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Translational research for better diagnosis and treatment of endometrial cancer

Edited by
Andrea Romano, Janina Tokarz, Dmytro Fishman, Andrzej Semczuk and Tea Lanisnik Rizner

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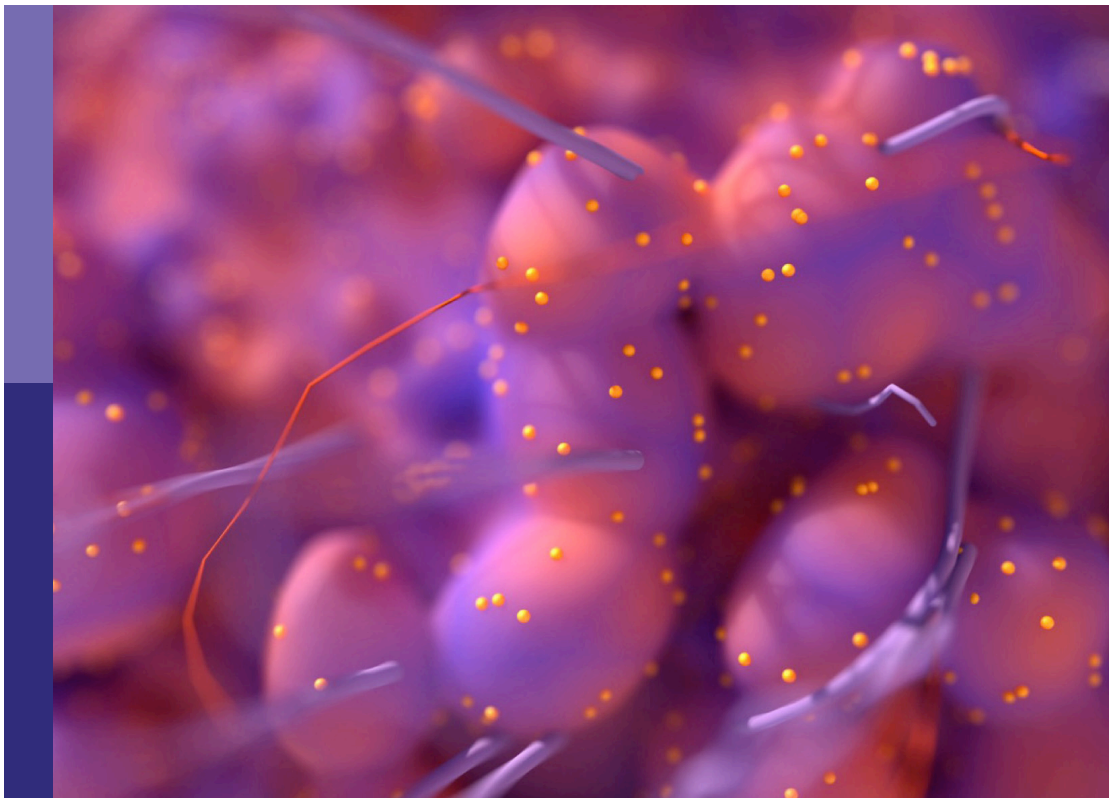

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Translational research for better diagnosis and treatment of endometrial cancer

Topic editors

Andrea Romano — Maastricht University, Netherlands
Janina Tokarz — Institute of Diabetes and Cancer, Helmholtz Center München, Helmholtz Association of German Research Centres (HZ), Germany
Dmytro Fishman — University of Tartu, Estonia
Andrzej Semczuk — Medical University of Lublin, Poland
Tea Lanisnik Rizner — University of Ljubljana, Slovenia

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EDITED AND REVIEWED BY
Sophia George,
University of Miami, United States

*CORRESPONDENCE
Andrea Romano
a.romano@maastrichtuniversity.nl
Tea Lanišnik Rižner
tea.lanisnik-rizner@mf.uni-lj.si

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Editorial: Translational research for better diagnosis and treatment of endometrial cancer

Andrea Romano[1,2*], Andrzej Semczuk[3], Janina Tokarz[4,5] and Tea Lanišnik Rižner[6*]

[1]Department of Gynaecology, Maastricht University Medical Centre (MUMC), Maastricht, Maastricht, Netherlands, [2]GROW – School for Oncology and Reproduction, Maastricht University, Maastricht, Netherlands, [3]Department of Gynaecology, Lublin Medical University, Lublin, Poland, [4]Helmholtz Zentrum München, German Research Center for Environmental Health, Institute for Diabetes and Cancer, Neuherberg, Germany, [5]German Center for Diabetes Research (DZD), Neuherberg, Germany, [6]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

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Editorial on the Research Topic
Translational research for better diagnosis and treatment of endometrial cancer

Endometrial cancer (EC) is the most frequent gynaecological malignancy in developed countries and represents a clinical challenge, especially in terms of early diagnosis and risk stratification of patients. Early diagnosis is fundamental to ensure a good prognosis, long survival and good quality of life, whereas an accurate (and ideally) pre-operative stratification of patients based on risk of recurrence is a prerequisite to appropriately decide on the extent of surgery and on the adjuvant care.

Currently, both these aspects are not optimal. An invasive endometrial histology is the gold standard for diagnosis, and there are no valid non-invasive methods; also, patient stratification is based on histopathology and surgical findings. To tackle these limitations and to develop non-invasive diagnostic/prognostic tools, the BioEndoCar project was launched in 2018 (funded by EU framework programme Horizon2020). Six European partners and five collaborating centres joined forces to prospectively collect blood specimens from patients and controls, to perform metabolomics and proteomics analyses in plasma samples in search for minimally invasive diagnostic and prognostic biomarkers, and to model the data for the development of prediction algorithms. The BioEndoCar project concluded with a two-day international symposium that was held in Portorož (Slovenia, March 2022) focussing on state-of-the-art *omics* technologies, biobanking, translational research and clinical management in the context of EC.

This symposium was the birth of the Research Topic for Frontiers on Oncology aiming to transfer the current challenges and discussions on EC into a dedicated collection of articles. The Research Topic focussed on translational research to improve diagnosis/prognosis and treatment of EC. We collected high quality Original Research articles, Systematic Reviews and Narrative Reviews describing different aspects of translational research, with particular emphasis on *omics* profiling and multi-*omics* in tissues and physiological fluids. Over 120 authors from China, Europe (Czech Republic, Germany,

Poland, Slovenia, The Netherlands), India, Switzerland, UK and United States contributed to the Topic with 15 papers.

In the present entitled '*Translational research for better diagnosis and treatment of endometrial cancer*', we coherently organised these 15 papers in four sections: 1. Pathogenesis, classification and treatment; 2. Comorbidities; 3. Diagnostic/prognostic models based on histological and blood biomarkers; and 4. Diagnostic/prognostic models based on imaging data.

The first Section (Pathogenesis, classification and treatment) consists of five contributions (one review and four original papers). The review by Zhang et al. summarises the expression, regulation, and functions of glucose transporters (GLUTs) in human EC. The authors review the upstream regulators of GLUTs, and briefly discuss their functions in tumour growth and invasion. The impact of GLUTs in the context of treatments and ongoing clinical trials is also discussed. The authors conclude that GLUTs overexpression may be implicated in insensitiveness to hormone therapy or resistance to chemoradiotherapy.

The original articles focus on intracellular signalling and tumour immune microenvironment. Ledinek et al. analyse the possible interconnection between Wnt signalling and epithelial-to-mesenchymal-transition (EMT) among 64 EC specimens. Markers of Wnt signalling and EMT correlate significantly with hormone receptor status, although no further correlation was found with clinic-pathological features or integrated molecular subgroups. The authors conclude that the correlation between hormone receptors, Wnt signalling and EMT confirms the intimacy between these pathways in EC. Dai et al. explore the immune cell infiltration in tumour samples from a small cohort of EC patients that were classified into four molecular subtypes (according to transPORTEC). The profiles of infiltrating immune cells differed between tumours with distinct molecular subtypes, implying distinct immune reactions (normal responses, absence or suppressed responses), and potentially explaining the differences in prognosis and therapy efficiency among different EC cancer subtypes.

The last two papers describe less-common forms of EC, specifically clear cell and serous carcinoma. In the first study, Cui et al. develop nomograms to predict overall survival (OS) at 3-, 5-, and 10-year after diagnosis using a retrospective cohort of 1778 cases. Age at diagnosis, marital status, stage, tumour size and surgery were independent predictors for OS among women with FIGO stage I/II. Age at diagnosis, stage, lymph node involvement, distant metastasis, tumour size, surgery, radio- and chemo-therapy were all independent OS predictors for FIGO stage III/IV. The authors conclude that the predictive models they built may be valuable tools in clinical practice.

The second study focus on serous carcinoma, an aggressive subtype of endometrial carcinoma. Alessandrino et al. examined associations between genomics and metastatic patterns in 67 patients (including Hispanic and black subjects) and observe lower overall survival in patients with presence or recurrence of metastases to the liver and *AKR1D1A* mutations. This study underscores the importance of genomic studies for individualised treatment of these patients.

Section 2 focuses on comorbidities associated with EC and features one review on the impact of adipose tissue and one original paper on the impact of type 2 diabetes on EC. The systematic review of van den Bosch et al. explores the association between patient characteristics and the distribution of adipose tissue. Eleven retrospective studies are included and indicate that the distribution of adipose tissue (visceral versus subcutaneous) significantly correlates with obesity, cancer histology, metastasis, sex steroid levels and survival. The work by Njoku et al. aims to investigate whether pre-existing diabetes can affect survival outcomes in patients with EC. The authors included over 500 subjects and demonstrated that pre-existing type 2 diabetes confers an increased risk of death among EC patients.

Section 3 features studies (one review and three original contributions) on diagnostic or prognostic models that included histological and/or blood biomarkers. The systematic review by Romano et al. describes the current state-of-the-art in diagnostic and prognostic biomarkers for EC. The review provides a brief description of technological and data analyses aspects, and continues by describing all studies that used proteomics and/or metabolomics for diagnostic and prognostic biomarker discovery. Vinklerová et al. address in their study the problem of preoperative risk stratification. The authors validate a previously developed Bayesian network model for preoperative risk stratification of EC patients (ENDORISK) developed within the ENITEC network (European Network of Individualized Treatment of Endometrial Cancer). In a cohort of 445 patients, ENDORISK, focusing on lymph node metastases and disease-specific survival, has good predictive value for low-risk but underestimates the risk among high-risk patients. This confirms that further improvements of the model are needed by including additional preoperative features (molecular classification, myometrial, cervical invasion, distant metastases, etc.) before its implementation in clinical practice. The original article by Roškar et al. includes 202 subjects (91 cases and 111 controls) and shows that plasma levels of leptin are significantly higher in patients with type 1 EC than in control patients, whereas IL-8 is higher in type 2 ECs versus control patients. The authors further develop a model based on age, IL-8, leptin, and the angiogenic factor G-CSF with good diagnostic accuracy. This section concludes with the study by He et al., who, through mining the TCGA database combined with *in vitro* investigations, explore the relation between KNL1 expression, patient prognosis and the effect on cell proliferation, invasion and metastatic potential. The authors conclude that KNL1 can be a prognostic and diagnostic biomarker in patients with EC.

Section 4 includes one review and three original papers where imaging techniques are used to develop diagnostic or prognostic models. The molecular classification of EC subordinates the histologic subtype to the molecular class. Fremond et al. suggest that Deep Learning (DL) could open a new door to refining the current EC classification by integrating histologic and molecular data. To date two studies have provided proof of principle for the prediction of molecular classes from H/E slide images by DL, albeit with relatively poor performance, that should improve with dataset size and quality and advances in DL technology. Automated DL

models could provide a cost-effective alternative, accelerate the diagnostic process and advance treatment. The study by Zhang et al. explores atypical endometrial hyperplasia (AEH), which is considered a direct precursor of EC, with concurrent EC diagnosed in approximately 40% of patients undergoing hysterectomy for AEH. The authors develop and annotate a multimodality MRI-based radiomic-clinical model to noninvasively distinguish EC from AEH. This model includes nulliparity status, endometrial thickness, and a combined radiomicroscopic signature with excellent performance. Further validation of this model in multicentre studies is needed, and the properties of the model can be further improved by combining it with genomic data. The diagnosis of EC relies currently on a combination of pre-operatively collected data such as age, BMI, blood-based tumour markers or imaging results, which are semi-structured or unstructured data. Feng et al. developed a clinical decision support system based on machine learning algorithms that include 16 features to assist physicians in classifying histology, stage, and grade of EC patients. The models showed different performances depending on the algorithm, and have highest accuracy if combined with a physician's judgement. Precise pre-operative EC tumour grade prediction is essential for risk stratification and treatment. Thus, Yue et al. use multiparametric magnetic resonance imaging (MRI) to determine radiomics features, which are the basis for calculating a radiomics score used to design a nomogram. The nomogram could improve the accuracy of recognizing a high-grade tumour prior to surgery in comparison to dilation and curettage and had a good net benefit according to decision curve analysis.

In conclusion, this e-book presents an up-to-date overview of the current diagnostic and prognostic tools that are under development in translational research and that hopefully will find their way to a clinical applicability in the near future. The editors hope that this e-book and the studies described herein will represent milestones in research and inspiration for all scientists and clinicians working in the field of EC to improve the care of women with this disease in the future.

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REVIEW
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Current and Emerging Prognostic Biomarkers in Endometrial Cancer

Kelechi Njoku[1,2,3], *Chloe E. Barr*[1,3] *and Emma J. Crosbie*[1,3]*

[1] Division of Cancer Sciences, University of Manchester, Manchester, United Kingdom, [2] Stoller Biomarker Discovery Centre, University of Manchester, Manchester, United Kingdom, [3] Department of Obstetrics and Gynaecology, St Mary's Hospital, Manchester, University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom

Endometrial cancer is the most common gynaecological malignancy in high income countries and its incidence is rising. Whilst most women with endometrial cancer are diagnosed with highly curable disease and have good outcomes, a significant minority present with adverse clinico-pathological characteristics that herald a poor prognosis. Prognostic biomarkers that reliably select those at greatest risk of disease recurrence and death can guide management strategies to ensure that patients receive appropriate evidence-based and personalised care. The Cancer Genome Atlas substantially advanced our understanding of the molecular diversity of endometrial cancer and informed the development of simplified, pragmatic and cost-effective classifiers with prognostic implications and potential for clinical translation. Several blood-based biomarkers including proteins, metabolites, circulating tumour cells, circulating tumour DNA and inflammatory parameters have also shown promise for endometrial cancer risk assessment. This review provides an update on the established and emerging prognostic biomarkers in endometrial cancer.

Keywords: endometrial cancer, prognosis, biomarkers, risk stratification, treatment

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Edited by:
Andrea Romano,
Maastricht University, Netherlands

Reviewed by:
Vit Weinberger,
University Hospital Brno, Czechia
Anna Myriam Perrone,
Sant'Orsola-Malpighi Polyclinic, Italy

***Correspondence:**
Emma J. Crosbie
emma.crosbie@manchester.ac.uk

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INTRODUCTION

Endometrial cancer is the sixth most frequently diagnosed cancer in females and the gynaecological malignancy with the greatest incidence in high-income countries. In 2020, there were an estimated 417,000 incident cases and 97,000 deaths from the disease worldwide (1). The incidence of endometrial cancer is rising alongside the growing obesity epidemic (2). In the United Kingdom (UK), there are around 9,700 cases and 2,400 endometrial cancer-associated deaths every year (3). Over the last decade, deaths have increased by 25%, a trend that has been reported in other high income countries. It is projected that mortality rates for endometrial cancer will rise by a further 19% in the UK between 2014 and 2035, despite improvements in overall survival (3).

