.

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**Supplementary Table S1: Search strategy for Pubmed and OVID Embase**

|  |  |
| --- | --- |
| **PUBMED** |  |
|  | **Endometrial cancer AND proteomics** |
|  | **((("Endometrial Neoplasms"[Mesh] OR "Endometrial Neoplasms"[tiab] OR "Endometrial Carcinoma\*"[tiab] OR "Endometrial Cancer\*"[tiab] OR "Endometrium Cancer\*"[tiab] OR "Cancer of the Endometrium"[tiab] OR "Carcinoma of Endometrium"[tiab] OR "Cancer of Endometrium"[tiab]) OR ("Uterine Neoplasms"[Mesh] OR "Uterine Neoplasm\*"[tiab] OR "Uterus Neoplasm\*"[tiab] OR "Cancer of Uterus"[tiab] OR "Uterus Cancer\*"[tiab] OR "Cancer of the Uterus"[tiab] OR "Uterine Cancer\*"[tiab] OR "Uterine Carcinoma\*"[tiab] ) OR ("Endometrial Neoplasms"[Mesh] OR "Endometrial Neoplasm\*"[tiab] OR "Endometrial Carcinoma\*"[tiab] OR "Endometrial Cancer\*"[tiab] OR "Cancer of the Endometrium\*"[tiab] OR "Carcinoma of Endometrium\*"[tiab] OR "Endometrium Carcinoma\*"[tiab] OR "Cancer of Endometrium\*"[tiab] OR "Endometrium Carcinoma\*"[tiab] OR "Endometrium Cancer\*"[tiab])) AND** ("Proteomics"[Mesh] OR "Proteomic\*"[tiab])) |
|  | **Endometrial cancer AND metabolomics** |
|  | **((("Endometrial Neoplasms"[Mesh] OR "Endometrial Neoplasms"[tiab] OR "Endometrial Carcinoma\*"[tiab] OR "Endometrial Cancer\*"[tiab] OR "Endometrium Cancer\*"[tiab] OR "Cancer of the Endometrium"[tiab] OR "Carcinoma of Endometrium"[tiab] OR "Cancer of Endometrium"[tiab]) OR ("Uterine Neoplasms"[Mesh] OR "Uterine Neoplasm\*"[tiab] OR "Uterus Neoplasm\*"[tiab] OR "Cancer of Uterus"[tiab] OR "Uterus Cancer\*"[tiab] OR "Cancer of the Uterus"[tiab] OR "Uterine Cancer\*"[tiab] OR "Uterine Carcinoma\*"[tiab] ) OR ("Endometrial Neoplasms"[Mesh] OR "Endometrial Neoplasm\*"[tiab] OR "Endometrial Carcinoma\*"[tiab] OR "Endometrial Cancer\*"[tiab] OR "Cancer of the Endometrium\*"[tiab] OR "Carcinoma of Endometrium\*"[tiab] OR "Endometrium Carcinoma\*"[tiab] OR "Cancer of Endometrium\*"[tiab] OR "Endometrium Carcinoma\*"[tiab] OR "Endometrium Cancer\*"[tiab])) AND** ("Metabolomics"[Mesh] OR "Metabolomic\*"[tiab])) |
| **OVID EMBASE** |  |
|  | **Endometrial cancer AND proteomics** |
|  | ***1:* exp endometrium tumor/**

|  |  |
| --- | --- |
| ***2:***  | ((endometrial or endometrium) adj3 (tumo?r$ or carcinoma$ or cancer$)).ti,ab.  |

***3:* 1 OR 2** ***4:* exp proteomics/*****5:* proteomic$.ti,ab.*****6:* 4 or 5** ***7:* 3 and 6** |
|  | **Endometrial cancer AND metabolomics** |
|  | ***1:* exp endometrium tumor/**

|  |  |
| --- | --- |
| ***2:***  | ((endometrial or endometrium) adj3 (tumo?r$ or carcinoma$ or cancer$)).ti,ab.  |

***3:* 1 OR 2**

|  |  |  |
| --- | --- | --- |
| ***4:* exp metabolomics/** |  |  |
| ***5:* metabolomic$.ti,ab.** |  |  |

***6:* 4 or 5** ***7:* 3 and 6** |

**Supplementary Table S2**: Selected signaling questions to assess the quality of the selected manuscripts.

|  |  |
| --- | --- |
|  | **QUADOMICS signaling questions** |
| **1** | **Was selection criteria clearly described?***Inclusion/exclusion criteria,**detailed information on sources of samples**(flow diagram not needed)* |
| **2** | **Was the spectrum of patients representative?***Target population that would need diagnostic or prognostic test.* |
| **3A** | **Was the type of sample fully described?***Type of sample (serum, plasma, tissue sample, etc.)**(for plasma: EDTA, heparin, citrate), time before centrifugation for serum!**centrifugation time and g (not rpm)**how were tissue sample obtained* |
| **3B** | **Was the collection procedure of sample fully described?***time of sample collection (morning, during the day, …)**time between blood flow and centrifugation (delay in processing)**time between sample acquisition and storage**freeze-thaw cycles**for tissues: time between collection and freezing* |
| **4** | **Were the procedures of biological sample collection with respect to clinical factors described with enough detail?***Clinical and physiological factors?* *Age, fasting status, BMI, menstrual phase (if applicable), menopausal status* |
| **5** | **Were handling and pre-analytical procedures reported in sufficient detail and similar for the whole group?***If differences in procedures were reported, was their effect on the results assessed?**Detailed description of pre-analytical procedures: temperature of storage, procedure of metabolite/protein extraction.* |
| **6** | **Is the time between the reference standard and the index test short enough to guarantee that the target condition did not change between the two tests?***Samples are usually obtained before or during surgery, which is considered a reference standard.* |
| **7** | **Did the whole sample or a random selection of the sample receive verification using a reference standard of diagnosis?***In the case/control studies healthy controls did not undergo surgical treatment.* |
| **8** | **Was the execution of the index test described in sufficient detail to permit replication of the test?***Metabolomics analysis: description of the MS or NMR* ***method****,* ***control procedures****, (calibration and randomization only for MS)* |
| **9** | **Was statistical analysis of the index test described in sufficient detail?***Statistical methods, reproducibility assessment, normalization, transformation and cross-validation (leave-one-out, bootstrap, jackknife and permutation tests, independent training and test set)* *Validation test performed:* ***yes****/no OR**Other approaches for overfitting:* ***yes****/no* |

**Supplementary Table S3**: QUADOMICS scoring of the included proteomic studies.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study/QUADOMICS** | **1** | **2** | **3A1** | **3B2** | **43** | **5** | **6** | **7** | **8** | **9** | **comments** |
| **Proteomics** |  |  |  |  |  |  |  |  |  |  |  |
| Yoshizaki 2005 tissue | Yes | No\* | Yes\*\*  | NC$ | No$$  | No | Yes | NC# | NC## | Yes | \* Controls pre-menopausal; \*\*tissue with confirmation adjacent FFPE;$ immediatey, no mention to a max time allowed; $$ No BMI information; # unclear if all women underwent hysterectomy; ##protein extraction: protein concentration detection method not named, ProteinChip array analysis: no info if samples were pooled or not before application on the array -> one array per sample?, SELDI analysis: calibration just named as `routine calibration` without explanation |
| DeSouza 2007 tissue | No\* | No\*\* | Yes | Yes | No | Yes | Yes  | Yes | NC$ | Yes  | \* Samples selected from biobank, no further info; \*\* Controls pre-menopausal; $ protein extraction: centrifugation details not described, labelling: no info how randomization in iTRAQ sets |
| Voisin 2011 tissue | No\*  | No\*\*  | No | No | No | No | No | No | NC$ | Yes | \* No mention; 5 samples were analysed in a previous study (most probably De Souza 2007), but there is no reference; \*\*Controls pre-menopausal; $ protein extraction: centrifugation details not described, labelling: no info how randomization in iTRAQ sets |
| Shan 2016 tissue | Yes | Yes | Yes | No | No | No | No | Yes | No | No  |  |
| Ceylan 2020 tissue | Yes | Yes | Yes | yes | yes  | yes | yes | yes | yes | No  |  |
| Mauland 2017 tissue | yes | yes | yes | yes | yes | Yes | yes | yes | Yes\* | yes | \* details missing like protein extraction procedure, but authors refer to previous studies |
| Akkour 2022 tissue | yes | yes | yes | no  | no | no | yes  | Yes | NC\*  | yes | \* protein extraction: no info on homogenization time, no info on how were contaminants removed (but referred to previous publications). Protein separation by 2-D electrophoresis: separated gels for identification of different spots and analysis by MS/MS: authors largely referred to previous publications |
| Kurimchak tissue | yes | yes | yes | yes | no | yes | yes | yes | yes | Yes |  |
| DeSouza 2010 tissue | No | No\* | Yes | Yes | No | yes | no | NC | Yes | Yes | \* proliferative endometrium as controls |
| Aboulaurd 2021 tissue | yes | yes | yes | Yes | yes | yes | yes | Yes | yes | Yes |  |
| Janakova 2021 tissue | Yes | Yes\* | yes | Yes | Yes | yes | yes | yes | yes | Yes | \* differences in stage and grade between the two groups |
| Zhu 2006 serum | yes | yes | No | No\* | No | no | yes | No | Yes | yes | \* serum was stored for max 48 h at 2°C before -80°C, no further details |
| Kikuchi 2007 serum | yes | Yes\* | yes | yes | Yes | yes | yes | yes | yes | yes | \* differences in mean ages between groups; (2) No validation, basic statistics |
| Zhu 2008 serum | yes | yes | no | No\* | no | no | yes | no  | yes | yes | \* serum was stored for max 48 h at 2°C before -80°C, no further details |
| Wang 2011 serum | yes | yes | NC\* | No | Yes | yes | yes | yes | yes | Yes | \* no clotting time, rpm and no x g; |
| Qiu 2010 serum | yes | yes | Yes\* | yes | no | yes | yes | yes | yes | Yes$ | \* Serum was let sediment (at least 30 min, immediately aliquoted and frozen; $ algorithms not fully explained |
| Enroth 2018 plasma | yes | yes | no | no | NC\* | yes | yes | yes | yes $ | yes | \* age only; $ not many details given but the authors refer to the Olink web page |
| Tarney 2019 serum | yes | yes | Yes | Yes\* | yes | yes | yes | yes | yes | Yes | \* Only study reporting the number of freeze-thaw cycles (no more than two) |
| Ura 2021 serum | yes | yes | yes | yes  | yes | yes | no | yes | yes | yes |  |
| Celsi 2022 serum | yes | yes | yes  | yes | yes | yes | no | yes | yes | yes |  |
| Ura 2022 serum | yes | Yes\* | yes | yes | no | yes | yes | yes | Yes$ | yes | \* mean age difference between cases and controls; $ procedures were performed at Olink® Proteomic (Dag Hammarskjölds väg 52B, SE-752 37 Uppsala, Sweden |
| Martinez-Garcia 2016 uterine aspirate | yes | NC\* | yes | yes | yes | yes | yes | yes | yes | yes | \* supplementary table with patient info could not be found on the journal website |
| Martinez-Garcia 2017 uterine aspirate | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes |   |

1: Although indicated as ‘yes’, no study on plasma reported on EDTA, heparin, citrate and one study only reported the time of serum sedimentation.

2: Although indicated as ‘yes’, no study reported the time of sample collection, and few studies reported other info (as indicated in the table).

3: Although indicated as ‘yes’, no studies reported on the fasting status, and not always BMI was reported (as indicated in Tables).