Most endometrial cancers are sporadic, with an estimated 5% occurring in the context of a hereditary predisposition, most commonly Lynch syndrome (4). Lynch syndrome is an autosomal dominant condition that arises from a defect in the DNA mismatch repair (MMR) system, predisposing to a constellation of malignancies, including endometrial cancer (5). There are currently no evidence–based screening options for endometrial cancer in either the general population or in high-risk women (6). Most women are diagnosed following routine investigations for post-menopausal bleeding, the cardinal symptom of the disease. In current

clinical practice, symptomatic women are investigated by sequential tests that include transvaginal ultrasound scan, endometrial biopsy and hysteroscopy (7). Most women with endometrial cancer are diagnosed at an early stage and have highly curable disease, reflected in excellent 5-year survival rates (3). A significant minority present with adverse clinico-pathological characteristics including biologically aggressive endometrial cancer phenotypes, and have a poor prognosis. The management of endometrial cancer is primarily surgery, with total hysterectomy and bilateral salpingo-oophorectomy as standard of care worldwide. Women with high-risk features are offered adjuvant therapy with chemotherapy and/or radiotherapy, aimed at reducing risk of recurrence (8). A significant minority are managed conservatively including those of reproductive age or those for whom surgery carries considerable risk such as the frail or medically unfit (7).

Identifying those with endometrial cancer at highest risk of recurrence and cancer-related death is important to ensure women receive appropriate evidence-based care whilst avoiding the harms and costs of unnecessary treatments for those at lowest risk. Clinical, sociodemographic, histopathological and molecular factors all impact on endometrial cancer outcomes (9). A validated risk-stratification model that accurately defines risk of disease recurrence and death will guide clinical care by allowing for treatment de-escalation for those at lowest risk and intensification for those at high risk (10). Such a model may also help define the optimal follow-up programme for recurrence and guide decisions regarding alternative primary treatments for the fraction of women who are managed conservatively. This review provides an update of the current and emerging prognostic biomarkers and risk-stratification algorithms in endometrial cancer. Further, we highlight the challenges in clinical translation and offer fresh perspectives on endometrial cancer biomarker research.

CURRENT ENDOMETRIAL CANCER PROGNOSTIC BIOMARKERS

What Are Prognostic Biomarkers?

Prognostic biomarkers are clinical or biological characteristics that can be objectively assessed and evaluated to predict the course of a disease regardless of therapy (11). Prognostic biomarkers are used in clinical practice to identify the likelihood of a clinical event (mortality, disease recurrence or progression) occurring amongst those with the condition of interest (12, 13). Examples of prognostic biomarkers include clinical, tumour specific molecular and histopathological characteristics.

Bokhman Dualistic Model of Endometrial Cancer

In 1983, Bokhman proposed a dualistic model of endometrial cancer based on clinical, epidemiologic and prognostic features (14). Type I tumours are by far the most common and are low-grade, oestrogen driven tumours that are associated with obesity and have a favourable prognosis. By contrast, type II tumours are relatively rare, high-grade, biologically aggressive tumours that are more common in healthy weight women and act independently of oestrogen (14). This model was of value several decades ago but has been shown to lack sufficient discriminatory ability to justify its continued use in the classification and management of endometrial cancers today (15). For example, ~20% of women with type I endometrial cancer experience a relapse while ~50% of those with type II do not, suggesting that the precision with which this dualistic model guides receipt of adjuvant therapy is moderate at best (16).

Histopathological Biomarkers and Current Risk Stratification Algorithms

Histological subtype, FIGO stage, disease grade, presence of lympho-vascular space invasion (LVSI) and deep myometrial invasion are established prognostic biomarkers in endometrial cancer (17) (**Figure 1**). The histological subtypes of endometrial cancer include endometrioid tumours, which have a favourable prognosis, and non-endometrioid tumours (serous, clear cell, carcinosarcomas and mixed), which are biologically aggressive and associated with poor outcomes. Endometrioid tumours make up over 80% of newly diagnosed endometrial cancers, while serous, clear cell and carcinosarcomas make up 10%, 3% and <2% respectively (18, 19). Low grade endometrioid tumours are type I and high grade endometrioid and non-endometrioid histological subtypes are type II tumours. The mutational profiles of the different histological subtypes vary. *PTEN* mutations portend a favourable prognosis are more common in endometrioid endometrial cancers, while *TP53* mutations are associated with a poor prognosis and are common in serous tumours (20). Surgical staging provides important prognostic information in the management of endometrial cancer and is based on the 2009 International Federation of Gynecology and Obstetrics (FIGO) staging system (21) (**Table 1**). Women with early stage (FIGO I/II) endometrial cancer have a favourable prognosis compared to those with advanced disease (FIGO III/IV). The 5-year survival rate is >90% in early stage disease and <20% in late stage disease (17, 21). Disease grade is also an important prognostic parameter (22). Studies have been consistent in suggesting a correlation between tumour grade and depth of myometrial invasion, presence of extra-uterine disease and lymph node metastasis (23). Depth of myometrial invasion is a component of FIGO staging for stage I tumours and is an independent predictor of endometrial cancer outcomes across all stages. A recent meta-analysis of 79 studies involving 68,870 women concluded that deep myometrial invasion is associated with high endometrial cancer recurrence risk and poor outcomes (24). LVSI is also an important prognostic parameter, being linked to an increased risk of nodal spread, disease recurrence and poor outcomes (25, 26).

Current endometrial cancer risk stratification is based on a consensus algorithm by the three major endometrial cancer consortiums: European Society for Medical Oncology, European Society of Gynaecological Oncology, and European Society for radiotherapy & Oncology (ESMO, ESTRO and ESGO) (8). This was recently updated by ESGO, ESTRO and the European Society

TABLE 1 | FIGO staging of endometrial cancer (21).

FIGO Staging	Carcinoma of the endometrium
Stage I	Tumour confined to the uterus
IA	No or <50% myometrial invasion
1B	≥50% myometrial invasion
Stage II	Cervical stromal invasion, but not beyond the uterus
Stage III	Local and/or regional tumour spread
IIIA	Tumour invades serosa and/or adnexa
IIIB	Vaginal and/or parametrial involvement
IIIC	Metastases to pelvic and/or para-aortic lymph nodes
IIIC1	Pelvic node involvement
IIIC2	Para-aortic lymph node involvement ± positive pelvic lymph nodes
Stage IV	Tumour invades bladder and/or bowel, and/or distant metastases
IVA	Tumour invasion of bladder and/bowel mucosa
IVB	Distant metastases including abdominal metastases and/inguinal nodes

Adapted based on the 2009 revised staging by the FIGO Committee on Gynecologic Oncology.

of Pathology (ESP) to also include prognostic risk groups where endometrial cancer molecular classification information(described in detail in section 3.0) is known (27). Women are classed as low, intermediate, high-intermediate, high -risk and advanced metastatic based on histological subtype, FIGO stage, and grade, depth of myometrial invasion, presence of LVSI and molecular grouping (27) (**Table 2**). The classification system based on histopathological parameters is used to guide receipt of adjuvant treatment but has been shown to have sub-optimal ability in defining endometrial cancer outcomes (9, 28). Histological subtype and grade have poor reproducibility even amongst expert pathologists, while FIGO stage and LVSI are only available post-hysterectomy, and thus cannot inform decisions regarding surgical management (29–31). A pathology review of patients with high-risk endometrial cancer as part of the PORTEC-3 trial found significant disagreement in the assignment of several risk defining parameters including histological subtype, grade, cervical stromal invasion, LVSI and depth of myometrial invasion (32). It is therefore not surprising that the currently used risk-stratification algorithm leads to imprecise estimation of the risk of recurrence and death in women with endometrial cancer (33). Furthermore, a small minority of women with endometrial cancer are managed conservatively for fertility-sparing and surgical fitness reasons, and so cannot be surgically staged. Imaging with MRI +/-CT are limited in their ability to define risk stratifiers. Novel prognostic biomarkers that guide decisions regarding the type and suitability of alternative primary treatments in this group of women has the potential to transform patient care.

EMERGING ENDOMETRIAL CANCER PROGNOSTIC BIOMARKERS

TCGA Endometrial Cancer Molecular Classification

Molecular subtyping offers a more objective and reproducible classification of endometrial cancer when compared with histopathological evaluation and has the potential to revolutionise patient care (33). Recently, the TCGA proposed four distinct endometrial cancer molecular subgroups based on mutational burden, microsatellite instability and copy number alterations observed in 373 endometrial cancer cases: copy number high, copy number low, MSI hypermutated, and *POLE* ultra-mutated (34) (**Table 3**). This classification has been validated in subsequent studies and shown to have prognostic and therapeutic implications (29, 38–41).

The copy number high (serous-like) cancers have the worst progression-free survival and are characterised by widespread genomic alterations with extensive copy number aberrations (34, 35). Patients in this subgroup have mostly high-grade and

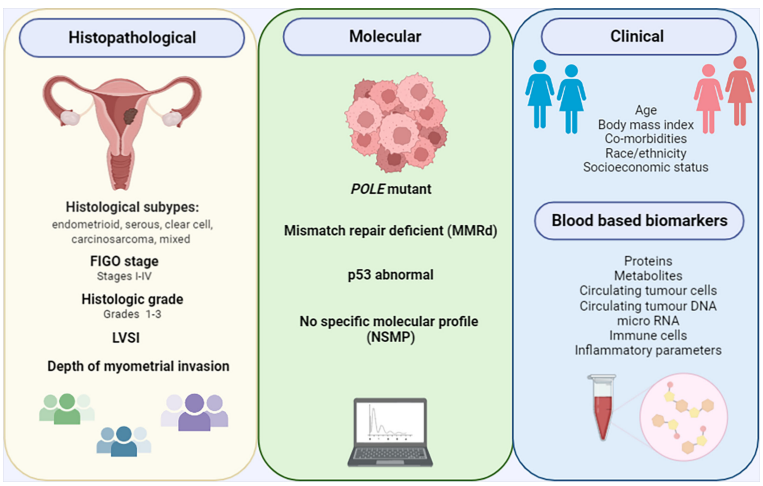

FIGURE 1 | Current and emerging endometrial cancer prognostic biomarkers.

TABLE 2 | Updated ESMO, ESTRO and ESGO endometrial cancer risk stratification algorithm (27).

Risk group	Molecular classification unknown	Molecular classification known
Low	• Stage IA endometrioid + low-grade + LVSI negative or focal	• Stage I–II ***POLE***-mutant endometrial carcinoma, no residual disease • Stage IA **MMRd/NSMP** endometrioid carcinoma + low-grade + LVSI negative or focal
Intermediate	• Stage IB endometrioid + low-grade + LVSI negative or focal • Stage IA endometrioid + high-grade + LVSI negative or focal • Stage IA non-endometrioid (serous, clear cell, undifferentiated carcinoma, carcinosarcoma, mixed) without myometrial invasion	• Stage IB **MMRd/NSMP** endometrioid carcinoma + low-grade + LVSI negative or focal • Stage IA **MMRd/NSMP** endometrioid carcinoma + high-grade + LVSI negative or focal • Stage IA **p53abn** and/or non-endometrioid (serous, clear cell, undifferentiated carcinoma, carcinosarcoma, mixed) without myometrial invasion
High-intermediate	• Stage I endometrioid + substantial LVSI regardless of grade and depth of invasion • Stage IB endometrioid high-grade regardless of LVSI status • Stage II	• Stage I **MMRd/NSMP** endometrioid carcinoma + substantial LVSI regardless of grade and depth of invasion • Stage IB **MMRd/NSMP** endometrioid carcinoma high-grade regardless of LVSI status • Stage II **MMRd/NSMP** endometrioid carcinoma
High	• Stage III–IVA with no residual disease • Stage I–IVA non-endometrioid (serous, clear cell, undifferentiated carcinoma, carcinosarcoma, mixed) with myometrial invasion, and with no residual disease	• Stage III–IVA **MMRd/NSMP** endometrioid carcinoma with no residual disease • Stage I–IVA **p53abn** endometrial carcinoma with myometrial invasion, with no residual disease • Stage I–IVA **NSMP/MMRd** serous, undifferentiated carcinoma, carcinosarcoma with myometrial invasion, with no residual disease
Advanced metastatic	• Stage III–IVA with residual disease • Stage IVB	• Stage III–IVA with residual disease of any molecular type • Stage IVB of any molecular type

Focal LVSI refers to the presence of a single focus around the tumour. Key: p53abn, p53-abnormal; MMRd, MMR-deficient; NSMP, no specific molecular profile.

biologically aggressive tumours including serous endometrial cancers and 25% of the grade 3 endometrioid tumours (34, 35). Mutations commonly observed in copy number high tumours include those in *TP53* and *PIK3CA*. Other mutations involving *FBXW7* and *PPP2RIA* are unique to copy number high tumours (34). Amplifications of *CCNE1* and *ERBB2* are also commonly observed (42, 43).

Copy number low endometrial cancers have few copy number aberrations and no increased mutation burden.They comprise low grade, microsatellite stable, endometrioid tumours (34, 35). Whilst tumours in this subgroup generally have a favourable prognosis, they have specific unique molecular features that are associated with poor prognosis, namely *CTNNB1* mutations and amplification of chromosome arm1q, thus making the group an interesting one for future stratified clinical trials (44, 45).

Microsatellite instable endometrial cancers have mismatch repair deficiency (MMR-d), high mutation rates and few copy number aberrations (34). They are characterised by mutations or epigenetic silencing affecting the MMR genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Other commonly mutated genes in this subgroup include *PTEN*, *ARIDIA*, *PIK3CA*, *PIK3RI*, and *RPL22* (34, 35). These tumours are usually endometrioid although their histological morphology can be unusual, making characterisation challenging (35).

The final subgroup of the TCGA classification is the *POLE* ultra-mutated group. This subgroup is characterised by high mutation rates and hotspot mutations in the *POLE* exonuclease domain (EDM) of polymerase-ε (34). *POLE* ultra-mutated tumours exhibit few copy number aberrations and have mutations in *PTEN, PIK3RI, PIK3CA, FBXW7* and *KRAS* genes. These tumours have an excellent prognosis with the best progression free survival (46). They are characterised by dense immune cell infiltrates. Whilst previously thought not to recur, there is emerging evidence that the *POLE* tumours can recur but at a much lower rate compared to other molecular subtypes (35, 46). The recent proteogenomic characterisation of endometrial cancer by the National Cancer Institute's Clinical Proteomic Tumour Analysis Consortium (CPTAC) provides further insights into the proteomic markers of endometrial cancer clinical and genomic tumour subgroups (47).

Whilst the TCGA classification substantially advanced our understanding of the molecular diversity of endometrial cancer and the associated prognostic implications, its clinical applicability in terms of refining surgical staging, guiding decisions about adjuvant therapy and intensity of post-treatment surveillance is limited (35). Barriers include the need for fresh-frozen tumour specimens, high costs and technical and methodological complexities.

Simplified and Pragmatic Endometrial Cancer Molecular Classifiers

Novel molecular classification tools have been developed and validated based on the use of surrogate markers to define four distinct subgroups of endometrial cancer that are analogous but not identical to the TGCA classification (40). The classifiers include the TransPORTEC (48) and ProMisE models (49).These novel classifiers utilise immunohistochemistry to identify MMR and p53 abnormalities and targeted sequencing to identify *POLE* mutations (40, 48). In contrast to the fresh-frozen tumour specimens required for TCGA classification, these pragmatic classifiers can be used on formalin-fixed, paraffin-embedded tumour materials, thus enhancing their clinical utility (29). There is good evidence to support their potential applicability to endometrial biopsy and curettage diagnostic specimens (50–

52) and the inter laboratory concordance is high (51). Studies have been consistent in confirming the prognostic value and potential clinical utility of these classifiers across unselected patient populations (53–56).

In the TransPORTEC initiative, the four molecular subgroups are p53-abnormal, MSI-high, *POLE*-mutant and those with no specific molecular profile (NSMP) (48) (**Figure 2A**). Of 116 high-risk endometrial cancer specimens analyzed by the TransPORTEC group, p53-abnormal (n=36) and NSMP (n=44) subgroups had significantly higher rates of distant metastases and lower 5-year relapse free survival than MSI-high (n=19) and *POLE*-mutant (n=14) tumours (48) (**Table 4**). The 5-year recurrence-free survival rates were 93% and 95% for the *POLE*-mutant and MSI-high subgroups respectively, compared with 42% (p53-abnormal) and 52% (NSMP) (48). A refined version of the TransPORTEC classifier has since been developed that incorporates the presence of LVSI and other molecular parameters such as L1CAM expression and the presence of *CTNNB1* mutation (57). This model is being prospectively tested in a cohort of women with high-to-intermediate risk endometrial cancer as part of the PORTEC-4a trial.

ProMisE stratifies women with endometrial cancer based on sequential molecular testing for aberrations in the order of MMR-D, *POLE* mutation and p53 status (**Figure 2B**). The four molecular groupings based on ProMisE are MMR-deficient (MMRd; analogous to MSI-high subgroup), *POLE EDM* (analogous to *POLE* ultramutated), p53-abnormal (p53 abn, analogous to the copy number high group) and p53-wild type (p53 wt, analogous to the copy number low group) (40, 49). These molecular subgroupings have also been shown to correlate with disease-free and overall survival even after adjusting for known risk parameters (35, 49). Women in the p53 abn group have the worst prognosis with a 3- to -5 fold higher risk of mortality or progressive/recurrent disease than the p53 wt group, and a 2-fold higher risk following adjustment for clinico-pathological parameters (35, 49). Those in the MMR-D subgroup have a 1.5 to 2-fold increase in mortality compared with the p53 wt subgroup; the survival benefit was non-significant following adjustment for confounding. The *POLE* EDM subgroup have the best prognosis and are least influenced by clinico-pathological features (35, 49).

Other Molecular Prognostic Parameters and Risk Algorithms

Other molecular parameters that are prognostic in endometrial cancer include overexpression of L1CAM and loss of oestrogen (ER) and/or progesterone receptors (PR), both of which are linked to a higher risk of recurrence and death (58–61). L1CAM expression strongly correlates with non-endometrioid histology, LVSI and lymph node metastasis (58). Loss of ER/PR expression is linked to high-grade disease, deep myometrial invasion and lymph node metastasis (62). DJ-1 protein distinguishes low-grade from high-grade endometrial cancer (63) while *CTNNBI* mutations have shown potential in identifying those low-grade, early stage, endometrial cancers at higher risk of recurrence and death (44).

A number of risk-prediction models, incorporating clinical, histological and molecular parameters, have been developed to aid prediction of survival outcomes in endometrial cancer. ENDORISK, a validated risk algorithm based on four pre-operative molecular markers, namely L1CAM, PR, ER, and p53 status, predicted risk of lymph node metastasis and survival in a multi-centric cohort of 763 women with endometrial cancer

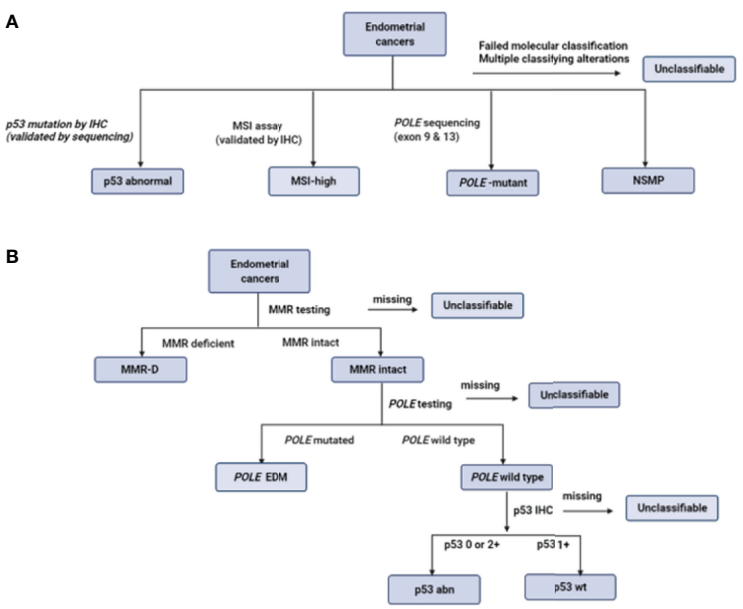

FIGURE 2 | Defining the molecular subgroups of endometrial cancer based on the TransPORTEC classifier **(A)** and ProMisE **(B)**. Adapted from (48, 49, 51).

TABLE 3 | Characteristics of the TCGA molecular classification of endometrial cancer.

Type	*POLE* (ultramutated)	MSI (hypermutated)	Copy number low (endometrioid)	Copy number high (serous like)
Prevalence	7%	28%	39%	26%
Mutation frequency	Very high (>100 mutations/Mb)	High 100-10 mutations/Mb	Low <10 mutations/Mb	Low <10 mutations/Mb
Commonly mutated genes	*POLE* (100%), *PTEN* (94%)	*PTEN* (88%) *PIK3CA* (54%)	*PTEN* (77%) *CTNNB (52%)*	*TP53* (92%) *PIK3CA (47%)*
Copy number aberrations	Very low	Low	Low	High
MSI/*MLH1* methylation	Mixed high and low MSI, stable	High MSI (*MLH1*, *PMS2*, *MSH2*, and/or *MSH6* deficiency)	MSI stable	MSI stable
Histological subtype	Endometrioid	Mostly endometrioid	Endometrioid	Serous, 25% high-grade endometrioid and mixed
Grade	G1-3	G1-3	G1-2	G3
Other features	Ambiguous histo-morphology Dense immune infiltrates	Display tumour-infiltrating lymphocytes	*CTNNB* mutations are associated with poor prognosis Subgroup with amplification of chromosome arm 1q has poor prognosis	Similar to high-grade serous ovarian carcinoma L1 cell adhesion molecule (L1CAM) expression associated with poor prognosis
Prognosis	Good	moderate	moderate	Poor

Adapted from (35–37).

across Europe, and 2 independent cohorts from the Netherlands and Norway (64). In a similar study, a model incorporating L1CAM, PR, ER and p53 status demonstrated a 48% sensitivity and 89% specificity for high-risk endometrial cancer (65). Ravegnini and colleagues found better stratification of NSMP patients with *CTNNB1* mutation alongside miR-499a-5p status (66).

Therapeutic Implications and Additional Benefits of the Molecular Classification of Endometrial Cancer

The molecular classification of endometrial cancer has prognostic and therapeutic implications. The p53-abnormal endometrial cancers are the most biologically aggressive and would ideally be managed with complete/aggressive surgical treatment. These tumours generally require adjuvant treatment. A retrospective molecular analysis of the PORTEC-3 trial for high-risk endometrial cancer confirmed that women with p53-abnormal endometrial cancer had significantly improved recurrence-free survival when platinum-based chemotherapy was used alongside radiation, compared with radiation alone (67). This survival benefit was not observed in the other molecular categories, although the PORTEC-3 trial was not originally powered for these subgroup analyses (67). The finding of several molecular similarities between the TCGA p53 endometrial cancer group and both high grade serous tubo-ovarian cancer (HGSOC) and basal-like breast cancer, has sparked interest in the potential for therapeutics that target homologous recombination in these tumours (33, 34). A number of clinical trials assessing the efficacy of PARP inhibitors alone or in combination with anti-angiogenics/immune checkpoint inhibitors for recurrent or metastatic endometrial cancer are under way (68). The TransPORTEC Refining Adjuvant treatment IN endometrial cancer Based On molecular features (RAINBO) suite of clinical trials is evaluating the role of adjuvant chemo-radiation with or without a DNA

TABLE 4 | Prognostic performance of ProMisE and TransPORTEC classifiers, adapted from (49) and (48), respectively.

Subgroups	N (%)	Overall survival		Disease specific survival		Progression free survival	
ProMisE		**HR(95%CI)**	**LRT p**	**HR(95%CI)**	**LRT p**	**HR(95%CI)**	**LRT p**
p53 wt	139 (45.6%)	Comparator group					
MMR-D	64 (20.1%)	1.90 (0.88-4.04)	0.0211	1.32 (0.51-3.35)	0.0156	0.64 (0.25-1.60)	0.011
POLE EDM	30 (9.4%)	1.01(0.26-2.99)		0.42 (0.04-1.88)		0.19 (0.02-0.81)	
P53 abn	86 (27.0%)	2.61 (1.27-5.72)		2.28 (1.02-5.58)		1.75 (0.84-3.96)	
TransPORTEC		**5-year overall survival**		**Distant recurrence rates**		**5-year recurrence free survival**	
NSMP	44 (38%)	61%	<0.001	39%	<0.001	52%	<0.001
MSI-high	19 (16%)	63%		0%		95%	
POLE mutant	14 (12%)	93%		0%		93%	
p53 abnormal	39 (34%)	40%		50%		42%	

ProMisE data are based on multivariable analysis in a validation cohort of 319 cancers. Variables included in model are age, BMI, grade, histology, any treatment received. TransPORTEC data included 116 high risk endometrial cancer patients. HR, hazard ratio; LRP, likelihood ratio test.

damage response targeting agent in women with p53-abnormal endometrial cancer (39). Women with p53 wild type disease have lower metastatic potential and surgical treatment alone may suffice (69). Those with POLE mutant tumours have such a good prognosis that adjuvant treatment is unlikely to improve survival outcomes and de-escalation of therapy may be appropriate. The MMR-D molecular group is highly immunogenic, providing therapeutic opportunities for the use of immunotherapy. Marebella and colleagues, in the KEYNOTE-158 study reported an objective response rate of 57.1% in 49 endometrial cancer patients with previously treated unresectable or metastatic MMR-D disease who were treated with pembrolizumab (70). The GARNET trial, a phase 1b trial of anti-PD1 dostarlimab reported an objective response rate of 42.3% for women with recurrent or advanced MMR-D endometrial cancer that had progressed after treatment with platinum-based chemotherapy (71). Both pembrolizumab and dostarlimab have been FDA approved (71, 72).

The incorporation of endometrial cancer molecular testing into routine clinical care has several additional advantages. It will allow for the early identification of women with an inherited defect affecting one of the four MMR genes (Lynch syndrome) for whom cancer surveillance and aspirin chemoprevention may help to prevent future cancers, and cascade testing may identify other affected family members (5). For women of reproductive age who are considering non-surgical management, molecular classification of endometrial biopsy specimens can guide treatment decisions as p53 abnormal status would discourage a conservative approach to management (69).

BLOOD-BASED ENDOMETRIAL CANCER PROGNOSTIC BIOMARKERS

A blood-based prognostic biomarker has strong appeal to clinicians and patients alike. 'Can a blood test be used in predicting survivorship and/or recurrent disease?' ranked 5th most important research priority in the James Lind Alliance endometrial cancer priority setting partnership, representing the views of patients, clinicians, and members of the general public (73). A blood-based test that can accurately detect deep myometrial invasion and lymph node metastasis pre-operatively could inform surgical management. Such a test may also have utility in risk stratifying within endometrial cancer molecular groups, since women whose tumours fall within MMR-D or NSMP groupings have overlapping survival outcomes and adjuvant therapy may be beneficial for some but not all (74). Several blood-based biomarkers, including proteins, metabolites, circulating tumour cells, cell-free DNA, immune cells and inflammatory parameters have shown potential for refining endometrial cancer risk assessment. However, the evidence to enable clinical translation is limited.

The most commonly reported blood-based protein prognostic markers include cancer antigen 125 (CA125) and Human Epididymis protein 4 (HE4) (75, 76). Serum CA125 was first shown to be elevated in women with recurrent and advanced endometrial cancer by Niloff and colleagues in 1984 (77). Subsequent studies have been consistent in suggesting an association between serum CA125 concentration and adverse endometrial cancer clinico-pathological parameters and outcomes (78–82). Jiang and colleagues, in an analysis of 995 patients with endometrial cancer, found that elevated CA125 significantly correlated with lymph node metastasis, myometrial invasion, FIGO stage but not histological subtype, and was an independent prognostic factor (83). This study was limited by its retrospective design and selection bias, as almost 20% of endometrial cancer patients were excluded due to lack of pre-operative serum CA125 (83). There is good evidence of an association between serum HE4 levels and endometrial cancer outcomes. The meta-analysis by Dai and colleagues, involving 4235 patients, reported that elevated HE4 levels were significantly associated with worse overall, disease-free and progression-free survival (84). Serum HE4 has also been shown to correlate with adverse endometrial cancer histopathological parameters, although the evidence has been limited by marked heterogeneity across the various studies, small sample sizes and significant variation in the prognostic thresholds used (74). Several blood-based metabolites have also been linked to adverse endometrial cancer clinico-pathological factors and poor outcomes (13). As yet, none have been translated into routine clinical practice.

There is emerging evidence of a correlation between circulating cell-free tumour DNA levels and endometrial cancer prognosis (85–88). Cicchillitti and colleagues found elevated levels of cell-free DNA in grades 2 and 3 endometrial cancer compared to grade 1 disease (86). These findings align with the report by Vizza and colleagues of a significantly increased level of total cell-free DNA in high grade endometrial cancer (85). In addition, serum DNA integrity (the ratio between long and short cell free DNA fragments) was found to be higher in women with LVSI (85). Tanaka and colleagues, on the other hand, did not find a significant change in cell-free DNA by endometrial cancer grade or stage (89). Further studies are thus needed to confirm the potential prognostic utility of circulating tumour DNA in endometrial cancer. Circulating tumour DNA have also been suggested as potential tools for the early detection of recurrence in endometrial cancer (88, 90). The small pilot study by Moss and colleagues found that ctDNA could detect endometrial cancer recurrence and progression earlier than imaging or clinical presentation with a median lead time of 2.5 months (88). Specific blood-based tumour mutations have also been associated with endometrial cancer prognosis. Dobrzycka and colleagues found an association between circulating cell-free DNA p53 antibody and *KRAS* mutation status and high-grade endometrial cancer (87). Bolivar and colleagues found a significant association between the presence of plasma ctDNA mutation (*CTNNBI, KRAS, PTEN*, or *PIK3C*) and advanced stage, deep myometrial invasion, LVSI, and primary tumour size (91). Circulating tumour cells have also been linked to endometrial cancer prognosis. Lemech and colleagues, in a feasibility study of 30 patients with advanced endometrial cancer found an association

between circulating tumour cell positivity and non-endometrioid histology, tumour size, disease stage and survival (92). The small prospective study by Bogani and colleagues, involving 28 patients with grade 3 endometrial cancer reported a significant correlation between the presence of circulating tumour cells and deep myometrial invasion and lymph node positivity (93). Studies exploring how best to incorporate circulating tumour markers into routine clinical care are needed.