**Supplementary Table S4**: QUADOMICS scoring of the included metabolomic studies.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study/QUADOMICS** | **1** | **2** | **3A** | **3B** | **4** | **5** | **6** | **7** | **8** | **9** | **comments** |
| **Metabolomics** |  |  |  |  |  |  |  |  |  |  |  |
| Ihata 2014 plasma | yes | NC\* | No\*\* | NC$ | No\*\*\* | no | NC | no | no | no | \*BD and HW; \*\*rpm; \*\*\*no menopausal status, BMI; $no information on daytime of sample aqcquisition, timing of sample processing, and freeze-thaw cycles |
| Trousil 2014 tissue | No\* | no | No\*\* | NC$ | No\*\*\* | yes | yes | yes | yes | NC# | Normal tissue; \*almost no data; \*\*biopsy or sample after hysterectomy; \*\*\*no clinical data; \*\*\*\* not written-clear for tissue samples?; $no information on daytime of sample acquisition and freeze-thaw cycles; # no info on data transformation and scaling |
| Jove 2016 tissue | no | No\* | no | NC$ | No\*\* | no | yes | yes | NC# | no | \*reproductive age women in control group; \*\* no data about age, menopausal status, BMI...; $no information on daytime of sample acquisition and time between collection and storage; # no info on sample randomization and no QC samples used |
| Shao 2016 urine | yes | no | yes | NC$ | No\*\* | yes | yes | no | NC# | NC## | \*BD and HW; \*\*no clinical data, age, BMI, menopausal status; $no information on timing of sample processing; # no info on sample randomization; ## no info on sample-to-sample normalization, data transformation, and scaling |
| Altadill 2017 tissue | yes | NC | yes | NC$ | No\* | yes | yes | yes | NC# | no | Benign disease; \*age and BMI is missing; $no information on daytime of sample acquisition; # no info on sample randomization and type of QC sample |
| Audet-Delage 2018 (Front Pharm) serum | yes | nc | No\* | yes | NC\*\* | yes | yes | No\*\*\* | no | no | Benign disease; \*no data about collection and storage; \*\*fasting status; \*\*\* HW |
| Audet-Delage 2018 (JSBMB) serum | yes | yes | No\* | NC$ | yes | yes | yes | yes | NC# | no | \* no data about collection and storage; $no information on timing of sample processing; # no info on sample randomization |
| Troisi 2018 serum | yes | no | No\*\* | yes | yes | yes | NC | no | NC# | NC## | \*BD and HW; \*\*no data about centrifugation; # no info on sample randomization; ## no info on sample-to-sample normalization |
| Shi 2018 serum | yes | No\* | No\*\* | NC$ | No\*\*\* | yes | NC | no | NC# | NC## | \*HW; \*\*time before centrifugation; \*\*\* menopausal status; $no information on daytime of sample acquisition, timing of sample processing, and freeze-thaw cycles; # no info on sample randomization and QC samples; ## no info on sample-to-sample normalization, data transformation, scaling |
| Knific 2018 plasma | yes | NC | yes | NC$ | No\* | yes | yes | yes | NC# | yes | Benign diseases; \*fasting status; $no information on daytime of sample acquisition, timing of sample processing, and freeze-thaw cycles; # no info on sample randomization |
| Bahado-Singh 2018 serum | yes | No\* | No\*\* | NC$ | No\*\*\* | yes | yes | no | NC# | NC## | \*HW; \*\*no data about serum collection;\*\*\*menopausal status, fasting?; $no information on daytime of sample acquisition, timing of sample processing, and freeze-thaw cycles; # no info on sample randomization and QC samples; ## no info on sample-to-sample normalization and scaling |
| Cummings 2019 tissue | no | NC | No\* | NC$ | No\*\* | no# | yes | Yes  | NC## | no | Normal and benign tissue; \* no data about sample collection; \*\* no clinical data, no age for CW; $no information on daytime of sample acquisition; # no storage temperature reported; ## no info on sample randomization and QC samples |
| Strand 2019 plasma | yes | yes | yes | NC$ | No\* | yes | yes | yes | no | no | \*not fasting; $no information on daytime of sample acquisition, timing of sample processing, and freeze-thaw cycles |
| Cheng 2019 CV fluid | yes | NC | No\* | NC$ | No\*\* | yes# | NC | yes | yes | yes | Normal, benign diseases; \*not clear what was time between collection and storage, when in the menstrual, menopausal cycle has been collected; \*\* premenopausal and menopausal women; $no information on daytime of sample acquisition, timing of sample processing, and freeze-thaw cycles; # no sample storage reported |
| Lunde 2020 serum | Yes | Yes | No | No | No\* | No | Yes | Yes | No | No | Flow diagram available; \*no info about fasting status, age, BMI, menopausal status (matched on age and BMI) samples from Danish Cancer Biobank, prognosis of postoperative pain |
| Shafiee 2020 blood (serum/plasma), tissue | Yes | Yes | No | No | No\* | No | Yes | Yes\*\* | Yes | NC$ | \*no info about fasting status, collection and storage of blood samples; \*\*outpatient endometrial sampling for PCOS, hysterectomy or hysteroscopy for EC and C patients; $ no information on missing values imputation, data transformation and scaling |
| Troisi 2020 dried blood spots | Yes | Yes\* | Yes | NC$ | No\*\* | Yes | Yes | No\*\*\* | NC$$ | NC$$$ | Flow diagram available; \* not sufficient info about control patients; \*\*fasting status not available; \*\*\*not clear for C in test cohort, histology for suspected EC cases in prospective cohort; $ storage temperature not given; $$ no information on sample randominzation and quality control; $$$ no information on normalization, transformation, scaling, missing value treatment |
| Kozar 2021 serum | Yes | No\* | Yes | NC$ | Yes\*\* | Yes | Yes | No\*\*\* | NC$$ | Yes | \*difference in menopausal status, age, fasting, no alcohol, smoking, medication (time frame not clear); \*\*\* ultrasound for C patients; $ time of day not clear; $$ no information on sample randomization and MS parameters |
| Njoku 2021 plasma | Yes | Yes\* | NC$ | No | Yes\*\* | Yes | Yes | No\*\*\* | NC$$ | Yes | \*prognosis of EC in obese population; \*\*overnight fast; \*\*\* endometrial biopsy for C patients, biopsy or hysterectomy specimen for EC patients; $ centrifugal force not given; $$ no info on sample randomization and QC samples |
| Kliemann 2021 plasma and serum | Yes\* | Yes\*\* | NC | No | Yes | Yes | NC\*\*\* | No# | NC$ | Yes | \*no flow diagram; \*\* metabolic signatures typical for obesity and EC; \*\*\* time between reference and index test is not known; # HW; $ no information on sample randomization and QC samples |
| Dossus 2021 plasma | Yes\* | No\*\* | NC | No$ | Yes | Yes | NC\*\*\* | No# | Yes | Yes | \*no flow diagram; \*\* no info about the control group, probably HW; \*\*\* time is known but is long 8.3 (4.5); # HW?; $ timing of sample collection and storage temperature unclear |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Skorupa 2021 tissue | Yes\* | Yes\*\* | Yes | NC$ | Yes\*\*\* | Yes | Yes | Yes | NC$$ | NC$$$ | \*no flow diagram; \*\* patients with different grades of EC; \*\*\* fasting status not available (not needed for tissue metabolomics); $ time of day of sample collection not given; $$ no information on metabolite identification; $$$ no information on missing value treatment |
| Gu 2021 serum | Yes\* | Yes\*\* | No | Yes | No\*\*\* | Yes | Yes# | Yes## | NC$ | NC$$ | \*no flow diagram;\*\* patients with different stages of EC; \*\*\*no age, BMI…; # samples collected on the morning of surgery, all patients underwent hysterectomy; $ no information on sample randomization, use of QC samples and metabolite identification; $$ no information on missing value imputation, transformation and scaling |
| Yan et al. 2022, serum | Yes\* | Yes\*\* | NC$ | NC$$ | NO\*\*\* | NC$ | NC# | NC## | NC$$$ | NC$$$$ | \*no flow diagram; \*\*patients with hyperplasia, polyps; \*\*\* no info about fasting status and BMI; #no info about the time of blood collection; ## not clear what kind of diagnosis was used for EP and EH; $ centrifugal force not given in x g; $$ time of day of sample collection not given; $$$ no information on sample randomization; $$$$ no information on data transformation and scaling |
| Schuhn 2022 serum | Yes\* | Yes\*\* | Yes | NC$ | NO\*\*\* | Yes | NC# | No## | NC$$ | No$$$ | \*no flow diagram; \*\*other cancers (breast) benign endometrial diseases, HW; \*\*\* no fasting and menopausal status; #no info about the time of blood collection; ## breast cancer, control women and healthy women did not have the same diagnostic test; $ time of day of sample collection not given; $$ no information on sample randomization and QC samples; $$$ no information on sample-to-sample normalization, transformation, scaling, model calculation, crossvalidation and approaches for overfitting |
| Düz 2022 tissue | Yes\* | Yes\*\* | Yes | No$ | NO\*\*\* | No$$ | Yes# | Yes## | Yes | NC$$$ | \*no flow diagram; \*\* benign endometrial pathologies versus cancer; \*\*\* no info about BMI, grade, stage of cancer, menopausal status (however, authors state the samples were matched for age, BMI and menopausal status); # tissue samples were obtained during hysterectomy; ## all patients underwent hysterectomy; $ timing unclear; $$ buffer compostion and centrifugation details missing; $$$ no information on missing value treatment, transformation; permutation test number too low |
| Yie 2022 tissue, urine, brushing samples | Yes\* | NC\*\* | Yes | NC$ | NO\*\*\* | NC$$ | Yes# | NC## | Yes | NC$$$ | \*no flow diagram; \*\* control group included also patients with cervix diseases, no info about number of different pathologies; \*\*\* BMI, age menopausal status availabe, but there was no data about menstrual phase for premenopausal women, fasting not available, but not relevant; # tissue samples were obtained during hysterectomy and urine one day before surgery, not clear for intrauterine brushing; ## not clear whether hysterectomy with histology has been performed for all patients; $ time of day and time between collection and storage unclear; $$ centrifugal forces not given in x g; $$$ no information on missing value treatment |
| Gatius 2022 tissue | Yes\* | Yes\*\* | No# | No# | NO\*\*\* | Yes | NC# | Yes## | NC$ | No | \*no flow diagram; \*\*separation between endometrioid and serous EC; \*\*\* no data about BMI and menopausal status; # no info provided on samples from biobank; ## no info; ## it is logical, but is not described; $ no information on sample randomization and QC samples |
| Breeur 2022 blood | Yes\* | Yes\*\* | Yes | NC$ | No\*\*\* | NC$$ | Yes# | No## | NC$$$ | Yes | \*no flow diagram; \*\*for epidemiological study, diagnostic biomarkers specific fof EC in whole population; \*\*\* no data for menopausal status; # info provided, relatively long 8.4 years after blood collection; ## only EC patients; $ no more information than »standardized protocols«; $$ sample preparation not described; $$$ no information on sample randomization, QC samples and measurements |