Systemic inflammatory parameters have shown potential as prognostic biomarkers in endometrial cancer (94). Chronic low-grade inflammation is one of the biological mechanisms underpinning endometrial carcinogenesis. Inflammation is known to damage DNA and potentiates pro-proliferative and anti-apoptotic processes that contribute to tumour development and progression. A recent study from our group found that women with elevated CRP at a decision threshold of 5.5mg/L had a two-fold increase in cancer-specific mortality risk (95). These findings need to be validated in an independent cohort prior to clinical translation. Other inflammatory parameters that are prognostic in endometrial cancer include neutrophil to lymphocyte ratio, monocyte to lymphocyte ratio, systemic inflammatory index, and Glasgow prognostic score (**Table 5**). However, there is insufficient evidence to enable clinical translation at present.

RADIOMIC PROGNOSTIC PROFILING OF ENDOMETRIAL CANCER

Radiomic-based risk-stratification models are emerging prognostic systems in endometrial cancer (118). Radiomics deals with the high-throughput mining of quantitative tomographic image parameters and their application in clinical decision making (119). There is growing evidence for the potential utility of radiomic techniques in improving cancer diagnostic, prognostic and predictive accuracy across various tumour sites (118, 119). This has been made possible by the advances in artificial intelligence and machine learning techniques, thus allowing for an in-depth tumour characterisation. Studies have been consistent in suggesting the potential utility of radiomic signatures in endometrial cancer risk-stratification and prediction of outcomes (118, 120–123). Increasingly, radiomics is combined with genomic data (radiogenomics) to aid the prediction of genetic variants including microsatellite instability. Veeraravaghan and colleagues proposed an integrated radiomic-clinical classification algorithm that distinguishes MMR-D endometrial tumours from copy number low and copy number high tumours with an AUC of 0.78 (121). Chen and colleagues found that an MRI-based radiomic model had better discrimination than clinical and conventional MRI parameters in predicting low risk endometrial cancer (124). Yan and colleagues showed that radiomic based models can aid the prediction of pelvic lymph node metastasis in endometrial cancer (120). A high-quality, robust and generalizable radiomic risk-prediction model is dependent on the optimal collection and integration of data from multimodal sources and rigor in model development and implementation (119, 125).

CLINICAL PARAMETERS AND ENDOMETRIAL CANCER PROGNOSIS

Several clinical parameters have been associated with endometrial cancer survival outcomes. They include age at diagnosis, body mass index (BMI) and the presence of comorbidities (126). Age at diagnosis is universally accepted as prognostic for most adult cancers, with older patients having worse outcomes. In the UK, endometrial cancer mortality rates were highest in women aged 85 to 89 between 2016 and 2018, with over 50% of all endometrial cancer deaths occurring in those aged 75 and over (3). An important consideration is whether this association is purely related to age or other unfavourable prognostic factors that are associated with age (126). Studies have been consistent in reporting an association between advancing age and the presence of adverse tumour related parameters (127–129). For example, Lachance and colleagues studied 396 women with endometrial cancer and reported a higher prevalence of aggressive disease, specifically higher grade, late stage, non-endometrioid endometrial cancers in those >65 years of age (129). In a retrospective analysis of 551 endometrial cancer patients, Son and colleagues found that age ≤40 years was associated with non-invasive cancers, less lympho-vascular space invasion and a higher body mass index (130). Lee et al, in a study of over 15,000 women with endometrial cancer, reported a higher rate of serous histology in those >40 years and a 5-year disease-specific survival rate of 86.4% compared to 93.2% in women <40 years (127). Following adjustment for histology and adjuvant therapy, the survival disadvantage persisted. Other factors including differential treatment and treatment-related morbidity may be contributory to these trends. Koul and colleagues found that older women (≥75 years) were less likely to be offered adjuvant therapy and had a significantly lower 5-year cancer-specific survival rate compared to those <75 years (128). Zeng and colleagues reported a higher rate of post-operative morbidity in elderly endometrial cancer patients undergoing robotic surgery (131). These findings are consistent with previously published data where age has been reported to independently impact on endometrial cancer outcomes, including risk of recurrence (130, 132–135).

Obesity is the most important modifiable risk factor in endometrial cancer, with every 5kg/m^2 increase in BMI conferring a 60% increased risk of the disease (136). Obesity-driven endometrial cancers are usually low grade, early stage, endometrioid tumours with a favourable prognosis when compared with the biologically aggressive non-endometrioid endometrial cancer phenotypes (136–138). Despite the survival advantages offered by favourable tumour biology, obesity is associated with higher all-cause mortality due to comorbid health conditions, particularly cardiovascular disease (139). Indeed, cardiovascular disease is the leading cause of death among endometrial cancer survivors (140). Arem and colleagues found that women with BMI ≥35kg/m^2 had an almost 5-fold higher risk of cardiovascular-related mortality 10 years post diagnosis compared with those with BMI <25kg/m^2

TABLE 5 | Circulating endometrial cancer prognostic biomarkers.

Category	Biomarker	Prognostic features
Proteins	Elevated CA125	Linked to poor survival (96, 97) Higher stage (83, 98) Higher grade (83, 98) Deep myometrial invasion (83, 98) Lymph node metastasis (83, 98) LVSI (98)
	Elevated HE4	Poor overall, disease-specific and recurrence free survival (74, 84) Deep myometrial invasion (99, 100) Advanced stage (100–102) Presence of LVSI (66, 103) Tumour size (100) Lymph node metastasis (99, 103) Recurrence (103)
	High Estriol (E3) High Estrone sufate (E1-S)	Non-myoinvasive tumours, low risk of recurrence and improved overall survival (104) Increased relapse (104)
Metabolites	Bradykinin, heme, lactic acid, homocysteine, myristic acid, valine, progesterone, threonine, stearic acid, sarcosine, glycine etc	Associated with histological subtype (13, 105, 106)
	Hydroxysphingomyelins, phospatidylcholines, estrogen metabolites	Associated with deep myometrial invasion (13, 106–108)
	Hexadecadienyl carnitine, phosphatidylcholines	Associated with LVSI (13, 107)
	Spermine, acylcholines, sphingolipids, linoleic acid, myristic acid, polyamines, ceramides	Associated with recurrence (13, 105)
	Methionine sulfoxide	Poor survival (109)
Circulating tumour cells (CTC)	Detection of CTC	Poor progression-free survival (92) Association with non-endometrioid cancer (92) Large tumour size (>5cm) (92) Lymph node involvement (93) Deep myometrial invasion (93)
Circulating tumour DNA (ctDNA)	Presence of ctDNA	Associated with type II tumours (87). Elevated in grades 2 and 3 endometrial cancer (85, 86)
	Serum ctDNA integrity	Elevated in LVSI (85)
	Plasma p53 antibody	Linked to serous tumours (87) Linked to higher grade in Type I tumours (87)
	Plasma *KRAS* mutation	Elevated in grade 2 of type I tumours (87)
	Presence of plasma mutation (*CTNNBI, KRAS, PTEN*, or *PIK3CA*)	Linked to tumour stage (91) Deep myometrial invasion (91) LVSI (91) Large tumour size (91)
Immune/inflammatory parameters	Elevated CRP	Associated with poor overall and cancer-specific survival (65, 80, 81, 110) Stage (111, 112) Lymph node involvement (112)
	Glasgow prognostic score	Survival and recurrence (113)
	Inflammatory parameters (NLR,MLR,PLR,SII etc)	Adverse clinico-pathological features and outcomes (94, 95, 114–117)

(141). Secord and colleagues, in a meta-analysis involving 665,694 endometrial cancer cases reported significantly higher odds of all-cause mortality with increasing BMI, with the highest risk for those with class III obesity (BMI≥40kg/m^2) (139). Obesity may also influence cancer-specific mortality from treatment-related factors (142). As an example, women with class III obesity are less likely to be offered hysterectomy, have a higher risk of perioperative morbidity and are more likely to receive suboptimal doses of chemotherapy from dose capping (142–146). Obesity may also impact on the optimal delivery of adjuvant radiation due to physical, technical and dosimetric constraints, thus contributing to poorer outcomes (147). Whilst obesity certainly impacts on endometrial outcomes, it is unclear whether weight loss interventions can improve survival and work in this space is on-going (148).

Studies have shown that women with a higher Age-adjusted Charlson-Comorbidity (AAC) index scores are at a greater risk of overall mortality, but not cancer-specific mortality or disease recurrence (149). Robbins and colleagues, in an analysis of 671 patients with FIGO stage I-II endometrioid endometrial cancer, report that high AAC scores independently predict short overall survival (149). It remains unclear whether lifestyle changes, including weight loss and dietary modifications, can reduce cardiovascular risk in endometrial cancer survivors, although

this is a tantalizing concept our group seeks to explore further. There is growing evidence that thyroid dysfunction may be linked to survival outcomes in endometrial cancer. The small study by Seebacher and colleagues reported poor disease-specific survival in women with TSH>2.5 mU/L (150). Our group recently found that endometrial cancer patients with comorbid hypothyroidism have significantly improved overall, cancer-specific and recurrence-free survival than those who are euthyroid (151). A prospective validation of these findings is warranted and the underlying mechanisms will need to be elucidated prior to clinical translation. Whether type 2 diabetes mellitus (T2DM) status impacts endometrial cancer survival outcomes is unclear. The meta-analysis by Zhang and colleagues involving 12,195 endometrial cancer cases and 575 deaths found no evidence of an association between T2DM status and endometrial cancer mortality (152). A more recent meta-analysis of five cohort studies by Laio and colleagues concluded that the data linking T2DM status and endometrial cancer-specific mortality are inconsistent. This analysis was limited by considerable clinical and methodological heterogeneity of included studies (153). In two of the included studies, a pooled relative risk of 1.32 (95% CI 1.10, 1.60. p=0.003) was reported. One study reported a hazard ratio of 1.64 (95% CI 0.17, 9.60, p=0.58) while the other three studies reported SMRs that could not be quantitatively synthesized (153). Further research is needed to clarify the prognostic impact of T2DM status on endometrial cancer outcomes.

SOCIODEMOGRAPHIC ASSOCIATIONS WITH PROGNOSIS

There is good evidence to suggest that ethnicity affects outcomes from endometrial cancer (154, 155). In the USA, Black women are more likely to be diagnosed with late stage disease and biologically aggressive endometrial cancer phenotypes (high grade, non-endometrioid cancers) than women of White ethnicity (154, 156–159). Park and colleagues found that non-Hispanic Black women had significantly shorter overall survival than non-Hispanic White women in an equal access healthcare system, despite correcting for traditional clinico-pathological characteristics, suggesting that other factors including molecular phenotypic differences might be contributing (155). It has been postulated that differential expression of specific tumour markers such as p53, *PTEN*, *HER2/neu* and *PIK3R1* mutations may explain some of the racial disparities (160, 161). *PTEN* mutation portends a favourable prognosis and has been reported to be less common in Black women compared to White women (162). *TP53* mutations, on the other hand, portend an unfavourable prognosis and are more common in Black women (163). Studies have also shown that women of Black ethnicity are less likely to undergo hysterectomy (160, 164) or receive adjuvant therapy than their White counterparts (165, 166). A review of the US National Cancer Database found that 47% of the 19,594 endometrial cancer patients who met the criteria for adjuvant radiation failed to receive radiation. The omission of adjuvant radiation was more common amongst Black, Asian and Hispanic women as well as those of lower socioeconomic status (166). Differences in comorbid conditions may also contribute to racial disparities in outcomes. Studies have been consistent in suggesting a higher comorbidity burden amongst Black women compared to women of White ethnicity (167, 168). Tarney and colleagues found that Black women <65 years with endometrial cancer are more likely to die from non-cancer related causes than White women (169).

Socioeconomic status has been linked with endometrial cancer outcomes too. Factors such as differential access to health care, level of income, educational status and areal-level economic deprivation may be contributory. Bedir and colleagues analyzed data on 21,602 German women with endometrial cancer and found differences in survival according to district level socioeconomic deprivation (170). In a Swedish study, women from the higher social groups were less likely to be diagnosed with advanced stage disease and non-endometrioid cancers, and had more favourable outcomes than women from the lower social groups (171). These findings are consistent with those reported in several high-income countries (149, 164, 172, 173). In the UK, results have been conflicting (174–176). Donkers and colleagues found no evidence of a socioeconomic disparity in survival after adjusting for confounding factors (175). Using the English multiple indices of deprivation, Njoku and colleagues found that women from more deprived neighbourhoods were more likely to present with fatal recurrence than those from less deprived areas (176). Further research is needed to confirm these findings and identify modifiable contributing factors.

CONCLUSION

Several clinical, sociodemographic and tumour specific parameters have emerged as important endometrial cancer prognostic biomarkers. The Cancer Genome Atlas and subsequent clinically translatable molecular classification systems, in particular, hold great promise to refine current endometrial cancer risk stratification systems. The clinical utility of endometrial cancer molecular classification in guiding adjuvant therapy and recurrence monitoring is yet to be defined and must now be prioritised. Blood-based markers including systemic inflammatory parameters, proteins and metabolites, and circulating tumour cells have also shown potential to refine endometrial cancer risk stratification algorithms and their prospective validation in larger study cohorts is warranted. The impact of socioeconomic status and ethnicity on endometrial cancer outcomes is becoming more apparent and studies exploring the factors underlying these disparities are urgently needed.

AUTHOR CONTRIBUTIONS

Conceptualization- KN and EJC. Writing- original draft preparation KN. Writing- review and editing KN, CEB, and

EJC. Supervision- EJC. Funding acquisition- EJC. All authors read and approved the final version for submission.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660
2. Crosbie E, Morrison J. The Emerging Epidemic of Endometrial Cancer: Time to Take Action. *Cochrane Database Syst Rev* (2014) 12:1465–858. doi: 10.1002/14651858.ED000095
3. CRUK. *Uterine Cancer Incidence Statistics*. CRUK (2020). CRUK. Available at: www.cancerresearchuk.org; http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/uterine-cancer/incidence#heading-One (Accessed 1/6/2020).
4. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial Cancer. *Lancet* (2016) 387(10023):1094–108. doi: 10.1016/S0140-6736(15)00130-0
5. Ryan NAJ, McMahon R, Tobi S, Snowsill T, Esquibel S, Wallace AJ, et al. The Proportion of Endometrial Tumours Associated With Lynch Syndrome (PETALS): A Prospective Cross-Sectional Study. *PloS Med* (2020) 17(9): e1003263. doi: 10.1371/journal.pmed.1003263
6. Njoku K, Abiola J, Russell J, Crosbie EJ. Endometrial Cancer Prevention in High-Risk Women. *Best Pract Res Clin Obstet Gynaecol* (2020) 65:66–78. doi: 10.1016/j.bpobgyn.2019.12.005
7. Jones ER, O'Flynn H, Njoku K, Crosbie EJ. Detecting Endometrial Cancer. *Obstet Gynaecol* (2021) 23(2):103–12. doi: 10.1111/tog.12722
8. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: Diagnosis, Treatment and Follow-Up. *Int J Gynecol Cancer* (2016) 26(1):2–30. doi: 10.1097/IGC.0000000000000609
9. McAlpine JN, Temkin SM, Mackay HJ. Endometrial Cancer: Not Your Grandmother's Cancer. *Cancer* (2016) 122(18):2787–98. doi: 10.1002/cncr.30094
10. Vermij L, Smit V, Nout R, Bosse T. Incorporation of Molecular Characteristics Into Endometrial Cancer Management. *Histopathology* (2020) 76(1):52–63. doi: 10.1111/his.14015
11. Sechidis K, Papangelou K, Metcalfe PD, Svensson D, Weatherall J, Brown G. Distinguishing Prognostic and Predictive Biomarkers: An Information Theoretic Approach. *Bioinformatics* (2018) 34(19):3365–76. doi: 10.1093/bioinformatics/bty357
12. Group F-NBW. *BEST (Biomarkers, EndpointS, and Other Tools) Resource [Internet]*. Silver Spring (MD): Food and Drug Administration (US) (2018). 2016-Co-published by National Institutes of Health (US), Bethesda (MD).
13. Njoku K, Sutton CJ, Whetton AD, Crosbie EJ. Metabolomic Biomarkers for Detection, Prognosis and Identifying Recurrence in Endometrial Cancer. *Metabolites* (2020) 10(8):314. doi: 10.3390/metabo10080314
14. Bokhman JV. Two Pathogenetic Types of Endometrial Carcinoma. *Gynecol Oncol* (1983) 15(1):10–7. doi: 10.1016/0090-8258(83)90111-7
15. Murali R, Soslow RA, Weigelt B. Classification of Endometrial Carcinoma: More Than Two Types. *Lancet Oncol* (2014) 15(7):e268–78. doi: 10.1016/S1470-2045(13)70591-6
16. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial Cancer. *Lancet* (2005) 366(9484):491–505. doi: 10.1016/S0140-6736(05)67063-8
17. Morrison J, Balega J, Buckley L, Clamp A, Crosbie E, Drew Y, et al. British Gynaecological Cancer Society (BGCS) Uterine Cancer Guidelines: Recommendations for Practice. *Eur J Obstet Gynecol Reprod Biol* (2022) 270:50–89. doi: 10.1016/j.ejogrb.2021.11.423
18. Setiawan VW, Yang HP, Pike MC, McCann SE, Yu H, Xiang Y-B, et al. Type I and II Endometrial Cancers: Have They Different Risk Factors? *J Clin Oncol* (2013) 31(20):2607. doi: 10.1200/jco.2012.48.2596
19. Dedes KJ, Wetterskog D, Ashworth A, Kaye SB, Reis-Filho JS. Emerging Therapeutic Targets in Endometrial Cancer. *Nat Rev Clin Oncol* (2011) 8 (5):261–71. doi: 10.1038/nrclinonc.2010.216
20. Urick ME, Bell DW. Clinical Actionability of Molecular Targets in Endometrial Cancer. *Nat Rev Cancer* (2019) 19(9):510–21. doi: 10.1038/s41568-019-0177-x
21. Pecorelli S. Revised FIGO Staging for Carcinoma of the Vulva, Cervix, and Endometrium. *Int J Gynecol Obstet* (2009) 105(2):103–4. doi: 10.1016/j.ijgo.2009.02.012
22. Soslow RA, Tornos C, Park KJ, Malpica A, Matias-Guiu X, Oliva E, et al. Endometrial Carcinoma Diagnosis: Use of FIGO Grading and Genomic Subcategories in Clinical Practice: Recommendations of the International Society of Gynecological Pathologists. *Int J Gynecol Pathol* (2019) 38(1 Suppl 1):S64. doi: 10.1097/PGP.0000000000000518
23. Uharček P. Prognostic Factors in Endometrial Carcinoma. *J Obstet Gynaecol Res* (2008) 34(5):776–83. doi: 10.1111/j.1447-0756.2008.00796.x
24. Wang J, Xu P, Yang X, Yu Q, Xu X, Zou G, et al. Association of Myometrial Invasion With Lymphovascular Space Invasion, Lymph Node Metastasis, Recurrence, and Overall Survival in Endometrial Cancer: A Meta-Analysis of 79 Studies With 68,870 Patients. *Front Oncol* (2021) 11:762329. doi: 10.3389/fonc.2021.762329
25. Moatasim A, Hameed Z, Ahmad I. Assessment of Lymphovascular Invasion in Early Stage Endometrial Carcinoma-a Retrospective Study. *Surg Exp Pathol* (2021) 4(1):1–6. doi: 10.1186/s42047-021-00091-6
26. Cusano E, Myers V, Samant R, Sudai T, Keller A, Le T, et al. Prognostic Significance of Lymphovascular Space Invasion in the Absence of Lymph Node Metastases in Early-Stage Endometrial Cancer. *Int J Gynecol Cancer* (2018) 28(5):890–94. doi: 10.1097/IGC.0000000000001229
27. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP Guidelines for the Management of Patients With Endometrial Carcinoma. *Int J Gynecol Cancer* (2021) 31(1):12–39. doi: 10.1136/ijgc-2020-002230
28. Bendifallah S, Canlorbe G, Collinet P, Arsene E, Huguet F, Coutant C, et al. Just How Accurate Are the Major Risk Stratification Systems for Early-Stage Endometrial Cancer? *Br J Cancer* (2015) 112(5):793–801. doi: 10.1038/bjc.2015.35
29. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, et al. Final Validation of the ProMisE Molecular Classifier for Endometrial Carcinoma in a Large Population-Based Case Series. *Ann Oncol* (2018) 29 (5):1180–8. doi: 10.1093/annonc/mdy058
30. Gilks CB, Oliva E, Soslow RA. Poor Interobserver Reproducibility in the Diagnosis of High-Grade Endometrial Carcinoma. *Am J Surg Pathol* (2013) 37(6):874–81. doi: 10.1097/PAS.0b013e31827f576a
31. Thomas S, Hussein Y, Bandyopadhyay S, Cote M, Hassan O, Abdulfatah E, et al. Interobserver Variability in the Diagnosis of Uterine High-Grade Endometrioid Carcinoma. *Arch Pathol Lab Med* (2016) 140(8):836–43. doi: 10.5858/arpa.2015-0220-OA
32. De Boer SM, Wortman BG, Bosse T, Powell ME, Singh N, Hollema H, et al. Clinical Consequences of Upfront Pathology Review in the Randomised PORTEC-3 Trial for High-Risk Endometrial Cancer. *Ann Oncol* (2018) 29 (2):424–30. doi: 10.1093/annonc/mdx753
33. Jamieson A, Bosse T, McAlpine JN. The Emerging Role of Molecular Pathology in Directing the Systemic Treatment of Endometrial Cancer.

Ther Adv Med Oncol (2021) 13:17588359211035960. doi: 10.1177/17588359211035959
34. Levine DANetwork CGAR. Integrated Genomic Characterization of Endometrial Carcinoma. *Nature* (2013) 497(7447):67–73. doi: 10.1038/nature12113
35. Vicky M, MacKay H, Ray-Coquard I, Levine DA, Westin SN, Daisuke A, et al. Endometrial Cancer (Primer). *Nat Rev Dis Primers* (2021) 7(1):88. doi: 10.1038/s41572-021-00324-8
36. McAlpine J, Leon-Castillo A, Bosse T. The Rise of a Novel Classification System for Endometrial Carcinoma; Integration of Molecular Subclasses. *J Pathol* (2018) 244(5):538–49. doi: 10.1002/path.5034
37. Stubert J, Gerber B. Current Issues in the Diagnosis and Treatment of Endometrial Carcinoma. *Geburtshilfe Frauenheilkd* (2016) 76(02):170–5. doi: 10.1055/s-0035-1558230
38. Raffone A, Travaglino A, Gabrielli O, Micheli M, Zuccalà V, Bitonti G, et al. Clinical Features of ProMisE Groups Identify Different Phenotypes of Patients With Endometrial Cancer. *Arch Gynecol Obstet* (2021) 303(6):1393–400. doi: 10.1007/s00404-021-06028-4
39. Bosse T, Powell M, Crosbie E, Leary A, Kroep J, Han K, et al. 595 Implementation of Collaborative Translational Research (TransPORTEC) Findings in an International Endometrial Cancer Clinical Trials Program (RAINBO). *BMJ Spec J* (2021) 31:A108–9. doi: 10.1136/ijgc-2021-ESGO.171
40. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A Clinically Applicable Molecular-Based Classification for Endometrial Cancers. *Br J Cancer* (2015) 113(2):299–310. doi: 10.1038/bjc.2015.190
41. Raffone A, Travaglino A, Mascolo M, Carbone L, Guida M, Insabato L, et al. TCGA Molecular Groups of Endometrial Cancer: Pooled Data About Prognosis. *Gynecol Oncol* (2019) 155(2):374–83. doi: 10.1016/j.ygyno.2019.08.019
42. Kuhn E, Bahadirli-Talbott A, Shih I-M. Frequent CCNE1 Amplification in Endometrial Intraepithelial Carcinoma and Uterine Serous Carcinoma. *Mod Pathol* (2014) 27(7):1014–9. doi: 10.1038/modpathol.2013.209
43. Fader AN, Roque DM, Siegel E, Buza N, Hui P, Abdelghany O, et al. Randomized Phase II Trial of Carboplatin–Paclitaxel Compared With Carboplatin–Paclitaxel–Trastuzumab in Advanced (Stage III–IV) or Recurrent Uterine Serous Carcinomas That Overexpress Her2/Neu (NCT01367002): Updated Overall Survival Analysis. *Clin Cancer Res* (2020) 26(15):3928–35. doi: 10.1158/1078-0432.CCR-20-0953
44. Kurnit KC, Kim GN, Fellman BM, Urbauer DL, Mills GB, Zhang W, et al. CTNNB1 (Beta-Catenin) Mutation Identifies Low Grade, Early Stage Endometrial Cancer Patients at Increased Risk of Recurrence. *Mod Pathol* (2017) 30(7):1032–41. doi: 10.1038/modpathol.2017.15
45. Depreeuw J, Stelloo E, Osse EM, Creutzberg CL, Nout RA, Moisse M, et al. Amplification of 1q32. 1 Refines the Molecular Classification of Endometrial Carcinoma. *Clin Cancer Res* (2017) 23(23):7232–41. doi: 10.1158/1078-0432.CCR-17-0566
46. McConechy MK, Talhouk A, Leung S, Chiu D, Yang W, Senz J, et al. Endometrial Carcinomas With POLE Exonuclease Domain Mutations Have a Favorable Prognosis. *Clin Cancer Res* (2016) 22(12):2865–73. doi: 10.1158/1078-0432.CCR-15-2233
47. Dou Y, Kawaler EA, Zhou DC, Gritsenko MA, Huang C, Blumenberg L, et al. Proteogenomic Characterization of Endometrial Carcinoma. *Cell* (2020) 180(4):729–48. doi: 10.1158/1538-7445.AM2020-6580
48. Stelloo E, Bosse T, Nout RA, MacKay HJ, Church DN, Nijman HW, et al. Refining Prognosis and Identifying Targetable Pathways for High-Risk Endometrial Cancer; a TransPORTEC Initiative. *Mod Pathol* (2015) 28(6):836–44. doi: 10.1038/modpathol.2015.43
49. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: A Simple, Genomics-Based Clinical Classifier for Endometrial Cancer. *Cancer* (2017) 123(5):802–13. doi: 10.1002/cncr.30496
50. Stelloo E, Nout RA, Naves LCLM, Ter Haar NT, Creutzberg CL, Smit VT, et al. High Concordance of Molecular Tumor Alterations Between Pre-Operative Curettage and Hysterectomy Specimens in Patients With Endometrial Carcinoma. *Gynecol Oncol* (2014) 133(2):197–204. doi: 10.1016/j.ygyno.2014.02.012
51. Talhouk A, Hoang LN, McConechy MK, Nakonechny Q, Leo J, Cheng A, et al. Molecular Classification of Endometrial Carcinoma on Diagnostic Specimens Is Highly Concordant With Final Hysterectomy: Earlier Prognostic Information to Guide Treatment. *Gynecol Oncol* (2016) 143(1):46–53. doi: 10.1016/j.ygyno.2016.07.090
52. Batista TP, Cavalcanti CLC, Tejo AAG, Bezerra ALR. Accuracy of Preoperative Endometrial Sampling Diagnosis for Predicting the Final Pathology Grading in Uterine Endometrioid Carcinoma. *Eur J Surg Oncol* (2016) 42(9):1367–71. doi: 10.1016/j.ejso.2016.03.009
53. Kim SR, Cloutier BT, Leung S, Cochrane D, Britton H, Pina A, et al. Molecular Subtypes of Clear Cell Carcinoma of the Endometrium: Opportunities for Prognostic and Predictive Stratification. *Gynecol Oncol* (2020) 158(1):3–11. doi: 10.1016/j.ygyno.2020.04.043
54. DeLair DF, Burke KA, Selenica P, Lim RS, Scott SN, Middha S, et al. The Genetic Landscape of Endometrial Clear Cell Carcinomas. *J Pathol* (2017) 243(2):230–41. doi: 10.1002/path.4947
55. Bosse T, Nout RA, McAlpine JN, McConechy MK, Britton H, Hussein Y, et al. Molecular Classification of Grade 3 Endometrioid Endometrial Cancers Identifies Distinct Prognostic Subgroups. *Am J Surg Pathol* (2018) 42(5):561. doi: 10.1097/PAS.0000000000001020
56. Brett MA, Atenafu EG, Singh N, Ghatage P, Clarke BA, Nelson GS, et al. Equivalent Survival of P53 Mutated Endometrial Endometrioid Carcinoma Grade 3 and Endometrial Serous Carcinoma. *Int J Gynecol Pathol* (2021) 40(2):116–23. doi: 10.1097/PGP.0000000000000674
57. Stelloo E, Nout RA, Osse EM, Jürgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, et al. Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-Stage Endometrial Cancer—Combined Analysis of the PORTEC Cohorts. *Clin Cancer Res* (2016) 22(16):4215–24. doi: 10.1158/1078-0432.CCR-15-2878
58. Zeimet AG, Reimer D, Huszar M, Winterhoff B, Puistola U, Abdel Azim S, et al. L1CAM in Early-Stage Type I Endometrial Cancer: Results of a Large Multicenter Evaluation. *J Natl Cancer Inst* (2013) 105(15):1142–50. doi: 10.1093/jnci/djt144
59. Van Der Putten LJM, Visser N, Van De Vijver K, Santacana M, Bronsert P, Bulten J, et al. L1CAM Expression in Endometrial Carcinomas: An ENITEC Collaboration Study. *Br J Cancer* (2016) 115(6):716–24. doi: 10.1038/bjc.2016.235
60. Hua T, Liu S, Xin X, Jin Z, Liu Q, Chi S, et al. Prognostic Significance of L1 Cell Adhesion Molecule in Cancer Patients: A Systematic Review and Meta-Analysis. *Oncotarget* (2016) 7(51):85196. doi: 10.18632/oncotarget.13236
61. Visser N, Bulten J, van der Wurff AAM, Boss EA, Bronkhorst CM, Feijen HWH, et al. PIpelle Prospective ENDOmetrial Carcinoma (PIPENDO) Study, Pre-Operative Recognition of High Risk Endometrial Carcinoma: A Multicentre Prospective Cohort Study. *BMC Cancer* (2015) 15(1):1–6. doi: 10.1186/s12885-015-1487-3
62. Trovik J, Wik E, Werner HMJ, Krakstad C, Helland H, Vandenput I, et al. Hormone Receptor Loss in Endometrial Carcinoma Curettage Predicts Lymph Node Metastasis and Poor Outcome in Prospective Multicentre Trial. *Eur J Cancer* (2013) 49(16):3431–41. doi: 10.1016/j.ejca.2013.06.016
63. Morelli M, Scumaci D, Di Cello A, Venturella R, Donato G, Faniello MC, et al. DJ-1 in Endometrial Cancer: A Possible Biomarker to Improve Differential Diagnosis Between Subtypes. *Int J Gynecol Cancer* (2014) 24(4):649–58. doi: 10.1097/IGC.0000000000000102
64. Reijnen C, Gogou E, Visser NCM, Engerud H, Ramjith J, van der Putten LJM, et al. Preoperative Risk Stratification in Endometrial Cancer (ENDORISK) by a Bayesian Network Model: A Development and Validation Study. *PloS Med* (2020) 17(5):e1003111. doi: 10.1371/journal.pmed.1003111
65. Weinberger V, Bednarikova M, Hausnerova J, Ovesna P, Vinklerova P, Minar L, et al. A Novel Approach to Preoperative Risk Stratification in Endometrial Cancer: The Added Value of Immunohistochemical Markers. *Front Oncol* (2019) 9:265. doi: 10.3389/fonc.2019.00265
66. Ravegnini G, De Leo A, Coada C, Gorini F, de Biase D, Ceccarelli C, et al. Identification of MiR-499a-5p as a Potential Novel Biomarker for Risk Stratification in Endometrial Cancer. *Front Oncol* (2021) 4321. doi: 10.3389/fonc.2021.757678
67. León-Castillo A, de Boer SM, Powell ME, Mileshkin LR, Mackay HJ, Leary A, et al. Molecular Classification of the PORTEC-3 Trial for High-Risk Endometrial Cancer: Impact on Prognosis and Benefit From Adjuvant Therapy. *J Clin Oncol* (2020) 38(29):3388–97. doi: 10.1200/JCO.20.00549
68. Rimel BJ. A Randomized, Phase II Study Comparing Single-Agent Olaparib, Single Agent Cediranib, and the Combination of Cediranib/Olaparib in