**Supplementary Table S5:** Additional quality checks and potential biases

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study** | **Country** | **Funding** | **COI** | **Open science** |
| (Yoshizaki, Enomoto et al. 2005) | Japan | No mention | No mention | No mention |
| (DeSouza, Grigull et al. 2007) | Canada | National Cancer Institute of Canada;Ontario Genomics Institute and Genome Canada | No mention | No mention |
| (Voisin, Krakovska et al. 2011) | Canada | Support by Canadian Cancer Society Research Institute and MITACS Accelerate Fellowship, Ontario | No mention | No mention |
| (Shan, Zhou et al. 2016) | China | No mention | No mention | No mention |
| (Ceylan, Akpinar et al. 2020) | Turkey | No mention | No mention | No mention |
| (Mauland, Ju et al. 2017) | Norway | University of Bergen;Western Norwegian Regional Health Authority; Research Council of Norway;Norwegian Cancer Society. | None | No mention |
| (Akkour, Alanazi et al. 2022) | Saudi Arabia | Funding: Dallah Hospital Group in Riyadh | None | No mention |
| (Kurimchak, Kumar et al. 2068) |  | National Institutes of Health CORE Grant (Fox Chase Cancer Center)R01National Institutes of Health Donations from Peggy’s Pathway for Women’s Cancer Care Cancer Kinome Initiative (CKI) at FCCCDonation from Don Morel | None | No mention |
| (DeSouza, Krakovska et al. 2010) | Canada | Canadian Cancer Society Research InstituteApplied Biosystems/MDS Analytical Technologies | NoneAuthors acknowledge company support  | No mention |
| (Aboulouard, Wisztorski et al. 2021) | France, Lebanon | Ministere de L’Education Nationale, de L’Enseignement Superieur et de la RechercheSIRIC ONCOLilleINCa-DGOS-INSERM | None | No mention |
| (Janacova, Faktor et al. 2617) | Switzerland, Czech RepublicIreland | Czech Science Foundation | None | Deposited data ProteomeXchange Consortium / PRIDE partner repository (PXD017217) |
| (Zhu, Zhang et al. 2006) | China | No mention | No mention | No mention |
| (Kikuchi, Honda et al. 2007) | Japan | Foundation for the Promotion of Cancer Research (Tokyo, Japan)Ministry of Health, Labor and Welfare of JapanMinistry of Education, Culture, Sports, Science and Technology of JapanNational Institute of Biomedical Innovation of JapanNaito Foundation | No mention | No mention |
| (Zhu, Zhang et al. 2008) | China | Research Grants from Peking University | No mention | No mention |
| (Qiu, Gao et al. 2010) | China, USA | Ministry of Science and Technology of China | No mention | No mention |
| (Wang, Cao et al. 2011) | China | Shanghai Leading Academic Discipline ProjectShanghai fundamental research emphasis project | None | No mention |
| (Enroth, Berggrund et al. 2018) | Sweden | The Swedish Cancer Foundation Vinnova (SWELIFE)The Foundation for Strategic Research (SSF)Assar Gabrielsson Foundation | None | Authors declare that data would be available upon publication |
| (Tarney, Wang et al. 2019) | USA | Uniformed Services University of the Health Sciences from the Defense Health Program | None | No mention |
| (Ura, Biffi et al. 2021) | Italy | Italian Health Ministry | None | Data available on request (for ethical reasons) |
| (Celsi, Monasta et al. 2022) | Italy | Italian Health Ministry | None | Data available on request |
| (Ura, Capaci et al. 2022) | Italy | Italian Health Ministry | None | Data available on request (for ethical reasons) |
| (Martinez-Garcia, Lesur et al. 2016) | Spain, USA, Luxemburg | Spanish Ministry of HealthSpanish Ministry of Economy and CompetitivitySpanish Ministry of Education, Culture and SportFondo Europeo de Desarrollo Regional - FEDERGrupos Estables de Investigacion 2011 – AECCFundació La Marató TV3CIRIT Generalitat de CatalunyaEuropean Commission, 7th Framework Programme, IRSES"Fonds National de la Recherche du Luxembourg" (FNR)AFR grant | None | No mention |
| (Martinez-Garcia, Lesur et al. 2017) | Spain, USA, Luxemburg | Spanish Ministry of HealthSpanish Ministry of Economy and CompetitivityFondo Europeo de Desarrollo RegionalGrupos Estables de Investigacion 2011-AECCFundació La Marató TV3CIRIT Generalitat de Catalunya Fundación DEXEUS Salud de la MujerSpanish Ministry of Economy and Competitiveness PERIS grant (Generalitat de Catalunya)Fonds National de la Recherche du Luxembourg" (FNR)AFR grant  | None |  |
| (Ihata, Miyagi et al. 2014) | Japan | Not mentioned | Several authors are employed by Ajinomoto Co. | No |
| (Trousil, Lee et al. 2014) | UK | National | No | No |
| (Jove, Gatius et al. 2016) | Spain | National, Eu grants | No | no |
| (Shao, Wang et al. 2016) | China | National | no | no |
| (Altadill, Dowdy et al. 2017) | Spain | National, Eu grants | no | yes |
| (Audet-Delage, Villeneuve et al. 2018) | Canada | national | Not mentioned | yes |
| (Audet-Delage, Grégoire et al. 2018) | Canada | national | no | no |
| (Troisi, Sarno et al. 2018) | Italy | Theoreo Srl spin-off University of Salerno | Applied for a patent | no |
| Shi et al. 2018  | China | National | no | no |
| (Knific, Vouk et al. 2018) | Slovenia | National | no | no |
| (Bahado-Singh, Lugade et al. 2017) | USA | Not mentioned | no | no |
| (Cummings, Massey et al. 2019) | UK | National | Not mentioned | No |
| (Strand, Tangen et al. 2019) | NorwayNetherlands | National | No | Yes |
| (Cheng, Chen et al. 2019) | Taiwan | national | no | No |
| (Lunde, Nguyen et al. 2020) | Denmark | National funding  | no | yes |
| (Shafiee, Ortori et al. 2020) | UK, Malaysia, | National funding | no | yes |
| (Troisi, Raffone et al. 2020) | Italy | National funding, | YesCEO Theoreo, patent owners,Hosmotic, Theoreo | yes |
| (Kozar, Kruusmaa et al. 2021) | Slovenia | Universal DX  | YesEmployees ofUniversal DX |  |
| (Njoku, Campbell et al. 2021)  | UK | Natinal Funding | No | Yes |
| (Kliemann, Viallon et al. 2021) | France | European and national funding | No | Yes |
| (Dossus, Kouloura et al. 2021) | UK | National funding | no | no |
| (Skorupa, Ponski et al. 2021) | Poland | national funding | No | Yes |
| (Gu, Chen et al. 2021) | China | National funding | Not mentioned | no |
| (Yan, Zhao et al. 2022) | China |  National funding |  no | no |
| (Schuhn, Tobar et al. 2022) | Germany | National funding | Patent applicaton | yes |
| (Arda Düz, Mumcu et al. 2022) | Turkey  | National funding | No | No |
| (Yi, Xie et al. 2022) | China | National funding | Yes; employees of Shanghai Omicsolution ltd. | yes |
| (Gatius, Jove et al. 2022) | Spain | National funding  | no | Yes |
| (Breeur, Ferrari et al. 2022) | France | National funding | no | yes |

**Supplementary Table S6:** Proteomic studies with less than 10 samples

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study****Aim** | **Samples** | **Study design****Verification** | **Method** | **Control group** | **Case group** | **Note/findings** |
| (DeSouza, Diehl et al. 2005)Diagnostic Biomarkers | Frozen tissue | Case - Control | iTRAQ / cICAT | N=3**iTRAQ**1 proliferative endometrium 1 secretory endometrium**cICAT**1 proliferative endometrium | N=5**iTRAQ**2 ECs**cICAT**3 ECs | histology confired on mirror face of the tissue block |
| (Li, Huang et al. 2008, Li, Zhao et al. 2008, Li, Min et al. 2010)\*Diagnostic & Prognsotic Biomarkers | Frozen tissue | Case – ControlProteomics on test set + HIC verification | MALDI-Q-TOF MS | N=0 for proteomicsValidation by HIC on FFPE: 29 endometrial intraepithelial neoplasia, 39 premonopausal endometria | N=8 for proteomicsEC + adjacent tissue45.6±3.4y (41-50y)Stage 1: 7Stage 2: 1Validation: HIC on FFPE (n=84) | Candidate potential biomarkers identified, including epidermal fatty acid-binding protein, calcyphosine, and cyclophilin A |
| (Attarha, Andersson et al. 2013)Biomarkers predictive of individualised tomor features | Frozen tissue | Cases onlyProteomics on test set + HIC verification (TMA) | DIGE-MALDI-TOF  | N=0 for proteomicsValidation by HIC 168 ECs and controls (not further specified) | N=3 for proteomicsEC + adjecent tissueValidation by HIC 168 ECs and controls (not further specified) | PKN1 and MST1 associated with aggressive cancer features |
| (Lintel, Luebker et al. 2018)Diagnostic Biomarkers (leiomyoma vs leiomyosarcoma) | FFPE Tissue | Case – ControlProteomics on test set + HIC verification (TMA) | LC-ESI-MS/MS | N=5 for proteomicsLeiomyomaValidation by HIC on TMA: 15 Leiomyoma (including those used for proteomics)  | N=5 for proteomicsLeiomyosarcomaValidation by HIC on TMA: 8 Leiomyosarcoma (including those used for proteomics) | Samples from FFPE archive592 proteins quantified, 10 DEPMVP (Major vault protein)Sensitivity: 50%Specificity: 100%COMT (Catechol O-methyltransferase)Sensitivity: 38%Specificity: 88% |
| (Liu, Hong et al. 2020)Diagnostic Biomarkers | Tissue (probably fresh froze, not mentioned) | Case – ControlProteomics on test set + HIC verification | 2D LC-MS/MS quantitative proteomics | N=5 for proteomicsValidation by HIC: 30 normal endometria (including those used for proteomics) | N=5 for proteomicsValidation by HIC: 30 ECs (including those used for proteomics) | 2521 proteins quantified, 619 DEPPI3K/AKT/mTOR pathway-related molecules (including PI3K, mTOR, ERK, SPP1, ANGPT2) associated with cancer |
| (Uyar, Huang et al. 2021)Diagnostic biomarkers by comparing the proteome before and after surgery in patients | serum | Case – controlConfirmation by western blotting, in vitro and in the TCGA dataset | 1D PAGE + LC-MS/MS | N=4benign conditions undergoing hsyterectomy | N=8EC, prior and after surgery | several DEP indeitifiedFAM83D confirmed by western blotting, in vitro and in the TCGA dataset (association with grade) |
| (Mu, Lim et al. 2012)Diagnostic Biomarkers | Urine | Case - Control | DIGE/MALDI TOF MS/MS | N=11Age matched | N=7Newly diagnosedStages IB-IIIB | DEP: 🡩 alpha-1 acid glycoprotein (AAG), zinc alpha-2 glycoprotein (ZAG) 🡫CD59 |
| (Mu, Lim et al. 2016)Test setDiagnostic Biomarkers (glycopeptides) | 2016 | UrineMorning midstream urine (50mL) | SELDI-TOF | N=4Heatly volunteers | N=4ECs | Potential candicate biomarkers (m/z peaks) identified |
|  (Ura, Monasta et al. 2017)Diagnostic & Prognostic Biomarkers | uterine aspirate | Case – ControlValidation by western blotting | DIGE-MS | N=6 | N=10 | 25 DEPIncluding ABRACL, PGAM2, FGB, ANXA3, validated by western blotting |
| (Alonso-Alconada, Santacana et al. 2015)Prognostic Biomarkers | Frozen tissue | Cases onlyDiscoveryIHC verificationBlood verification | DIGE-MS | N=0 for protoemicsIHC: 38 Normal EndometriaSerum: 27 age matched controls | N=12 for proteomicsPrimary / matched recrrent ECIHC: cohort 1: 140 EC (115 primary and 25 postradioation recurrecne);Cohort 2: 131 primary EC (93 EEC of which 50 reccurring and 43 non-recurrent; 38 NEserum: 34 EC (from Grade 3 Stage IB to tage IV / recurrences); 27 age matched controls | macroscopically dissected endometrial primary lesionsANXA2 by IHC (threshold od 190)Sensitity: 76% (62–87% 95% CI)Specificity: 67% (51–81% 95% CI)AUC: 74% (ROC analysis) |
| (Huang, Hao et al. 2021)Diagnostic biomarkers | Frozen tiessue | Cases onlyVerification by IHC (on controls as well) | label‐free quantification (LFQ) (LC‐MS/MS) | N=0 for proteomicsFFPE cohort (retrospective): 30 controls50.9y (46‐58y) | N=3 for proteomics (prospective)EC + adjacent normal tissueFFPE cohort (retrospective):75 EC 51.7y (42‐76y)Stage I-II: 54Stage III-IV: 21 | 3245 proteins identified, 925 DEP 🡩: IFIT3, pPARP9, SLC34A2, CYB5R1, PTPN1🡫: DPT, SLPI |
| (Mittal, Klingler-Hoffmann et al. 2016, Mittal, Klingler-Hoffmann et al. 2017)\*Biomarkers for patient startification (prediction LN metastasis) | FFPE tissue | Cases onlyVerification by IHC | MALDI-MSIon TMA, LC-ESI-MS/MS | - | N=10 for proteomics5 with LN metastasis, 5 withoutValidation by HIC (including those used for proteomics):Without LN metstasis: 27With LN metastases: 16Stage I: 27Stage III: 16Grade 1: 20Grade 2: 14Grade 3: 9 | histologyic confirmation on serial slidesbased on 19 peaks m/z, 88% of the ptients were correctly cliassified.Plectin, alpha-Actin-2, validated with LC-MS/MS DIA and immunohistochemistry |
| (Ura, Monasta et al. 2017)Diagnostic & Prognostic Biomarkers | uterine aspirate | Case - Control | 2D-DIGE-MS | N=6 | N=10 | Candidate biomarkers were identified |

\* It seems that authors used the same study cohort/data, but this is not clearly stated in the papers.

**Supplementary Table S7**: Proteomics studies in endometrial cancer.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study** **Aim** | **samples** | **Study design****Verification (1)(2)**  | **Method** | **Control group (2)** | **Case group (2)** | **Findings (3)** |
| **Blood (serum, plasma), urine and other fluids** |
| Zhu, 2006 (Zhu, Zhang et al. 2006)Diagnostic Biomarkers | Serum (pre-surgical) | Case - Control | SELDI-TOF-MS | N=30age-matched volunteers | N=40 EC Stage I: 33; Stage II: 2Stage III: 4; Stage IV: 157 y (34–78) | SN: 92.5%; SP: 100%Based on 13 biomarkers, with total coincidence of 95.7% |
| Kikuchi, 2007 (Kikuchi, Honda et al. 2007)Diagnostic Biomarkers | Serum | Case - Control | MALDI-TOF-MS | N=107Metroptosis patients (n=16), 65.8±9.8yMyoma uteri (n = 74), 48.8±4.1yhealthy volunteers (n = 17) 36.7±12.4y | N=92Stage 0: 6; Stage I: 63Stage II: 13; Stage IV: 259.4±10.5y | 507 protein peaks identified, and 3 m/z peaks distinguished ECs/controlscut-off values of mean of controls ±2SD:m/z 4769: SN=42.4%; SP=100%m/z 6254: SN=38.0%; SP=97.0%m/z 11792: SN=47.8%; SP: 97.0%Three peaks together:SN: 65.2% (60/92)SP: 93.9% (31/33) |
| Zhu, 2008 (Zhu, Zhang et al. 2008)Diagnostic Biomarkers | Serum | Case - Control |  | N=40Discovery cohort (n=30)Age-matched volunteersSame as (same as in Zhu 2006)\*\* not clearly stated by the authorsValidation cohort (n=10) | N=60Discovery cohort (n=40). Same as in Zhu 2006)\*\* not clearly stated by the authorsValidation cohort (n=20)Stage I: 18; Stage II: 2 | Discovery cohort:SP: 100%; SN: 92.5%Validation cohortSP: 60%; SN: 75%Based on 4 protein peaks |
| Qiu, 2010 (Qiu, Gao et al. 2010)Diagnostic Biomarkers | Serum(pre-operative) | Case - Control | Magnetic bead separation, MALDI-TOF-MS | N=3047y | N=30EC, 50y | Algorithm ClinProTool:SN: 97.62%; SP: 100%Based on 4 protein m/z peaks (2902.49, 5068.89, 6052.9, 7010.58)Algorithm SNNSN: 92.02%; SP: 100%Based on 10 protein peaksAlgorithm QCSN: 92.86%; SP: 93.75%Based on 2 protein m/z peaks (1012.6, 6093.12) |
| Wang, 2011 (Wang, Cao et al. 2011)Diagnostic Biomarkers | Serum | Cases only(pre-malignant to malignant conditions) | iTRAQ labelling, 2D LC−MS/MS | - | N=16Simple EH: 6; 46y (43-52y)Complex EH: 4; 40y (28-46y) Atypia EH: 4; 33y (29-40y)Stage I EC: 6; 53y (44-62y) | potential markers:SERPINA3; SERPINC1; APOA4; APOC2; APOE; HP; HRG; IGFBP4; ITIH4; ORM1; SAA1; SAA2 |
| Enroth, 2018 (Enroth, Berggrund et al. 2018)Diagnostic Biomarkers | Plasma | Case - Control | Proximity Extension Assays (PEA) | N=223Discovery cohort (n=105)Benign tumours; 60y (16-88y)Validation cohort (n=118)Benign tumours (n=61); 76y (22-88y)Healthy controls (n=57); 57y (28-86y) | N=302Discovery cohort (n=74)Stage I: 50; Stage II: 8Stage III: 10; Stage IV: 560y (29-86y) Validation cohort (n=228)Stage I: 109; Stage II: 13Stage III: 122; Stage IV: 11Unknown: 7368y (29-90y)  | DiscoveryEC vs Benign conditionsSN: 70%; SP: 67%; AUC: 0.72Based on 16 DEPValidationEC vs Benign conditionsSN: 77%; SP: 79%; AUC:0.83EC vs healthy controls SN: 69%; SP: 78%; AUC: 0.71Based on 9 DEP (PRSS8, MK, WFDC2 (HE4), ADM, MMP-7, ST2, VEGF-A, IL-6, HGF) |
| Tarney, 2019 (Tarney, Wang et al. 2019)Diagnostic Biomarkers | Serum (prediagnostic) | Nested case-control study from larger trial  | tandem-mass tag (TMT)isobaric labelling, LC-MS/MS  | N=112matched 1:1 on age (mean, 62.1y), race, study site, year of blood draw, and year of randomization  | N=112Incident cases, 62.3yEEC: 97Garde 1: 52; Grade: 32Grade 3: 5; Unknown: 8Mixed or type 1: 15 | 6 proteins (CFB, TF, CAT, PSMB4, B2M, PCDH18)AUC: 0.80 - 95% CI: 0.72-0.88**Cut-off of 0.5**SN: 45.2%; SP: 96.4%NPV: 86.4%; PPV: 77.8% |
| Ura, 2021 (Ura, Biffi et al. 2021)Diagnostic biomarkers | Serum | Case - Control | 2D-DIGE, LC-MS/MS | N=15non-cancer patients | N=1545y (36-48y)Type 1 EC | 🡩 6 proteins (CLU, SERPINC1, ITIH4, C1R, APOC3, DSC1)🡫 6 proteins (APCS, C9, APOA1, ALB, ITIH2, APOA4, ITIH2, CFHR1, ITIH2, ACTB) |
| Celsi, 2022 (Celsi, Monasta et al. 2022)Diagnostic Biomarkers | Serum | Case - Control | 2D-DIGE, LC-MS/MSProteomics on 10 ECs + 10 controls (serum)Validation by WB in 30 ECs + 30 controls | N=60non-cancer controls42y (32-77y)Uterine leiomyomas (hysterectomy tissue available); 30Serum only: 30 | N=4445y (33- 56y) | 🡩 7 proteins (fold change ≥1.5; (APOC3, APOC2, APOE, SERPINC1, C1R, SERPINA1, A2M)🡫 17 proteins (fold change ≤0.6; APOA1, APOA1, APCS, APOE, CLU, CD5L, CFHR1, VTN, C9, C8A, ALB, C4BPA, IGHM, ITIH2, C1R, SERPINA1, FLG2, SBSN, APOA4, CPS1)Further focus on SBSN |
| Ura, 2022 (Ura, Capaci et al. 2022)Diagnostic Biomarkers | Serum | Case - Control | Proximity Extension Assays (PEA)Panel: Immuno-oncologyPanel: Target 96 Oncology III | N=4467y (22-77y)non-cancer controls | N=4467y (44-81y)Type 1 ECs | **DEP**: Gal-9, Gal-1, MMP7, FASLG, CGB3, HSPB6, CDHR2, NCS1 DCTPP1 LMLN ALPP SCLY CD300E rfng LACTB2 CCT5 GFER VPS37A VAT1 PSMD9 KLK4 CPVL FLT3 HMBS UBAC1 HLA-E AFP COL9A1, CDHR2, NCS1, MLN, FLT3, COL9A1**Model 1 -** Immuno-oncology panelPseudo R-squared: 0.605AUC: 0.954% (95% CI 0.91–0.993)SN: 97.67%; SP: 74.42%based on 4 markers (Gal-9, Gal-1, MMP7, FASLG) **Model 2 -** Target 96 Oncology IIIPseudo R-squared: 0.436AUC: 0.889% (0.821–0.956)SN: 95.45%; SP: 69.77%based on 5 markers (CDHR2 NCS1, MLN, FLT3, COL9A1)**Model 3 -** Models 1 & 2 + COL9A1Pseudo R-squared: 0.691AUC: 0.969% (0.939–0.999)SN: 97.67%; SP: 83.72%  |
| Martinez-Garcia, 2016 (Martinez-Garcia, Lesur et al. 2016)Diagnostic Biomarkers | uterine aspirate | Case - Control | LC-MS/MS, LC-PRM (parallel reaction monitoring, 52 proteins measured) | N=18Non-cancer controlsPostmenopausal subjects>50y | N=20Postmenopausal subjects>50y | 🡩 (in EC) 26 proteins (PERM, CADH1, SPIT1, ENOA, MMP9, LDHA, CASP3, KPYM, PRDX1, OSTP, PDIA1, NAMPT, MIF, CTNB1, K2C8, ANXA2, CAPG, FABP5, MUC1, CAYP1, XPO2, NGAL, SG2A1, ANXA1, HSPB1, PIGR)ROC: 0.75 - 0.97AUC: >0.9 (for 10 markers) As single markersSN: >80%; SP: 95%4 proteins as single markers (PERM, CADH1, SPIT1, OSTP isoform A)  |