Women With Recurrent, Persistent or Metastatic Endometrial Cancer. *Gynecol Oncol* (2021) 162:S43–4. doi: 10.1016/S0090-8258(21)00727-7
69. Talhouk A, McAlpine JN. New Classification of Endometrial Cancers: The Development and Potential Applications of Genomic-Based Classification in Research and Clinical Care. *Gynecol Oncol Res Pract* (2016) 3(1):1–12. doi: 10.1186/s40661-016-0035-4
70. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord J-P, et al. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair–Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *J Clin Oncol* (2020) 38(1):1. doi: 10.1200/JCO.19.02105
71. Oaknin A, Tinker AV, Gilbert L, Samouëlian V, Mathews C, Brown J, et al. Clinical Activity and Safety of the Anti–Programmed Death 1 Monoclonal Antibody Dostarlimab for Patients With Recurrent or Advanced Mismatch Repair–Deficient Endometrial Cancer: A Nonrandomized Phase 1 Clinical Trial. *JAMA Oncol* (2020) 6(11):1766–72. doi: 10.1001/jamaoncol.2020.4515
72. Marabelle A, Fakih M, Lopez J, Shah M, Shapira-Frommer R, Nakagawa K, et al. Association of Tumour Mutational Burden With Outcomes in Patients With Advanced Solid Tumours Treated With Pembrolizumab: Prospective Biomarker Analysis of the Multicohort, Open-Label, Phase 2 KEYNOTE-158 Study. *Lancet Oncol* (2020) 21(10):1353–65. doi: 10.1016/S1470-2045(20)30445-9
73. Wan YL, Beverley-Stevenson R, Carlisle D, Clarke S, Edmondson RJ, Glover S, et al. Working Together to Shape the Endometrial Cancer Research Agenda: The Top Ten Unanswered Research Questions. *Gynecol Oncol* (2016) 143(2):287–93. doi: 10.1016/j.ygyno.2016.08.333
74. Behrouzi R, Barr CE, Crosbie EJ. HE4 as a Biomarker for Endometrial Cancer. *Cancers* (2021) 13(19):4764. doi: 10.3390/cancers13194764
75. Coll-de la Rubia E, Martinez-Garcia E, Dittmar G, Gil-Moreno A, Cabrera S, Colas E. Prognostic Biomarkers in Endometrial Cancer: A Systematic Review and Meta-Analysis. *J Clin Med* (2020) 9(6):1900. doi: 10.3390/jcm9061900
76. Njoku K, Chiasserini D, Whetton AD, Crosbie EJ. Proteomic Biomarkers for the Detection of Endometrial Cancer. *Cancers (Basel)* (2019) 11(10):1572. doi: 10.3390/cancers11101572
77. Niloff JM, Klug TL, Schaetzl E, Zurawski VR Jr, Knapp RC, Bast RC Jr. Elevation of Serum CA125 in Carcinomas of the Fallopian Tube, Endometrium, and Endocervix. *Am J Obstet Gynecol* (1984) 148(8):1057–8. doi: 10.1016/S0002-9378(84)90444-7
78. Kurihara T, Mizunuma H, Obara M, Andoh K, Ibuki Y, Nishimura T. Determination of a Normal Level of Serum CA125 in Postmenopausal Women as a Tool for Preoperative Evaluation and Postoperative Surveillance of Endometrial Carcinoma. *Gynecol Oncol* (1998) 69(3):192–6. doi: 10.1006/gyno.1998.5018
79. Sood AK, Buller RE, Burger RA, Dawson JD, Sorosky JI, Berman M. Value of Preoperative CA 125 Level in the Management of Uterine Cancer and Prediction of Clinical Outcome. *Obstet Gynecol* (1997) 90(3):441–7. doi: 10.1016/S0029-7844(97)00286-X
80. Dotters DJ. Preoperative CA 125 in Endometrial Cancer: Is It Useful? *Am J Obstet Gynecol* (2000) 182(6):1328–34. doi: 10.1067/mob.2000.106251
81. Yildiz A, Yetimalar H, Kasap B, Aydin C, Tatar S, Soylu F, et al. Preoperative Serum CA 125 Level in the Prediction of the Stage of Disease in Endometrial Carcinoma. *Eur J Obstet Gynecol Reprod Biol* (2012) 164(2):191–5. doi: 10.1016/j.ejogrb.2012.05.038
82. Han S, Lee SH, Kim DH, Kim JW, Park N, Kang S, et al. Evaluation of Preoperative Criteria Used to Predict Lymph Node Metastasis in Endometrial Cancer. *Acta Obstet Gynecol Scand* (2010) 89(2):168–74. doi: 10.3109/00016340903370114
83. Jiang T, Huang L, Zhang S. Preoperative Serum CA125: A Useful Marker for Surgical Management of Endometrial Cancer. *BMC Cancer* (2015) 15(1):1–8. doi: 10.1186/s12885-015-1260-7
84. Dai C, Zheng Y, Li Y, Tian T, Wang M, Xu P, et al. Prognostic Values of HE4 Expression in Patients With Cancer: A Meta-Analysis. *Cancer Manag Res* (2018) 10:4491. doi: 10.2147/CMAR.S178345
85. Vizza E, Corrado G, De Angeli M, Carosi M, Mancini E, Baiocco E, et al. Serum DNA Integrity Index as a Potential Molecular Biomarker in Endometrial Cancer. *J Exp Clin Cancer Res* (2018) 37(1):1–9. doi: 10.1186/s13046-018-0688-4
86. Cicchillitti L, Corrado G, De Angeli M, Mancini E, Baiocco E, Patrizi L, et al. Circulating Cell-Free DNA Content as Blood Based Biomarker in Endometrial Cancer. *Oncotarget* (2017) 8(70):115230. doi: 10.18632/oncotarget.23247
87. Dobrzycka B, Terlikowski SJ, Mazurek A, Kowalczuk O, Niklinska W, Chyczewski L, et al. Circulating free DNA, P53 Antibody and Mutations of KRAS Gene in Endometrial Cancer. *Int J Cancer* (2010) 127(3):612–21. doi: 10.1002/ijc.25077
88. Moss EL, Gorsia DN, Collins A, Sandhu P, Foreman N, Gore A, et al. Utility of Circulating Tumor DNA for Detection and Monitoring of Endometrial Cancer Recurrence and Progression. *Cancers (Basel)* (2020) 12(8):2231. doi: 10.3390/cancers12082231
89. Tanaka H, Tsuda H, Nishimura S, Nomura H, Kataoka F, Chiyoda T, et al. Role of Circulating Free Alu DNA in Endometrial Cancer. *Int J Gynecol Cancer* (2012) 22(1):82–6. doi: 10.1097/IGC.0b013e3182328c94
90. Pereira E, Camacho-Vanegas O, Anand S, Sebra R, Catalina Camacho S, Garnar-Wortzel L, et al. Personalized Circulating Tumor DNA Biomarkers Dynamically Predict Treatment Response and Survival in Gynecologic Cancers. *PloS One* (2015) 10(12):e0145754. doi: 10.1371/journal.pone.0145754
91. Bolivar AM, Luthra R, Mehrotra M, Chen W, Barkoh BA, Hu P, et al. Targeted Next-Generation Sequencing of Endometrial Cancer and Matched Circulating Tumor DNA: Identification of Plasma-Based, Tumor-Associated Mutations in Early Stage Patients. *Mod Pathol* (2019) 32(3):405–14. doi: 10.1038/s41379-018-0158-8
92. Lemech CR, Ensell L, Paterson JC, Eminowicz G, Lowe H, Arora R, et al. Enumeration and Molecular Characterisation of Circulating Tumour Cells in Endometrial Cancer. *Oncology* (2016) 91(1):48–54. doi: 10.1159/000445999
93. Bogani G, Liu MC, Dowdy SC, Cliby WA, Kerr SE, Kalli KR, et al. Detection of Circulating Tumor Cells in High-Risk Endometrial Cancer. *Anticancer Res* (2015) 35(2):683–7.
94. Ni L, Tao J, Xu J, Yuan X, Long Y, Yu N, et al. Prognostic Values of Pretreatment Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios in Endometrial Cancer: A Systematic Review and Meta-Analysis. *Arch Gynecol Obstet* (2020) 301(1):251–61. doi: 10.1007/s00404-019-05372-w
95. Njoku K, Ramchander NC, Wan YL, Barr CE, Crosbie EJ. Pre-Treatment Inflammatory Parameters Predict Survival From Endometrial Cancer: A Prospective Database Analysis. *Gynecol Oncol* (2021) 164(2022):146–53.. doi: 10.1016/j.ygyno.2021.11.009
96. Reijnen C, Visser NCM, Kasius JC, Boll D, Geomini PM, Ngo H, et al. Improved Preoperative Risk Stratification With CA-125 in Low-Grade Endometrial Cancer: A Multicenter Prospective Cohort Study. *J Gynecol Oncol* (2019) 30(5). doi: 10.3802/jgo.2019.30.e70
97. Huang GS, Chiu LG, Gebb JS, Gunter MJ, Sukumvanich P, Goldberg GL, et al. Serum CA125 Predicts Extrauterine Disease and Survival in Uterine Carcinosarcoma. *Gynecol Oncol* (2007) 107(3):513–7. doi: 10.1016/j.ygyno.2007.08.060
98. Chen Y, Huang C, Chien T, Huang S, Wu C, Ho C. Value of Pre-Operative Serum CA125 Level for Prediction of Prognosis in Patients With Endometrial Cancer. *Aust N Z J Obstet Gynaecol* (2011) 51(5):397–402. doi: 10.1111/j.1479-828X.2011.01325.x
99. Panyavaranant P, Manchana T. Preoperative Markers for the Prediction of High-Risk Features in Endometrial Cancer. *World J Clin Oncol* (2020) 11(6):378. doi: 10.5306/wjco.v11.i6.378
100. Espiau Romera A, Cuesta Guardiola T, Benito Vielba M, De Bonrostro Torralba C, Coronado Martín PJ, Baquedano Mainar L. HE 4 Tumor Marker as a Predictive Factor for Lymphatic Metastasis in Endometrial Cancer. *Int J Gynecol Obstet* (2020) 149(3):265–8. doi: 10.1002/ijgo.13140
101. Kemik P, Saatli B, Yıldırım N, Kemik VD, Deveci B, Terek MC, et al. Diagnostic and Prognostic Values of Preoperative Serum Levels of YKL-40, HE-4 and DKK-3 in Endometrial Cancer. *Gynecol Oncol* (2016) 140(1):64–9. doi: 10.1016/j.ygyno.2015.11.020
102. Stiekema A, Lok CAR, Korse CM, van Driel WJ, van der Noort V, Kenter GG, et al. Serum HE4 is Correlated to Prognostic Factors and Survival in Patients With Endometrial Cancer. *Virchows Arch* (2017) 470(6):655–64. doi: 10.1007/s00428-017-2115-1