| Martinez-Garcia, 2017 (Martinez-Garcia, Lesur et al. 2017)Diagnostic BiomarkersPrognostic Biomarkers | uterine aspirate | Case - Control | LC-PRM (52 proteins measured)Prospective training + Leave-one-out validationIndependent cohort (as in Martinez-Garcia 2016) | N= 47Non-cancer controls (women with suspicion of EC based on endometrial thickness )53y (30-80y) | N=69Stage I: 43; Stage II: 12Stage II: 10; Stage IV: 4EEC: 49; 67y (37-87y)Grade 1: 5; Grade 2: 33Grade 3: 10SEC: 20; 73y (51-93y) | 🡩 28 proteins (EC vs controls): LDHA, KPYM, aKPYM, MMP9, NAMPT, SPIT1, CADH1 , ENOA, PERM, CAPG, CH10, CTNB1, K2C8, CLIC1, PDIA1, PRDX1, CD44, MIF, FABP5, XPO2, TPIS, CASP3, GSTP1, ANXA1, NGAL, ANXA2, GTR1, OSTP: isoform A, B, D, MUC1AUC of 5 best individual biomarkers: LDHA: 0.91 (95% CI, 0.856–0.957)KPYM isoform M1-M2: 0.90 (95% CI, 0.841–0.953)MMP9: 0.89 (95% CI, 0.827– 0.950)NAMPT: 0.88 (95% CI, 0.824–0.942) SPIT1: 0.88 (95% CI, 0.814–0.948)Stage Ia (n=30) vs controls (n=47) AUC >0.84 based on 5 individual biomarkers (LDHA, KPYM, MMP9, NAMPT, SPIT1)EH (n=9) vs controls (n=38)AUC>0.85 based on 16 proteins**Diagnostic model (EC vs controls)**MMP9, KPYM**Diagnostic model (EC vs controls)**Training cohortSN: 94.2% (88.4-98.6); SP: 87.2% (76.6-95.7)AUC: 0.96 (95% CI, 0.94–0.99)Leave-one-out validationSN: 89.9%; SP: 85.1%Independent cohort validationSN: 100%; SP: 83.3% (66.7-100)AUC: 0.96 (95% CI, 0.89–100)Based on MMP9, KPYM**Prognostic model (EEC vs SEC)**Training cohortSN: 95% (95% CI, 85–100)SP: 95.9% (95% CI, 89.8–100) AUC: 0.99 (95% CI, 0.90–1.0)Leave-one-out validationSN: 95%; SP: 89.8%Based on CTNB1, XPO2, CAPG |
| **Studies on Frozen Tissue** |
| Yoshizaki, 2005 (Yoshizaki, Enomoto et al. 2005)Diagnostic Biomarkers | Frozen tissue | Case - Control | SELDI-TOF-MS | N=20premenopausal (adenomyosis, myoma, cervical cancer, benign ovarian tumours) | N=19All EEC, stage I-IIIgrade 1: n=4; grade 2: n=8grade 3: n=7 | one protein consistently up-regulated one consistently down-regulated |
| DeSouza, 2007 (DeSouza, Grigull et al. 2007)Diagnostic Biomarkers | Frozen tissue | Case - Control  | iTRAQ labelling, SCX separation, MD LC-MS/MS | N=20proliferative endometria: n=10secretory endometria: n=10 | N=20Type 1 EC: n=10Type 2 EC: n=10  | Model based on: AAT, PK, CPN10SN: 95%; SP: 95%AUC 0.96; PPV 0.95 |
| Voisin, 2011 (Voisin, Krakovska et al. 2011)Diagnostic, Prognostic, Therapeutic Biomarkers | Frozen tissue | Case - Control  | iTRAQ labelling, drill-down LC-MS/MS | N=10 proliferative endometria | N=10Type 1 EC (it is stated, 5 were analysed in a previous study, but no clear reference) | PPV: 1.0%; AUC: 1.0%Based on: CALU, KRT8, CAP-G |
| Shan, 2016 (Shan, Zhou et al. 2016)Diagnostic Biomarkers | Frozen tissue | Cases onlyVerification: qRT-PCR; WB; functional studies on cell lines (for HSPA8 only) | iTRAQ-MS | - | N=10 All EC stage I; adjacent non-cancerous tissue | **DEP**: CCT7, HSPA8, PCBP2, LONP1, PFN1, and EEF2 |
| Ceylan, 2020 (Ceylan, Akpinar et al. 2020)Diagnostic Biomarkers | Frozen tissue  | Case - Control  | 2D-DIGE, MALDI TOF/TOF-MS | N=13Benign disease with abnormal bleedingpre-menopausal: n=7 (46.5 y) atrophic endometria: (46-64 y)complex/atypia EH: n=5 (51-63 y) | N=18Stage IA: n=5; Stage IB: n=5Stage II: n=3; Stage III: n=5Age 40-78 y | EC vs EH: 19 DEPEC stage IA vs controls:🡩 GRP78, GSTP1, ACTG, PDIA3, ENOA🡫 ALBUEC stage IB vs controls:🡩 GSTP1, ACTB, ACTG, K2C8, ANXA1, ENOA🡫 TRFE EC stage II vs controls:🡩 GSTP1, PDIA3EC stage III vs controls:🡩GSTP1, ACTB, K2C8, PDIA3, TRFE, ENOA EH vs controls:🡩 HSPB1, EF-Tu, IDHC |
| Mauland, 2017 (Mauland, Ju et al. 2017)Prognostic Biomarker associated with obesity | Frozen tissue | Cases onlyVerification:mRNAIHC | RPPA (163 proteins measured) | - | N=518EECTraining Norway cohort (n=272); Stage I-IV; Grade 1-3; pre/postmenopausal; 65.2±11.5 y; BMI 29.4±6.9Validation cohort 1: Norway Test Cohort (n=68); Stage I-III; Grade 1-3; pre/postmenopausal; 66.9±10.9 y; BMI=29.1±6.0Validation cohort 2:MDACC, Houston, TX (n=178); Stage I-IV; Grade 1-3; pre/postmenopausal;60.5±12.9 y; BMI 36.3±11.2 | Low PI3K-activation in non-obese patients with FIGO stage 1 and ERα positive tumours: reduced disease specific survival |
| Akkour, 2022 (Akkour, Alanazi et al. 2022) Diagnostic Biomarkers | Frozen tissue | Case - Control | 2D-DIGE, MALDI-MS/MS | N=12undergoing hysterectomyAge-matched | N=2412 EC; 12 EH; 46–75y | **DEP** potentially associated with progression from EH to ECDES, PPIA, ZNF844, ALDOA, ENO1, KRT10 |
| Kurimchak, 2020 (Kurimchak, Kumar et al. 2020)Prognostic Biomarkers | Frozen tissue | Cases only Verification:CPTAC & TCGA IHC (n=57; SEC: 39; EEC: 18; Normal n=12)functional validation *in vitro* | LFQsuper-SILAC labelling,MIB-MS | - | N=20primary EC n=20(EEC=17; SEC: 3)normal endometrial tissues (adjacent to tumour) n=16 | SRPK1 associated with poor survival  |
| **FFPE Tissue** |  |  |  |  |  |  |
| DeSouza, 2010 (DeSouza, Krakovska et al. 2010)DiscoveryDiagnostic Biomarkers | FFPE tissue | Case - Control | mTRAQ labelling, LC–multiple reaction monitoring (MRM)-MS, Targeted proteomics (17 proteins) | N=15Proliferative endometria | N=10 | Feasibility of using FFPE samples |
| Aboulouard, 2021 (Aboulouard, Wisztorski et al. 2021)Prognostic Biomarkers(Lymph-node disease) | FFPE tissue  | Case - Control | LC−MS/MS | N=9 Healthy Endometrium: 6Normal SLN: 3 | N=1559-74 y; BMI 26.7-41.3; Caucasian32 samples (15 used for proteomics):Grade I EEC: 4; Grade II EEC: 8; Grade III EEC: 4; Grade I SNL: 4; Grade II SLN: 8 Grade III SNL: 4 | **DEP**: 1005pathways altered in cancer**DEP EC vs SLN**: PRSS3, PTX3, ASS1, ALDH2, ANXA1 (verified by IHC) |
| Janacova, 2020 (Janacova, Faktor et al. 2020)Prognostic Biomarkers in the context of tamoxifen users | FFPE tissue | Cases only EC with / without previous exposure to Tamoxifen  | LC−MS/MS in SWATH-MS mode DIA | - | N=36Tamoxifen user: 15; 67.2±8.8 yTamoxifen naïve: 21; 66.3±6.1 y45 samples (EC and adjacent myometrium) | STMN1 associated with poor survival |

Footnotes and abbreviations: (1) qRT-PCR: quantitative real-time PCR; WB: western blotting. (2) BMI: body mass index - kg/m2; y: years; EH: endometrial hyperplasia; EEC: endometrioid EC; SEC: serous EC; CCEE: clear cell EC. (3) 95% CI: 95% confidence interval; vs: *versus*; SN: sensitivity; SP: specificity; PPV: positive predictive value; NPV: negative predictive value; AUC: area under the curve; ROC: receiver operating characteristic; DEP: differentially expressed proteins.

**Supplementary Table S8**: Metabolomics studies in endometrial cancer.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study****Aim** | **Samples** | **Study design****Verification** | **Method** | **Control group** (1) | **Case group** (1) | **Findings** |
| **Blood (plasma or serum) samples** |  |  |  |  |  |  |
| Ihata, 2014 (Ihata, Miyagi et al. 2014)Diagnostic Biomarkers | Plasma | Case-control | HPLC-ESI-MSTargeted | N= 362benign gynecological diseases (n=122); 45y (32-82y)Leiomyoma: 54Adenomyosis: 7Endometrial cyst: 18Cystic teratoma: 14Mucinous cyst: 13Serous cyst adenoma: 1Fibroma: 4; Simple cyst: 9Others: 2Healthy women (n=240Age and BMI matched58y (32-82y)Training set: 120 healthy womenValidation set (n=242)120 healthy women + 122 patients with benign diseases | N=80EC patients; 58y (32-80y)Sage I: 48; Stage II: 9Stage III: 15; Stage IV: 8Grade 1: 40; Grade 2: 15Grade 3: 6; Unknown: 19EEC: 54; SEC: 6; CCEC: 3Adenosquamous EC: 6Mucinous: 1Carcinosarcoma: 1Squamous EC: 1Poorly differentiated EC: 1Training set: 40 EC patientsValidation set: 40 EC patients | Training:EC vs Healthy women:🡫 His, Trp, Val, Phe, Asp, Ser, Leu, and Met🡩 ornithine, Ile, Pro**LR models**:EC vs Healthy womenHis, Ile, Val and Pro: AUC: 0.94; SN: 60%; SP: 98.3%CA-125: AUC: 0.80Validation setEC vs Benign diseaseHis, Ile, Val, and Pro: AUC:0.83CA-125: AUC: 0.60EC stage I vs Healthy womenHis, Ile, Val, and Pro: AUC: 0.91CA-125: AUC: 0.79EC stage II-IV vs Healthy womenHis, Ile, Val, and Pro: AUC: 0.99CA-125: AUC: 0.83 |
| Knific, 2018 (Knific, Vouk et al. 2018)Diagnostic & Prognostic Biomarkers | Plasma | Case-controlTraining and test setsselected by splitting into equal sets 100 times | FIA-ESI-MS/MSAbsolute*IDQ*TM p150 kit (Biocrates Life Sciences)Targeted | N=65Patients with benign pathologiesprolapsed uterus or myoma63.2±9.4y; BMI: 28.3 ±4.7 | N=61EC patients65.1±8.7y; BMI: 32.1±7.3 LVI- or unknown: 52LVI+: 9; MI>½: 16no difference between groups in age, menopausal status, medication intake, diabetes, hypertension, smoking status | 🡫 3 metabolites: PCaa C40:1, PCaa C42:5, PCaa C42:6, 166 metabolite ratios🡩 total short-chain and long chain acylcarnitines, Pro/TyrLR modelEC vs controlsC16/PCae C40:1, Pro/Tyr, PCaa C42:0/PCae C44:5AUC: 0.84; SN: 85.3%; SP: 69.2%C16/PCae C40:1, Pro/Tyr, PCaa C42:0/PCae C44:5, smokingAUC: 0.86; SN: 77.0%; SP: 79.0%Detection of MI:SMOH C14:1/SMOH C24:1, PCaa C40:2/PCaa C42:6AUC: 0.86; SN: 81.3%; SP: 86.4%SMOH C14:1/SMOH C24:1, C16:2/lyso PCa C16:1AUC: 0.84; SN: 75%; SP: 72.7%SMOH C14:1/SMOH C24:1, PCaa C40:2/PCae C40:1AUC: 0.85; SN: 68.8%; SP: 97.7%SMOH C16:1/SMOH C24:1, PCaa C34:4/PCae C34:3AUC: 0.85; SN: 81.2%; SP: 77.3%Detection of LVI:PCaa C34:4/PCae C38:3, C16:2/PCaa C38:1AUC: 0.94; SN: 88.9%; SP: 84.3% |
| Strand, 2019 (Strand, Tangen et al. 2019)Prognostic Biomarkers | Plasma | Cases onlyCross-validation | TargetedLC-MS/MSAbsolute*IDQ*TM p180 kit(Biocrates Life Sciences) | - | N=40Group 1, short survival: 2075y (63.6-81.5y)EEC: 8; SEC: 5Carcinosarcoma: 5Non-endometrioid: 2Grade 1: 3; Grade 2: 2Grade 3: 3; Stage I: 18Stage II: 2Group 2, long survival: 2067y (56.0 -77.y)EEC: 7; CCEC: 3; SEC: 3Carcinosarcoma: 6Non-endometrioid: 1Grade 1: 3; Grade 2: 2Grade 3: 2; Stage I: 18Stage II: 2Group 1/2 matched histology, grade, age, BMI | Long/short survival:🡫 methionine sulfoxide (MetSO), hydroxypropionylcarnitine (C3-OH)**Model 1**: AUC: 0.82 (MetSO, serotonin, spermine, C3-OH, PCaa C36:5, SM C20:2)**Model 2:** AUC: 0.935 (MetSO, serotonin, spermine, C3-OH, PCaa C36:5, SM C20:2, spermidine, butenylcarnitine (C4:1), lyso PCa C18:2 and lyso PCa C24:0)**Model 3**: AUC: 0.965 (MetSO, serotonin, spermine, C3-OH, PCaa C36:5, SM C20:2, spermidine, C4:1, lyso PCaa C18:2, lyso PCaa C24:0, Asp, dimethylarginine, hexose, PCae C30:1) |
| Njoku, 2021 (Njoku, Campbell et al. 2021)Diagnostic BiomarkersPrognostic Biomarkers | Plasma | Case-controlstudyPatients with BMI > 30RF based on training and test sets | Non-targetedMetabolon Inc ®RP UHPLCMS/M; HILIC | N=69Control patients referred to weight loss managementNormal histologyPostmenopausal: 2146y (IQR 39.53y)BMI: 50 (IQR 46.55) | N=67EC patientsPostmenopausal: 56Grade 1: 47; Grade 2: 14Grade 3: 6; Stage I: 59Stage II: 2; Stage III:6LVI+: 12; >50% MI: 1263y (IQR 54.69y)BMI: 40 (IQR 34.46) | EC vs Controls**Univariate ROC analysis**1-lignoceroyl GPC (24:0), AUC: 0.911-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2), AUC = 0.851-linolenoyl-GPC (18:3) AUC: 0.843-hydroxybutyryl carnitine, AUC:0.833-hydroxybutyrateAUC; 0.82**RF algorithm**EC vs Controls (top 20 metabolites)Training (86% accuracy), *Test set* (93% accuracy)AUC: 0.95EC stage I vs Controls (top 20 metabolites); AUC: 0.98EC with/without LVI: AUC=0.832 (1-linoleoyl-GPE)EC with/without > 50% MI: AUC=0.74 (Homovanillate)-only postmenopausal women: AUC=0.83 (Tricosanoyl sphingomyelin) |
| Kliemann, 2021 (Kliemann, Viallon et al. 2021)Association | Plasma (EPIC)serum (Intercept) | Nested case-control study(EPIC, Intercept)Discovery metabolic signatures of BMI, waist circumference (WC) and waist/hip ratio (WHR)Validation associations with EC | Targeted LC-MS/MSAbsolute*IDQ* p180 kit | N=648Control patients53.99±7.9y; BMI: 25.9±4.2Matched for study recruitment center, age, menopausal status, time of blood collection, fasting status,No diabetes, no HRT | N=635EC patients53.96±7.9y; BMI: 28±5.4 | Metabolic signatures of body size (BMI, WC and WHR)🡩 Val, Ile, Gln, PCaa C38:3, PCaa C38:4,🡫 Asn, Gln, Gly, Ser, lysoPC C17:0, lysoPC C18:1, lysoPC C18:2, PCaa C42:0, PCaa C34:3, PCaa C40:5, PCaa C42:5EC associated with metabolic signatures of BMI, WC and WHC(Conditional LR models)BMI OR 1.5 (1.30-1.74)WC OR 1.46 (1.27-1.69)WHC OR 1.54 (1.33-1.79) |
| Dossus, 2021 (Dossus, Kouloura et al. 2021)Association | Plasma | Nested case-control study(EPIC) | Targeted LC-MS/MSAbsolute*IDQ* p180 kit | N=853Control patients54.7±7.5y; BMI: 25.7±4.1 | N=853EC patientsType 1: 761Type 2: 42Not known: 5054.7±7.5y; BMI: 27.7±5.4 | (Conditional LR models)28 metabolites associated with EC (12 GP, 2 acylcarnitines and 2 sphingolipids)Adjustment for BMI:SM C18:0: OR 1.18 (1.05-1.33)Gly, Ser, free carnitine (C0):OR 0.89 (0.80-0.99)OR 0.89 (0.79-1.00)OR 0.91 (0.81-1.00)Esterified/free carnitine OR 1.14 (1.02-1.28)Short chain/free acylcarnitines OR 1.12 (1.00-1.25) |
| Breeur, 2022 (Breeur, Ferrari et al. 2022)Association | Plasma and serum samples from EPIC study | Case-control studyPan- cancer studywith 11 656 participants | Targeted metabolomicsLC-MS/MSAbsolute*IDQ* p150, Absolute*IDQ* p180 | N = 689Control women:54.3 ±7.8y; BMI: 26.0 ±4.3  | N = 689EC patients:54.3 ±7.8y; BMI: 28.2 ±5.5Cases were matched with controls with regard to study centre, sex, age, time of the collection, fasting status, and exogenous hormones. | Different cancers (BC, colorectal, prostate cancer/control women)6 metabolites: 🡫 Gln, butyrylcarnitine (C4), lysoPCa C18:2, PCaa 32:2, PCaa 36:0, PCaa 36:1,🡩 Pro, decanoylcarnitine (C10), PCaa C28:1EC/control women🡩 SM\_C16:0 cluster OR 1.51 (1.19-1.93)OR 1.20 (0.97-1.47) adjusted for additional factors |
| Audet-Delage, 2018 (Audet-Delage, Villeneuve et al. 2018)DiscoveryDiagnosticprognostic | Serum | Case-controlexploratory | Metabolon platformRP-UPLC-MS/MSNon-targeted | N=18Patients with benign conditionspostmenopausalno HRT for the last 3 weeks58.9±10.4y; BMI: 27.5±7.2  | N=26EC patientsType 1: 24; Type 2: 12postmenopausalno HRT for the last 3 weeksnon-recurrent (NR) N= 18Type 1: 12; Type 2: 666.3±8.3y; BMI: 28.4±7.0 recurrent (R) N = 18Type 1: 12; Type 2: 667.5±9.4y; BMI: 28.0±6.4 | 1592 metabolites analysed,EC/C: 137 metabolites, 🡩115 (acylcholines, monoacylgycerols, acylcarnitines), 🡫22 (free fatty acids)Peptides and amino acids: spermine and isovalerate, glycylvaline, gamma-glutamyl-2-aminobutyrate AUC = 0.92 (0.84-1.00)Type 1/type 2: 98 metabolites, 🡩 30 (bradykinin, sulfated androgens)🡫 68 (heme, saturated long-chain acylcarnitine, choline, sarcosine, Gly)R/NR: 104 metabolites (80 involved in lipid metabolism)🡩 monoacylglycerols (16:1, 18:1, 20:5 and 22:6), docosahexaenoyl carnitine, 2-hydroxypalmitate, 2-hydroxystearate 🡫 Ser, ThrR/NR: 2-oleoylgycerol and TAG 42:2-FA12:0AUC = 0.90 (0.79-1.00)Type 1 R cases 🡫 bile acids (taurodeoxycholate, glycodeoxycholate and taurocholate) 🡩 phosphorylated fibrinogen cleavage peptideType 2 R cases 🡩 sphingolipids (ceramides, dihydroceramides, lactosylceramides) |
| Audet-Delage, 2018 (Audet-Delage, Grégoire et al. 2018)DiscoveryPrognosticDiagnostic | Serumpre-surgical &one month after surgery | Case-controlCases onlyTraining only | TargetedGC-MS (13 unconjugated steroids), RP-LC-MS/MS (14 conjugated steroids, catechol estrogens) | N=110healthy postmenopausal women,58.3±5.6yOC: 145 no, 91 yes, 10 missingHRT: 157 never, 80 ever, 9 missing | N=246EC patients65.1±8.9yType 1: 202; Type 2: 44Grade 1: 90; Grade 2: 94Grade 3: 61; Unknown: 197Stage I: 197; Stage II: 12Stage III: 28; Stage IV: 9MyomInv<50%: 187MyomInv>50%: 59LVI-: 183; LVI+: 58No relapse: 220Relapse: 265 year recurrence: 24(FW time: 65.5m)OC: 19 no, 91 yesHRT: 40 never, 70 ever | BMI: 🡩 E3, E1-S, E1, E2, 2MeO-E1MI 🡫 E3Recurrence (pre-operative):🡩 E1-S🡫 E3EC (after)/EC (before): 🡫 all steroids except 🡩 4MeO-E2EC (after) ≈ HW¸🡩 4MeO-E2EC (type 1 and type 2 before) vs healthy🡩 DHEA-S, DHEA, 5-diol,4-dione, testosterone, DHT, ADT-G, 3a-Diol-G, 3a-Diol-17G, E1-S, E1, E2EC (type 2, before) vs healthy🡩 DHEA, 5-diol, 4-dione, testosterone, ADT-G |