103. Abbink K, Zusterzeel PLM, Geurts-Moespot AJ, van Herwaarden AE, Pijnenborg JMA, Sweep FCGJ, et al. HE4 is Superior to CA125 in the Detection of Recurrent Disease in High-Risk Endometrial Cancer Patients. *Tumor Biol* (2018) 40(2):1010428318757103. doi: 10.1177/1010428318757103
104. Audet-Delage Y, Grégoire J, Caron P, Turcotte V, Plante M, Ayotte P, et al. Estradiol Metabolites as Biomarkers of Endometrial Cancer Prognosis After Surgery. *J Steroid Biochem Mol Biol* (2018) 178:45–54. doi: 10.1016/j.jsbmb.2017.10.021
105. Audet-Delage Y, Villeneuve L, Grégoire J, Plante M, Guillemette C. Identification of Metabolomic Biomarkers for Endometrial Cancer and its Recurrence After Surgery in Postmenopausal Women. *Front Endocrinol (Lausanne)* (2018) 9:87. doi: 10.3389/fendo.2018.00087
106. Raffone A, Troisi J, Boccia D, Travaglino A, Capuano G, Insabato L, et al. Metabolomics in Endometrial Cancer Diagnosis: A Systematic Review. *Acta Obstet Gynecol Scand* (2020) 99(9):1135–46. doi: 10.1111/aogs.13847
107. Knific T, Vouk K, Smrkolj Š, Prehn C, Adamski J, Rižner TL. Models Including Plasma Levels of Sphingomyelins and Phosphatidylcholines as Diagnostic and Prognostic Biomarkers of Endometrial Cancer. *J Steroid Biochem Mol Biol* (2018) 178:312–21. doi: 10.1016/j.jsbmb.2018.01.012
108. Audet-Walsh E, Lepine J, Gregoire J, Plante M, Caron P, Teˆtu B, et al. Profiling of Endogenous Estrogens, Their Precursors, and Metabolites in Endometrial Cancer Patients: Association With Risk and Relationship to Clinical Characteristics. *J Clin Endocrinol Metab* (2011) 96(2):E330–9. doi: 10.1210/jc.2010-2050
109. Strand E, Tangen IL, Fasmer KE, Jacob H, Halle MK, Hoivik EA, et al. Blood Metabolites Associate With Prognosis in Endometrial Cancer. *Metabolites* (2019) 9(12):302. doi: 10.3390/metabo9120302
110. Terlikowska KM, Dobrzycka B, Terlikowski R, Sienkiewicz A, Kinalski M, Terlikowski SJ. Clinical Value of Selected Markers of Angiogenesis, Inflammation, Insulin Resistance and Obesity in Type 1 Endometrial Cancer. *BMC Cancer* (2020) 20(1):1–10. doi: 10.1186/s12885-020-07415-x
111. Schmid M, Schneitter A, Hinterberger S, Seeber J, Reinthaller A, Hefler L. Association of Elevated C-Reactive Protein Levels With an Impaired Prognosis in Patients With Surgically Treated Endometrial Cancer. *Obstet Gynecol* (2007) 110(6):1231–6. doi: 10.1097/01.AOG.0000292085.50987.f2
112. Wang LJ, Zhou H, Lu HW, Li J, Lin ZQ. Prognostic Value of Preoperative Serum High Sensitivity C-Reactive Protein in Patients With Endometrial Cancer. *Zhonghua Yi Xue Za Zhi* (2011) 91(41):2927–30.
113. Saijo M, Nakamura K, Masuyama H, Ida N, Haruma T, Kusumoto T, et al. Glasgow Prognostic Score Is a Prognosis Predictor for Patients With Endometrial Cancer. *Eur J Obstet Gynecol Reprod Biol* (2017) 210:355–9. doi: 10.1016/j.ejogrb.2017.01.024
114. Mirili C, Bilici M. Inflammatory Prognostic Markers in Endometrial Carcinoma: Systemic Immune-Inflammation Index and Prognostic Nutritional Index. *Med Sci Discov* (2020) 7(1):351–9. doi: 10.36472/msd.v7i1.339
115. Aoyama T, Takano M, Miyamoto M, Yoshikawa T, Kato K, Sakamoto T, et al. Pretreatment Neutrophil-to-Lymphocyte Ratio was a Predictor of Lymph Node Metastasis in Endometrial Cancer Patients. *Oncology* (2019) 96(5):259–67. doi: 10.1159/000497184
116. Acikgoz AS, Cakmak B, Tuten A, Oncul M, Eskalen S, Demirkiran F, et al. Can Preoperative Neutrophil to Lymphocyte and Platelet to Lymphocyte Ratios Predict Cervical Stromal Involvement in Endometrioid Endometrial Adenocarcinoma? *Eur J Gynaecol Oncol* (2017) 38(1):20–4.
117. Pergialiotis V, Oikonomou M, Damaskou V, Kalantzis D, Chrelias C, Tsantes AE, et al. Platelet to Lymphocyte and Neutrophil to Lymphocyte Ratio as Predictive Indices of Endometrial Carcinoma: Findings From a Retrospective Series of Patients and Meta-Analysis. *J Gynecol Obstet Hum Reprod* (2018) 47(10):511–6. doi: 10.1016/j.jogoh.2018.08.016
118. Hoivik EA, Hodneland E, Dybvik JA, Wagner-Larsen KS, Fasmer KE, Berg HF, et al. A Radiogenomics Application for Prognostic Profiling of Endometrial Cancer. *Commun Biol* (2021) 4(1):1–12. doi: 10.1038/s42003-021-02894-5
119. Lambin P, Leijenaar RTH, Deist TM, Peerlings J, De Jong EEC, Van Timmeren J, et al. Radiomics: The Bridge Between Medical Imaging and Personalized Medicine. *Nat Rev Clin Oncol* (2017) 14(12):749–62. doi: 10.1038/nrclinonc.2017.141
120. Yan BC, Li Y, Ma FH, Zhang GF, Feng F, Sun MH, et al. Radiologists With MRI-Based Radiomics Aids to Predict the Pelvic Lymph Node Metastasis in Endometrial Cancer: A Multicenter Study. *Eur Radiol* (2021) 31(1):411–22. doi: 10.1007/s00330-020-07099-8
121. Veeraraghavan H, Friedman CF, DeLair DF, Ninčević J, Himoto Y, Bruni SG, et al. Machine Learning-Based Prediction of Microsatellite Instability and High Tumor Mutation Burden From Contrast-Enhanced Computed Tomography in Endometrial Cancers. *Sci Rep* (2020) 10(1):1–10. doi: 10.1038/s41598-020-72475-9
122. Xu X, Li H, Wang S, Fang M, Zhong L, Fan W, et al. Multiplanar MRI-Based Predictive Model for Preoperative Assessment of Lymph Node Metastasis in Endometrial Cancer. *Front Oncol* (2019) 9:1007. doi: 10.3389/fonc.2019.01007
123. Fasmer KE, Hodneland E, Dybvik JA, Wagner-Larsen K, Trovik J, Salvesen Ø, et al. Whole-Volume Tumor MRI Radiomics for Prognostic Modeling in Endometrial Cancer. *J Magn Reson Imaging* (2021) 53(3):928–37. doi: 10.1002/jmri.27444
124. Chen J, Gu H, Fan W, Wang Y, Chen S, Chen X, et al. MRI-Based Radiomic Model for Preoperative Risk Stratification in Stage I Endometrial Cancer. *J Cancer* (2021) 12(3):726. doi: 10.7150/jca.50872
125. van Dijk LV, Fuller CD. Artificial Intelligence and Radiomics in Head and Neck Cancer Care: Opportunities, Mechanics, and Challenges. *Am Soc Clin Oncol Educ B* (2021) 41:e225–35. doi: 10.1200/EDBK_320951
126. Binder PS, Mutch DG. Update on Prognostic Markers for Endometrial Cancer. *Womens Health* (2014) 10(3):277–88. doi: 10.2217/WHE.14.13
127. Lee NK, Cheung MK, Shin JY, Husain A, Teng NN, Berek JS, et al. Prognostic Factors for Uterine Cancer in Reproductive-Aged Women. *Obstet Gynecol* (2007) 109(3):655–62. doi: 10.1097/01.AOG.0000255980.88205.15
128. Koual M, Ngo C, Girault A, Lécuru F, Bats A-S. Endometrial Cancer in the Elderly: Does Age Influence Surgical Treatments, Outcomes, and Prognosis? *Menopause* (2018) 25(9):968–76. doi: 10.1097/GME.0000000000001119
129. Lachance JA, Everett EN, Greer B, Mandel L, Swisher E, Tamimi H, et al. The Effect of Age on Clinical/Pathologic Features, Surgical Morbidity, and Outcome in Patients With Endometrial Cancer. *Gynecol Oncol* (2006) 101(3):470–5. doi: 10.1016/j.ygyno.2005.11.009
130. Son J, Carr C, Yao M, Radeva M, Priyadarshini A, Marquard J, et al. Endometrial Cancer in Young Women: Prognostic Factors and Treatment Outcomes in Women Aged≤ 40 Years. *Int J Gynecol Cancer* (2020) 30(5):631–9. doi: 10.1136/ijgc-2019-001105
131. Zeng XZ, Lavoue V, Lau S, Press JZ, Abitbol J, Gotlieb R, et al. Outcome of Robotic Surgery for Endometrial Cancer as a Function of Patient Age. *Int J Gynecol Cancer* (2015) 25(4):637–44. doi: 10.1097/IGC.0000000000000411
132. Jolly S, Vargas CE, Kumar T, Weiner SA, Brabbins DS, Chen PY, et al. The Impact of Age on Long-Term Outcome in Patients With Endometrial Cancer Treated With Postoperative Radiation. *Gynecol Oncol* (2006) 103(1):87–93. doi: 10.1016/j.ygyno.2006.01.038
133. Zaino RJ, Kurman RJ, Diana KL, Morrow CP. Pathologic Models to Predict Outcome for Women With Endometrial Adenocarcinoma: The Importance of the Distinction Between Surgical Stage and Clinical Stage–A Gynecologic Oncology Group Study. *Cancer Interdiscip Int J Am Cancer Soc* (1996) 77(6):1115–21. doi: 10.1002/(SICI)1097-0142(19960315)77:6<1115::AID-CNCR17>3.0.CO;2-4
134. Keys HM, Roberts JA, Brunetto VL, Zaino RJ, Spirtos NM, Bloss JD, et al. A Phase III Trial of Surgery With or Without Adjunctive External Pelvic Radiation Therapy in Intermediate Risk Endometrial Adenocarcinoma: A Gynecologic Oncology Group Study. *Gynecol Oncol* (2004) 92(3):744–51. doi: 10.1016/j.ygyno.2003.11.048
135. Lurain JR, Rice BL, Rademaker AW, Poggensee LE, Schink JC, Miller DS. Prognostic Factors Associated With Recurrence in Clinical Stage I Adenocarcinoma of the Endometrium. *Obstet Gynecol* (1991) 78(1):63–9.
136. Crosbie EJ, Zwahlen M, Kitchener HC, Egger M, Renehan AG. Body Mass Index, Hormone Replacement Therapy, and Endometrial Cancer Risk: A Meta-Analysis. *Cancer Epidemiol Prev Biomarkers* (2010) 19(12):3119–30. doi: 10.1158/1055-9965.EPI-10-0832
137. Lundqvist EÅ. Classification of Endometrial Cancer. In: *Management of Endometrial Cancer*. Switzerland: Springer (2020). p. 3–6. doi: 10.1007/978-3-319-64513-1_1