| Troisi, 2018 (Troisi, Sarno et al. 2018)DiscoveryValidationDiagnosticPrognostic | Serum | Case-controlRecruitment IRecruitment II | Non-targetedGC-MSkit (Theoreo) | N=1301st group:80 Healthy Women60y (55-65y); BMI: 27.8 (24.2-29.0)2nd group:50 Healthy Women65y (59-69y); BMI: 27.1 (23.9-30.5)10 Patients with benign diseases (hyperplasia, polyps, abnormal bleeding) | N=1281st group: n=88Type I: 67; Type II: 21Grade 1: 2; Grade 2: 53Grade 3: 33; Stage I: 36Stage II: 45; Stage III: 768y (62-68y); BMI: 28.3 (25.1-30.3) 2nd group: n=30Type I: 23; Type II: 7Grade 1: 4; Grade 2: 22Grade 3: 4; Stage I: 12Stage II: 15; Stage III: 366y (61-72y); BMI 28.9 (26.3-31.1) | 259 metabolites determined consistentlyEnsemble modelsPLS-DA model (also LDA, NB, DT, RF, K-NN, ANN, SVM)EC/HW (Model I):🡩 lactic acid, homocysteine, 3-hydroxybutyrate🡫 linoleic acid, stearic acid, myristic acid, Thr, Val, progesteroneAccuracy: 0.99±0.0SN: 97±3%; SP: 98±2%type 1/ type 2 (Model II):🡩 lactic acid, Cys, Ser, malate, Glu, homocysteine,🡫 progesteroneAccuracy: 0.93±0.04SN: 96±4%; SP: 86±13%Ensemble models I and IIAccuracy: 1.00 ±0.0SN: 100 ±0.0 %; SP: 100 ±0.0 % |
| Shi, 2018 (Shi, Wang et al. 2018)DiscoveryExploratory | Serum | Case-controlCross validation with 100 random permutations | RP-UPLC-ESI-Q-TOF/MSNon-targeted | N=46Healthy women57 ±10y; BMI 25.8±3.1 | N=46Type 1 ECStage Ia: 27; Stage IIb: 19Grade 1: 20; Grade 2: 13Grade 3: 1352 ±8y; BMI 26.9±5.1 | PLS-DA and OPLS-DA model:7646 in positive mode, 2579 negative mode🡩 Phe, indoleacrylic acid, phosphocholine (PC), lyso-platelet-activating factor 16 |
| Bahado-Singh, 2017(Bahado-Singh, Lugade et al. 2017)Discovery (Training + test set)ValidationDiagnostic | Serum | Case –controlrandom stratification into test and training | Non-targetedNMR (32)TargetedAbsolute*IDQ*TMRP-LC-MS/MS (149)(Biocrates Life Sciences) | N=60Healthy women59.2±12.7yTraining + test set: 36Validation set: 24 | N=5659.1±12.8yStage I-II: 46; Stage III-IV: 10Training + test sets: 33Validation set: 23 | All EC/HWSignificant differences: 4/32; 36/149 (16 overlap)VIP: 3-hydroxybutyrate, C14:2, C6 (C4:1DC), C10, C18:2, L-Met, C8, 2-hydroxybutyrate, C7-DC, C18:1, C16, kynurenine, C14:1, PCae C40:1LR model (validation data)EC/HWC14:2, PCae C38:1, 3-hydroxybutyric acidAUC: 0.83 (0.70-0.95)SN: 82.6%; SP: 70.8%C18:2, PCae C40:1, C6, C4:1-DCAUC: 0.81 (0.69-0.94)SN: 82.6%; SP: 66.7%BMI, C14:2, PCae C40:1AUC: 0.80 (0.67-0.78)SN: 78.3%; SP: 62.5%EC stage I-II/ HWPCae C38:1, 3-hydroxybutyric acid, C14:2AUC: 0.82 (0.69-0.95)SN: 72.2%; SP: 79.2%BMI, C14:2, PCae C40:1AUC: 0.80 (0.67-0.93)SN: 72.2%; SP: 75.0% |
| Lunde, 2020 (Lunde, Nguyen et al. 2020)Exloratory studyPrediction of postoperative pain | SerumDanish Cancer Biobank | Cases onlyFrom a nested case-control studyreference test: hysterectomy,prognosis of pain according to VAS > 3five-fold cross-validation | Non-targeted NMR | - | N=78Low-risk: 50Intermediate-risk: 9High-risk: 19With chronic postoperative pain: 26Without pain: 52matched on age and BMI | 🡩 19 metabolitesbranched-chain amino acids, cholesterol, cholesteryl ester, linoleic acid, phospholipids, triglycerides🡫 glycerolEC with/without painIDL-TG, LDL-TG, L-LDL-TG, AUC = 0.8PLS-DA, LSVM, LR, RF models including 14 metabolites:AUC = 0.79-0.87 |
| Kozar, 2021 (Kozar, Kruusmaa et al. 2021)Discovery | Serum | Prospective observational studyCross validation with 50 iterations (training and test set) | Non-targetedHPLC-TQ/MS | N=21Control patients (pelvic floor disorders, endometriosis, benign ovarian cysts)Premenopausal: 15 Postmenopausal: 654± 19 yBMI:25±4  | N = 15EEC patientsPremenopausal: 3Postmenopausal: 12stage I: 9stage II: 2stage III: 3stage IV: 1Grade 1: 10Grade 2: 4Grade 3: 1Age: 64 ± 14 yBMI: 29± 7  | EC/CUnivariate analysisCer 34:1;2Cer 40:1;2AC 16:1-OH; 1-methyladenosine, AC 16:1, AC 14:1; AC 14:0AUC = 0.75-0.83; SEN: 60-86%, SP: 73-95%, accuracy: 0.22-0.81RF model (Cer 34:1;2, Cer 40:1;2, AC 16:1-OH and 1-methyladenosineAUC = 0.92 (0.91-0.95); SEN: 94%, SP: 75%, accuracy: 22-81% |
| Gu, 2021 (Gu, Chen et al. 2021)DiscoveryDiagnosticPrognostic | Serum | Case-controlOnly training | Non-targetedGC-MS | N=30postmenopausal patients indicated hysterectomyNo HRT (3 weeks before)40-85 y | N=60Postmenopausalno HRT (3 weeks before)Stage I: 30Stage II: 3040-85 y | EC stage I/C: 27 metabolites: 7🡩, 20🡫OPLS-DA(VIP> 1.5): urea, arachidonic acid, mannose, phosphoric acid, threose, GABA, 1-monopalmitoylgycerol, ethylamine, cholesterolEC stage II/C: 28 metabolites: 6🡩, 22🡫(VIP> 1.5): diphosphate, 3-oxaoct-4-en-11-imine, -D-allopyranose, Ser, L-Ile, Gly, arachidonic acid, 1-monopalmitoylglycerol, GABA, aminomalonic acid, oxalic acid, ureaEC stage I/stage II: 25 metabolites 7🡩, 18🡫(VIP> 1.5): D-galactose, phosphoric acid, threose, urea, 5-hydroxycaproic acid, cholesterol, mannose, GABA, -D-allopyranose |
| Yan, 2022 (Yan, Zhao et al. 2022)Discoveryand validationDiagnosticPrognostic | Serum | Case-controlDiscovery (95)validation (456) | Non-targetedUPLC-Q-TOF/MS | N=496Discovery (n=72)Healthy women: 3051.9 ±7.5 yPremonopausal: 13 Postmenopausal: 11Perimenopausal: 6 (stratification according to age)Endometrial polyps: 3051.9 ±10.9 yPostmenopausal: 16EH: 1246.8 ±8.4 yPostmenopausal: 1No HRT (3 months before)Validation (n=406)Healthy women: 195Endometrial polyps: 171EH: 40 EH | N=73Discovery (n=23)58.7 ±8.6 yEEC: 20SEC: 2Muellerian: 1Grade 1: 7Grade 2: 6Grade 3: 3Unknown: 7Stage Ia: 11Stage Ib: 5Stage II: 1Stage III: 3Stage IV: 2LVI-: 7LVI+: 9Postmenopausal: 21No HRT 3 months before surgery, no info about BMIValidation: 50 ECNo other data | EC/HWOPLS-DA model (VIP>1, p< 0.05) discovery + validation:🡩 LysoPC 20:2, Lyso PC 20:4,🡫DG 38:5; Cer(d18:0/18:0), PG 34:0, CE 16:0, PC 38:3, PC 15:1AUC: 0.737-0.882, SEN: 64%-92%, SP: 56.5%-91.3%Logistic regression model (4 EP specific biomarkers: 6-ketoPGF1, PA 37:4, LysoPC 20:1, PS 36:0)EP/EC (discovery/validation):AUC = 0.92, SEN: 100%, SP: 72.4%AUC = 0.90, SEN: 70.4%, SP: 94.1%EC stage I /EPAUC = 0.90, SEN: 94.8%, SP: 76.9% |
| Schuhn, 2022 (Schuhn, Tobar et al. 2022)DiscoveryDiagnostic | Serum | Case-controlTraining only | Targeted17 amino acids and 28 acylcarnitinesESI-MS/MS | N=171Healthy women: 15748 ±14 yBMI: 26.0±7.1N= 14Patients with benign pathologies (polyps, suspicious endometrium on imaging):56 ±12 yBMI: 28.0 ±7.6 | N=20EC patients62 ±9 yBMI: 30 ±6.2  | EC/C:🡫Thr, Arg, Met, 🡩 malonylcarnitineAUC = 0.73-0.85ThrAUC = 0.85 (0.72-0.98), SEN: 70%, SP: 92.9%EC/HW:malonylcarnitine, acetylcarnitine, carnitine, tetradecenoylcarnitineAUC = 0.75-0.82malonylcarnitineAUC = 0.82 (0.72-0.92), SEN: 80%, SP: 73.1% |
| Dried blood samples |  |  |  |  |
| Troisi, 2020 (Troisi, Raffone et al. 2020)Discovery and validationDiagnostic | Dried blood samples | A multicenter prospective cohort studydiscovery and validation | Non-targeted GC-MS | N= 70DiscoveryPatients without EC (matched age, years from menopause, tobacco use, comorbidities) postmenopausal, no HRT, no previous hysterectomy, no immunosuppressive therapyAge: 68.2 ± 11.7 yBMI: 27.6 ± 4.3 N = 1430Validation:Prospective cohort of postmenopausal women (16 women were diagnosed with EC, see next cell)  | N = 50DiscoveryEC (postmenopausal, FIGO I-III, G1-G3)N=16 EC (from the prospective cohort of 1430 subjects)Age: 69.4 ± 13.8 yBMI 29.3± 4.9 12 IA, 3 IB, 1 IIAge: 59.7± 7.7 yBMI: 26.8± 4.6  | EC/CClassification models:Decision tree, Naive Bayes, RF, k-Nearest neighbours, Artificial neural network, Linear discriminating analysis, SVM, linear regression, Deep Learning, Partial least squares-discriminant analysisSP: 96.3-100%, SEN: 50- 100 %; PPV: 89.7-100%, NPV: 80-100%, Accuracy: 83.3-100Ensemble Machine Learning algorithm (10 different classification modelsSP: 99.9%, SEN: 100 %; PPV: 88.9 (7.4), NPV: 100%, Accuracy: 99.9 % |
| Urine samples |  |  |  |  |  |
| Shao, 2016 (Shao, Wang et al. 2016)DiscoveryDiagnostic | Urine(morning) | Case-controlTraining and test set | RP-UPLC-ESI-Q-TOF-MSNon-targeted | N=35Healthy women (n=25)EH (n=10) | N=25EC patientsno significant difference in age and weight | PLS-DA model (all 60 patients)5 metabolites EC/HW+EH🡫 porphobilinogen, acetylcysteine🡩 N-Acetylserine, urocanic acid, isobutyrylglycineSVM modelEC/HW+ EH (2/3 training set, 1/3 test set) |
| Cervicovaginal fluid |  |  |  |  |
| Cheng, 2019 (Cheng, Chen et al. 2019)DiscoveryDiagnostic | Cervicovaginal fluidCollected in the middle of the menstrual cycle. | Case-controlNCT02528864Training and test set | Non-targeted1H NMRBruker Advance 600 MHz | N=33Patients with benign pathologies47 y (32-74 y)Fibroid: 17Endometrioma: 7Adenomyosis: 5Polyp: 4Pre-menopause: 26Post-menopause: 7Cases/controls: no differences in diabetes, metabolic syndrome, undergoing estroprogestinic therapy | N=21EC patients52 y (30-67 y)Stage I: 17Stage II: 1Stage III: 3Grade 1 or 2: 12Grade 3: 7Pre-menopause: 13Post-menopause: 8 | EC/CTraining data set: 17 cases, 28 controlsTest data set: 4 cases; 5 controls29 metabolites identifiedSignificant 🡩: choline, formate, fumarate, malate, phosphocholineSignificant 🡫: Asn, Asp, Ile, Phe, pyruvatePrediction models built upon phosphocholine, malate, AsnEC/CTraining:RF: AUC = 0.92 (0.80-0.99)SVM: AUC = 0.88 (0.76-0.97)PLS-DA: AUC = (0.89 (0.76-0.97)LR: AUC = 0.88 (0.70-0.97)ANN: AUC = 0.88 (0.82-0.92)Testing:RF: Acc. 0.78 (0.4-0.97); SEN 0.75 (0.19-0.99); SP. 0.8 (0.28-1.00)SVM: Acc. 0.78 (0.4-0.97); SEN. 0.75 (0.19-0.99); SP. 0.8 (0.28-1.00)PLS-DA: Acc. 0.67 (0.3-0.93); SEN 0.75 (0.19-0.99); SP 0.6 (0.15-0.95)LR: Acc. 0.67 (0.3-0.93); SEN 0.75 (0.19-0.999; SP 0.6 (0.15-0.95)ANN: Acc. 0.73 (0.63-0.8); SEN. 0.68 (0.55-0.74); SP0.64 (0.52-0.72) |
| Tissue samples |  |  |  |  |
| Jove, 2016 (Jove, Gatius et al. 2016)DiscoveryDiagnostic | Tissue | Case-control(permutation test) | RP-LC-ESI-QTOF-MS/MSNon-targeted | N=15Normal endometriumProliferative (n=10)Secretory (n=10) | N=27EECGrade 1: 6Grade 2: 13Grade 3: 8Two different samples:Surface endometrioid carcinoma (SEC)myometrial invasive front (MIF) | EC/NE:44 metabolites (4 identified)🡩 stearamide, monoolein,hypoxanthine, 1,2-dihexadecanoyl-sn-glycerolPLS-DAG III-IV/I-II:26 metabolites (3 identified)🡫 taurine, erythriol,🡩 oleamideSEC/MIF104 metabolites (14 identified):🡩 xanthine, lactamide, lactic acid, alpha-D-fucose, 3-mercaptopyruvate, ribitol, PC 32:0, eicosapentaenoic acid🡫 inosine, deoxycytidine, hypoxanthine, CDP-ethanolamine, 5-methylthioadenosine, monoolein |
| Altadill, 2017 (Altadill, Dowdy et al. 2017)DiscoveryDiagnosticPrognostic | Tissue | Case-controlTraining only | Non-targetedRP-UPLC-ESI-TOF-MSWaters SYNAPT G2 Si | N=17Benign diseases> 50 yPostmenopausalno treatment | N=39Stage Ia: 10Stage Ib: 9Stage II: 10Stage III: 10>50 yPostmenopausalno treatment | EC vs Controls80 metabolites, 42 identified mainly lipids 🡩 8 glycerophosphocholines (PC), 1 phosphatidylserine (PS), 1 phosphatidylglycerol (PG), 9 phospatidylethanolamines (PE), 4 phosphatidylinositol (PI); linoleic acid, 3-deoxyvitamin D3, UDP-N-Acetyl-D-galactosamine, 1-palmitoyl-2-linoleoyl PE🡫 Glu-Phe-Arg-Trp, palmic amide, stearamide, oleamide, 1 PA, 2 PE, PG, inosine, picolinic acid29 stage I/II EC vs 10 stage III🡩 PC (16:0/20:5), PE (22:6/P-18:1), UDP-N-acetyl-D-galactosamine, arachidonic acid, 🡫 PC (16:0/22:6), 2 PE (16:0/22:6), (18:1/22:6) |
| Trousil, 2014 (Trousil, Lee et al. 2014)DiscoveryDiagnostic | Tissue | Case-control(Seven fold cross-validation) | 1H-NMRBruker DRX600Non-targeted | N = 10Normal endometriumMedian age 47.8 y | N = 10EC patients G3Median age 65.8 y | EC/C🡩 Val, Leu, Ala, Pro, phosphocholine, Tyr🡫 glutathione, scyllo-inositol, myo-inositol, inosine/adenosine | PLS-DA modelAUC = 0.987 |
| Cummings, 2019 (Cummings, Massey et al. 2019)DiscoveryDiagnostic | Tissue | Case-controltraining | TargetedRP-LC-MS/MS | N=53Patientsundergoing hysterectomyProliferative: 13Secretory: 6Atrophic: 33AEH: 31 | N=10867 y (39-89 y)Stage I: 79Stage II: 7Stage III: 14Stage IV: 8 IVLVI-: 58LVI+: 50Type 1: 55Grades 1 and 2Type 2: 53Grade 3: 10SEC: 19CCEC: 5Mixed EC: 4Carcinosarcoma: 15 | EC/CDihydro-15-keto derivatives:🡫 type 1 and type 2 /NE13,14-dihydro-15-keto PGE2🡫 type 2 /NE13,14-dihydro-15-keto PGF2Type 2/ type 1 EC:🡫 12-HETE |
| Skorupa, 2021 (Skorupa, Ponski et al. 2021)DiscoveryDiagnostic | Tissue | Case-control(Seven fold cross-validation) | Non-targetedHR MAS NMR | N=10Patients with benign diseases (prolapse of uterus, leiomyomas, cystadenomas)62.0±7.3 yBMI: 27.0±2.2No HRT | N=64Grade 1: 1464.9±9.1 yBMI: 26.9±2.0Grade 2: 3370.2±7.4 yBMI: 30.5±5.6Grade 3: 1768.0±8.4 yBMI: 29.4±3.3No HRT | OPLS-DAEC/C: 🡩 Val, Leu, Ile, hypotaurine, Ser, Lys, ethanolamine, choline🡫 creatine, creatinine, glutathione, ascorbate, Gln, PE, scyllo-inositolEC G1/2/C: 🡩 taurineEC G1/3/C: 🡩 Gly, N-acetyl compound, lactateG1: 🡩 dimethylsulfone, phosphocholine, 🡫 glycerophosphocholine, GlnG2/3 🡫 myoinositolG3: 🡩 3-hydroxybutyrate, Ala, betaineG1/G2Dimethyl sulfone AUC = 0.95 (0.86-0.99)G2/G3Taurine AUC = 0.92 (0.84-0.98)Scyllo-inositol AUC = 0.92 (0.83-0.99)Choline AUC = 0.85 (0.73-0.94)G1/G3Choline AUC = 0.96 (0.86-1.0)3-hydroxybutyrate AUC = 0.93 (0.81-1.0)Taurine AUC = 0.95 (0.83-1.0) |
| Arda Düz, 2022 (Arda Düz, Mumcu et al. 2022)DiscoveryDiagnostic | Tissue | Case-controlStatistical validation (100 times permutation) | Nontargeted1H HR-MAS | N = 18Patients with benign diseasesAge: 49 ± 7.1 y(cystadenoma, prolapse) hysterectomy performed, histopathological normal endometriumNo HRTMatched in terms of age, BMI, menopausal status | N = 17EC patientsAge: 53.5 ± 7.9 yNo HRTMatched in terms of age, BMI, menopausal statusNo data about Grade, FIGO stage | EC/CPLS-DA models(VIP>1, p< 0.05)🡩lactate, Ala, choline/glycerophosphocholine/phosphocholine/O-phosphoetanolamine, Glu/Gln/Met, taurine, Glu, Leu/Ile, O-phosphoethanolamine, Ile ,Val, Gln, N-acetyltyrosine/Arg, Arg/LeuAUC for individual metabolites/combinations > 0.85Lactat: AUC= 0.88Glu/Gln/Met: AUC= 0.87Ala: AUC= 0.86Phe: AUC= 0.85Leu/Ile: AUC= 0.85 |
| Gatius, 2022 (Gatius, Jove et al. 2022)DiscoveryPrognosis | Tissue from Biobank | Cases onlytraining | Non-targeted LC-MS/MSLC-ESI-Q-TOF MS/MS |  | N = 31EEC = 20Grade 1: 6Grade 2:12,Grade 3: 2SEC = 11Grade 3 =11Age: 39-86 y (3 not available)RecurrenceEEC: 2Serous EC: 6 | PLS-DA model (VIP > 1, p< 0.05)EEC/serous EC: 232 different metabolitesmetabolites: 🡫 in serous EC:LysoPG 18:1, SM d43:2, SM d44:2, eicosadienoic acid, PA 48:0, tetracosatetraenoic acid, 3-hydroxypristanic acidMetabolites 🡩 in serous EC: adenosine-monophosphate, 2'-deoxyguanosine-5'-monophospate |
| Different samples |  |  |  |  |
| Shafiee, 2020 (Shafiee, Ortori et al. 2020)DiscoveryDiagnostic | Plasma andtissue samples | Cros-sectional studyPCOS based on Rotterdam criteria.Training only | Non-targetedLC-HRMS | N=68PCOS: 34 patientsSecretory: 3Proliferative: 23Unknown: 831.8± 6.0 yBMI: 29.3± 2.9 Control patients (uterine fibroids, benign ovarian cysts, tubal ligation): 34Premenopausal: 20Perimenopausal: 1443.7± 13.1 yBMI: 28.6± 2.6 similar WHR no HT | N=34Premenopausal: 4Perimenopausal: 13Postmenopausal: 17Moderately differentiated: 44.1%Poorly differentiated: 29.4%Well differentiated: 26.5%63.4±10.1 yBMI: 32.2± 5.7  | PCOS/C and EC/Cno metabolite changed in blood samplestissue samples:🡫 monoacylglycerol 24:0 and capric acidEC/C tissue samples🡩 hydroxyundecanoyl carnitine, phoshorylcholine, diglyceride (26:4e). phosphatidylcholine (PC) 36:6; phosphatidylethanolamine (PE) 38:2; ceramide d29:2; PE 36:6e, diglyceride 36:6, ceramide d34:O, phosphatidylglycerol 36:2, acylcarnitine 17:0, monoacylglycerol (MAG) 18:2, PC 16:1e🡫triglyceride 33:0; MAG 24:0, hexacosanoic acid, diacyglycerol 36:4, MAG 24:1, MAG 22:0, sterol (C27H48O5); MAG 22:4; oxotestosterone; triglyceride 28:0; MAG 22:2, MAG 24:4, DHT sulfate, triglyceride 24:0, capric acid |
| Yi, 2022 (Yi, Xie et al. 2022)DiscoveryDiagnostic | TissueUrineintrauterine brushing samples | Case-controlMonte CarloCross Validation | NontargetedESI Q-TOF MC combined with LC-20A HPLC | N=43Control patients (hysteromyoma, cyst, endometrial polyps and cervix diseases)Tissue samples: 1839.8 ± 5.3 yBMI: 23.0± 2.5 All premenopausalUrine samples: 1044.3± 9.1 yBMI; 22.4 ± 2.9 Postmenopausal: 25%Intrauterine brushing samples: 1148.2± 5.7 yBMI: 22.6± 3.0 Postmenopausal: 18% | N=44 (all Type 1 EC)Tissue samples: 2448.2± 13yearsBMI: 24.8 ± 3.9 kg/m2Postmenopausal: 33.3 %Grade 1: 24Stage Ia: 22Stage II: 1Stage IIIa: 1Urine samples: 1044.0± 11.6 yBMI. 27.1 ± 5.9 Postmenopausal: 30%Grade 1: 9Grade 2: 1Stage Ia: 10Intrauterine brushing samples: 1045.8± 9.9 yBMI: 24.8± 3.6 Postmenopausal: 20%Grade 1: 9Grade 2: 1Stage Ia: 10 | PLS-DA models (VIP > 1, p< 0.05)EC/Ctissue 74 metabolitesurine 285 metabolitesintrauterine brushing 122 metabolitesUrine (top 100 metabolites)N-acetylaspartylglutamate, pseudouridine, adenosylmethionine, xanthine, His, hydroxykynurenamineAUC = 0.81 (0.46-1.0) (100), 0.781 (0.40-1.0) (50)Intrauterine brushing (100 metabolites)Uridine, Ala, homoserine, Gln, N-acetylputrescine, formylkynurenineAUC = 0.85 (0.58-1.09 (100), 0.82(0.52-1.0) (50)Comparison tissue/urine/brushingTissue: 74 metabolites: 🡩 47, 🡫27Urine: 49/74 metabolites: 🡩 6, 🡫43Intrauterine brushing: 21/74 metabolites: 🡩 9, 🡫12 |

(1) BMI: body mass index - kg/m2.

**Abbreviations:** ANN, artificial neural network; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; E1, estrone; E2, estradiol; E3, estriol; E1-S, estrone-sulfate; ESI-MS/MS, electrospray ionisation tandem mass spectrometry; G, grade; HRT, hormone replacement therapy; IDL, intermediate-density lipoproteins; LDL, low density lipoproteins; L-LDL, large LDL; LVSI, lymphovascular space invasion; MeO, methoxy; FW time: follow up time; MD, missing data; MI, myometrial invasion; NA, not available; ND, not determined; OPLS-DA, orthogonal partial least squares discriminant analysis; OC, oral contraception; OR, odds ratio; PCA, Principal Component Analysis; PCae, PCaa, glycerophospholipids; P, proliferative phase; PC, phosphatidylcholine, PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PLS-DA, Partial Least Squares Discriminant Analysis; RF, random forest; S, secretory phase; SEN, sensitivity; SM, sphingomyelin; SMOH, hydroxysphingomyelin; SP, specificity; SVM; support vector machine; TG, triglycerides; VIP, variable importance in projection; QTOF, quadrupole time of flight; y, years.

(10X-Genomics® http://www.10xgenomics.com/technology/)

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