138. Kitson SJ, Crosbie EJ. Endometrial Cancer and Obesity. *Obstet Gynaecol* (2019) 21(4):237–45. doi: 10.1111/tog.12601
139. Secord AA, Hasselblad V, Von Gruenigen VE, Gehrig PA, Modesitt SC, Bae-Jump V, et al. Body Mass Index and Mortality in Endometrial Cancer: A Systematic Review and Meta-Analysis. *Gynecol Oncol* (2016) 140(1):184–90. doi: 10.1016/j.ygyno.2015.10.020
140. Ward KK, Shah NR, Saenz CC, McHale MT, Alvarez EA, Plaxe SC. Cardiovascular Disease Is the Leading Cause of Death Among Endometrial Cancer Patients. *Gynecol Oncol* (2012) 126(2):176–9. doi: 10.1016/j.ygyno.2012.04.013
141. Arem H, Park Y, Pelser C, Ballard-Barbash R, Irwin ML, Hollenbeck A, et al. Prediagnosis Body Mass Index, Physical Activity, and Mortality in Endometrial Cancer Patients. *J Natl Cancer Inst* (2013) 105(5):342–9. doi: 10.1093/jnci/djs530
142. Slawinski CGV, Barriuso J, Guo H, Renehan AG. Obesity and Cancer Treatment Outcomes: Interpreting the Complex Evidence. *Clin Oncol* (2020) 32(9):591–608. doi: 10.1016/j.clon.2020.05.004
143. Hunter RJ, Navo MA, Thaker PH, Bodurka DC, Wolf JK, Smith JA. Dosing Chemotherapy in Obese Patients: Actual Versus Assigned Body Surface Area (BSA). *Cancer Treat Rev* (2009) 35(1):69–78. doi: 10.1016/j.ctrv.2008.07.005
144. Simpson AN, Sutradhar R, Ferguson SE, Robertson D, Cheng SY, Li Q, et al. Perioperative Outcomes of Women With and Without Class III Obesity Undergoing Hysterectomy for Endometrioid Endometrial Cancer: A Population-Based Study. *Gynecol Oncol* (2020) 158(3):681–8. doi: 10.1016/j.ygyno.2020.06.480
145. Modesitt SC, Tian C, Kryscio R, Thigpen JT, Randall ME, Gallion HH, et al. Impact of Body Mass Index on Treatment Outcomes in Endometrial Cancer Patients Receiving Doxorubicin and Cisplatin: A Gynecologic Oncology Group Study. *Gynecol Oncol* (2007) 105(1):59–65. doi: 10.1016/j.ygyno.2006.10.045
146. Bouwman F, Smits A, Lopes A, Das N, Pollard A, Massuger L, et al. The Impact of BMI on Surgical Complications and Outcomes in Endometrial Cancer Surgery—An Institutional Study and Systematic Review of the Literature. *Gynecol Oncol* (2015) 139(2):369–76. doi: 10.1016/j.ygyno.2015.09.020
147. Pichon B, Thureau S, Delpon G, Barillot I, Mahé MA. Obesity and Radiation: Technical Difficulties, Toxicity and Efficacy. *Cancer Radiother* (2013) 17(5–6):543–8. doi: 10.1016/j.canrad.2013.06.034
148. Kitson S, Ryan N, MacKintosh ML, Edmondson R, Duffy JMN, Crosbie EJ. Interventions for Weight Reduction in Obesity to Improve Survival in Women With Endometrial Cancer. *Cochrane Database Syst Rev* (2018) 2:1465–858. doi: 10.1002/14651858.CD012513.pub2
149. Robbins JR, Gayar OH, Zaki M, Mahan M, Buekers T, Elshaikh MA. Impact of Age-Adjusted Charlson Comorbidity Score on Outcomes for Patients With Early-Stage Endometrial Cancer. *Gynecol Oncol* (2013) 131(3):593–7. doi: 10.1016/j.ygyno.2013.10.007
150. Seebacher V, Hofstetter G, Polterauer S, Reinthaller A, Grimm C, Schwameis R, et al. Does Thyroid-Stimulating Hormone Influence the Prognosis of Patients With Endometrial Cancer? A Multicentre Trial. *Br J Cancer* (2013) 109(1):215–8. doi: 10.1038/bjc.2013.282
151. Barr CE, Njoku K, Hotchkies L, Ryan NAJ, Wan YL, Davies DA, et al. Does Clinical and Biochemical Thyroid Dysfunction Impact on Endometrial Cancer Survival Outcomes? A Prospective Database Study. *Cancers (Basel)* (2021) 23(2):294–303. doi: 10.3390/cancers13215444
152. Zhang Z-H, Su P-Y, Hao J-H, Sun Y-H. The Role of Preexisting Diabetes Mellitus on Incidence and Mortality of Endometrial Cancer: A Meta-Analysis of Prospective Cohort Studies. *Int J Gynecol Cancer* (2013) 23(2):294–303. doi: 10.1097/IGC.0b013e31827b8430
153. Liao C, Zhang D, Mungo C, Tompkins DA, Zeidan AM. Is Diabetes Mellitus Associated With Increased Incidence and Disease-Specific Mortality in Endometrial Cancer? A Systematic Review and Meta-Analysis of Cohort Studies. *Gynecol Oncol* (2014) 135(1):163–71. doi: 10.1016/j.ygyno.2014.07.095
154. Felix AS, Weissfeld JL, Stone RA, Bowser R, Chivukula M, Edwards RP, et al. Factors Associated With Type I and Type II Endometrial Cancer. *Cancer Causes Control* (2010) 21(11):1851–6. doi: 10.1007/s10552-010-9612-8
155. Park AB, Darcy KM, Tian C, Casablanca Y, Schinkel JK, Enewold L, et al. Racial Disparities in Survival Among Women With Endometrial Cancer in an Equal Access System. *Gynecol Oncol* (2021) 163(1):125–9. doi: 10.1016/j.ygyno.2021.07.022
156. Cote ML, Ruterbusch JJ, Olson SH, Lu K, Ali-Fehmi R. The Growing Burden of Endometrial Cancer: A Major Racial Disparity Affecting Black Women. *Cancer Epidemiol Prev Biomarkers* (2015) 24(9):1407–15. doi: 10.1158/1055-9965.EPI-15-0316
157. Feinberg J, Albright B, Black J, Lu L, Passarelli R, Gysler S, et al. Ten-Year Comparison Study of Type 1 and 2 Endometrial Cancers: Risk Factors and Outcomes. *Gynecol Obstet Invest* (2019) 84(3):290–7. doi: 10.1159/000493132
158. Smotkin D, Nevadunsky NS, Harris K, Einstein MH, Yu Y, Goldberg GL. Histopathologic Differences Account for Racial Disparity in Uterine Cancer Survival. *Gynecol Oncol* (2012) 127(3):616–9. doi: 10.1016/j.ygyno.2012.08.025
159. Sherman ME, Devesa SS. Analysis of Racial Differences in Incidence, Survival, and Mortality for Malignant Tumors of the Uterine Corpus. *Cancer* (2003) 98(1):176–86. doi: 10.1002/cncr.11484
160. Long B, Liu FW, Bristow RE. Disparities in Uterine Cancer Epidemiology, Treatment, and Survival Among African Americans in the United States. *Gynecol Oncol* (2013) 130(3):652–9. doi: 10.1016/j.ygyno.2013.05.020
161. Althubiti MA. Mutation Frequencies in Endometrial Cancer Patients of Different Ethnicities and Tumor Grades: An Analytical Study. *Saudi J Med Med Sci* (2019) 7(1):16. doi: 10.4103/sjmms.sjmms_154_18
162. Maxwell GL, Risinger JI, Hayes KA, Alvarez AA, Dodge RK, Barrett JC, et al. Racial Disparity in the Frequency of PTEN Mutations, But Not Microsatellite Instability, in Advanced Endometrial Cancers. *Clin Cancer Res* (2000) 6(8):2999–3005.
163. Clifford SL, Kaminetsky CP, Cirisano FD, Dodge R, Soper JT, Clarke-Pearson DL. Racial Disparity in Overexpression of the P53 Tumor Suppressor Gene in Stage I Endometrial Cancer. *Am J Obstet Gynecol* (1997) 176(6):s229–32. doi: 10.1016/S0002-9378(97)70380-6
164. Madison T, Schottenfeld D, James SA, Schwartz AG, Gruber SB. Endometrial Cancer: Socioeconomic Status and Racial/Ethnic Differences in Stage at Diagnosis, Treatment, and Survival. *Am J Public Health* (2004) 94(12):2104–11. doi: 10.2105/AJPH.94.12.2104
165. Bain RP, Greenberg RS, Chung KC. Racial Differences in Survival of Women With Endometrlal Cancer. *Am J Obstet Gynecol* (1987) 157(4):914–23. doi: 10.1016/S0002-9378(87)80089-3
166. Luo LY, Aviki EM, Lee A, Kollmeier MA, Abu-Rustum NR, Tsai CJ, et al. Socioeconomic Inequality and Omission of Adjuvant Radiation Therapy in High-Risk, Early-Stage Endometrial Cancer. *Gynecol Oncol* (2021) 161(2):463–9. doi: 10.1016/j.ygyno.2021.01.041
167. Esnaola NF, Hall BL, Hosokawa PW, Ayanian JZ, Henderson WG, Khuri SF, et al. Race and Surgical Outcomes: It Is Not All Black and White. *Ann Surg* (2008) 248(4):647–55. doi: 10.1097/SLA.0b013e31818a159a
168. Nicholas Z, Hu N, Ying J, Soisson P, Dodson M, Gaffney DK. Impact of Comorbid Conditions on Survival in Endometrial Cancer. *Am J Clin Oncol* (2014) 37(2):131–4. doi: 10.1097/COC.0b013e318277d5f4
169. Tarney CM, Tian C, Wang G, Dubil EA, Bateman NW, Chan JK, et al. Impact of Age at Diagnosis on Racial Disparities in Endometrial Cancer Patients. *Gynecol Oncol* (2018) 149(1):12–21. doi: 10.1016/j.ygyno.2017.07.145
170. Bedir A, Abera SF, Vordermark D, Medenwald D. Socioeconomic Disparities in Endometrial Cancer Survival in Germany: A Survival Analysis Using Population-Based Cancer Registry Data. *J Cancer Res Clin Oncol* (2022) 1–9. doi: 10.1007/s00432-021-03908-9
171. Svanvik T, Marcickiewicz J, Sundfeldt K, Holmberg E, Strömberg U. Sociodemographic Disparities in Stage-Specific Incidences of Endometrial Cancer: A Registry-Based Study in West Sweden, 1995–2016. *Acta Oncol (Madr)* (2019) 58(6):845–51. doi: 10.1080/0284186X.2019.1581947
172. Fader AN, Habermann EB, Hanson KT, Lin JF, Grendys EC, Dowdy SC. Disparities in Treatment and Survival for Women With Endometrial Cancer: A Contemporary National Cancer Database Registry Analysis. *Gynecol Oncol* (2016) 143(1):98–104. doi: 10.1016/j.ygyno.2016.07.107
173. Cheung MR. African American Race and Low Income Neighborhoods Decrease Cause Specific Survival of Endometrial Cancer: A SEER Analysis. *Asian Pac J Cancer Prev* (2013) 14(4):2567–70. doi: 10.7314/APJCP.2013.14.4.2567
174. Gildea C, Nordin A, Hirschowitz L, Poole J. Thirty-Day Postoperative Mortality for Endometrial Carcinoma in England: A Population-Based Study. *BJOG* (2016) 123(11):1853–61. doi: 10.1111/1471-0528.13917
175. Donkers H, Bekkers R, Massuger L, Galaal K. Socioeconomic Deprivation and Survival in Endometrial Cancer: The Effect of BMI. *Gynecol Oncol* (2020) 156(1):178–84. doi: 10.1016/j.ygyno.2019.10.030

176. Njoku K, Barr CE, Hotchkies L, Quille N, Louise Wan Y, Crosbie EJ. Impact of Socioeconomic Deprivation on Endometrial Cancer Survival in the North West of England: A Prospective Database Analysis. *BJOG* (2020) 128 (7):1215–24. doi: 10.1111/1471-0528.16618

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Impact of Type 2 Diabetes Mellitus on Endometrial Cancer Survival: A Prospective Database Analysis

Kelechi Njoku[1,2], *Heather J. Agnew*[1] *and Emma J. Crosbie*[1,3*]

[1] Division of Cancer Sciences, School of Medical Sciences, Faculty of Biology, Medicine and Health, St Mary's Hospital, University of Manchester, Manchester, United Kingdom, [2] Stoller Biomarker Discovery Centre, Institute of Cancer Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom, [3] Department of Obstetrics and Gynaecology, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom

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***Correspondence:**
Emma J. Crosbie
emma.crosbie@manchester.ac.uk

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Purpose: Type 2 diabetes mellitus (T2DM) is an established risk factor for endometrial cancer but its impact on endometrial cancer survival outcomes is unclear. The aim of this study was to investigate whether pre-existing T2DM impacts survival outcomes in endometrial cancer.

Patients and Methods: Women diagnosed with endometrial cancer were recruited to a single centre prospective cohort study. Relevant sociodemographic and clinico-pathological data were recorded at baseline. T2DM status was based on clinical and biochemical assessment, verified by general practitioner records and analysed in relation to overall, cancer-specific and recurrence-free survival using Kaplan-Meier estimation and multivariable Cox-regression.

Results: In total, 533 women with median age and BMI of 66 years (Interquartile range (IQR), 56, 73) and 32kg/m^2 (IQR 26, 39) respectively, were included in the analysis. The majority had low-grade (67.3%), early-stage (85.1% stage I/II), endometrial cancer of endometrioid histological phenotype (74.7%). A total of 107 (20.1%) had pre-existing T2DM. Women with T2DM had a two-fold increase in overall mortality (adjusted HR 2.07, 95%CI 1.21-3.55, p=0.008), cancer-specific mortality (adjusted HR 2.15, 95% CI 1.05-4.39, p=0.035) and recurrence rates (adjusted HR 2.22, 95% CI 1.08-4.56, p=0.030), compared to those without, in multivariable analyses.

Conclusion: T2DM confers an increased risk of death in endometrial cancer patients. Well-designed longitudinal studies with large sample sizes are now needed to confirm these findings.

Keywords: endometrial cancer, prognosis, survival, type 2 diabetes mellitus, mortality

INTRODUCTION

Endometrial cancer is the sixth most common cancer in women globally and the most common gynaecological malignancy in high-income countries. Worldwide, there were an estimated 417,000 incident cases and 97,000 deaths in 2020 (1). The incidence of endometrial cancer is rising year on year, in line with the obesity epidemic (2). Deaths from endometrial cancer are also rising, albeit at a slower rate, despite improvements in overall survival (3, 4). Although most women with endometrial cancer are diagnosed with highly curable disease and have a favourable prognosis, a significant minority present with adverse clinico-pathological characteristics that portend poor outcomes (5).

Identifying women with endometrial cancer who are at a higher risk of relapse and cancer-related mortality is fundamental to ensuring women receive appropriate evidence-based management whilst minimising the side effects and costs of unnecessary interventions for those at lowest risk (6). In current clinical practice, endometrial cancer risk assessment is based on clinico-pathological parameters including International Federation of Gynecology and Obstetrics (FIGO) surgical stage, tumour grade and histological subtype, lymphovascular space invasion and depth of myometrial invasion. The molecular classification of endometrial cancer offers a more objective and reproducible endometrial cancer risk assessment compared with traditional histopathological evaluation (7, 8). Age, body mass index (BMI) and comorbidity status are other predictors of outcomes that are often taken into consideration in treatment algorithms (9). A retrospective analysis of 671 patients with FIGO stage I-II endometrioid endometrial cancer found that higher age-adjusted comorbidity scores are associated with worse outcomes (10). Indeed, cardiovascular events are the leading cause of death amongst endometrial cancer survivors (11).

Type 2 diabetes mellitus (T2DM) is an important risk factor and a common comorbidity in women with endometrial cancer (12). A meta-analysis of 13 primary studies adjusting for BMI concluded that women with T2DM have a 62% increase in the risk of endometrial cancer, independent of obesity (13). Mechanistically, insulin resistance and the resultant hyperinsulinemia promotes endometrial carcinogenesis and progression by the direct pro-proliferative and anti-apoptotic effect of insulin and insulin growth factor (IGF-1) on endometrial cells (14, 15). Whether T2DM also impacts on outcomes following diagnosis and treatment for endometrial cancer is unclear. The meta-analysis of six prospective cohort studies by Zhang and colleagues reported that there was insufficient evidence for an association between T2DM status and endometrial cancer mortality (16). A more recent meta-analysis of five cohort studies by Liao and colleagues concluded that the data linking T2DM and endometrial cancer-specific mortality are inconsistent and the association uncertain (17).

The aim of this study was to investigate whether pre-existing T2DM impacts on survival outcomes in endometrial cancer patients in a large prospective database study.

METHODS

Study Population

Women with a diagnosis of endometrial cancer who were treated between 2010 and 2016 at St Mary's Hospital, a regional specialist centre for the management of gynaecological malignancies, were eligible for inclusion. All study participants gave written informed consent for their pseudo-anonymised data to be used for future research. We collected relevant sociodemographic and clinico-pathological data, including age, BMI, T2DM status, socioeconomic quintile, histological subtype, tumour grade and stage, depth of myometrial invasion, lymphovascular space invasion (LVSI) and baseline serum C-reactive protein (CRP). Age at diagnosis was dichotomised into <65 and ≥65 years, consistent with previous studies, and women were classed as underweight (BMI<18.5 kg/m^2), normal weight (BMI 18.5-24.9kg/m^2), overweight (BMI 25-29.9kg/m^2) or obese (BMI≥30kg/m^2) in line with the World Health Organisation BMI groupings. Endometrial cancers were classified according to histological subtype (endometrioid, serous, clear cell, carcinosarcoma) based on expert histopathology review by two specialist gynaecological pathologists, reporting according to the UK Royal College of Pathology standards and using FIGO 2009 surgical staging classification.

The primary treatment for most women was surgical with total hysterectomy and bilateral salpingo-oophorectomy. Women with intermediate and high-risk disease were offered adjuvant therapy in accordance with national and international guidelines (9, 18). A small minority of women with grade 1 stage IA endometrial cancer who wished to preserve their fertility, or who were medically unfit for surgery, were managed conservatively with primary hormonal therapy (+/-delayed hysterectomy). A few women received primary palliative radiotherapy.

All cases were reviewed in follow-up clinics at 3-month (for 3 years), 6-month (for 1 year) and 12-month intervals for a total duration of 5 years, or until relapse or death, whichever was sooner. Where women had completed routine hospital-based follow up or moved away from Manchester, general practitioners were contacted to ascertain their current status. Women who relapsed during follow up were managed according to national and international guidelines (9, 18). Those with local pelvic recurrence were treated surgically or with radiotherapy as appropriate, whereas those with wide-spread metastatic or distant recurrent disease were managed with palliative hormone therapy, chemotherapy +/- radiotherapy. The cause of death was based on information obtained from death certificates.

Statistical Analysis

The study end-points were overall, cancer-specific and recurrence free survival. Overall survival was calculated from primary treatment initiation to death from any cause or the last day of availability of survival data. Cancer-specific survival was calculated from initiation of primary treatment to death from endometrial cancer or the date of last follow-up, and censored on date of death from other causes. Recurrence-free survival was calculated from primary treatment initiation to the first record of disease recurrence, death or date of last follow-up, whichever was

sooner. Chi-square (X^2) and Fisher's exact tests were used to test for associations between categorical variables, as appropriate. Student's t-test and one-way or two-way ANOVA was used to test for statistical significance for continuous variables as indicated. We used the Kaplan–Meier method to compute survival rates and the log-rank test was used to assess survival differences between groups. Cox regression multivariable modelling was used to evaluate the association between T2DM status and the study end-points while adjusting for confounding and effect modifications. We computed hazard ratios (HRs) with 95% confidence intervals (95% CIs) for both univariable and multivariable analyses. The confounding variables adjusted for in the models were age at diagnosis, BMI, FIGO stage, histological subtype, grade, LVSI, depth of myometrial invasion, socioeconomic quintile and baseline CRP. We assessed for confounding by evaluating the changes in hazard coefficients following the introduction of these variables to the Cox regression models. We assessed for the assumptions of proportional hazards which was met for all models. A p-value of <0.05 was considered statistically significant. All analyses were conducted using the statistical package STATA 16.0 (https://www.stata.com).

RESULTS

Descriptive Characteristics of the Study Population

In total, 533 women with histologically confirmed endometrial cancer were included in this analysis (**Table 1**). Their median age and BMI were 66 years (Interquartile range (IQR), 56, 73) and 32kg/m^2 (IQR 26, 39) respectively. Most women were overweight or obese (83.5%) and aged ≥65 years (54.4%). One-fifth of the study population (20.1%) had pre-existing T2DM. The modal socioeconomic quintile was quintile I (most deprived) and accounted for 37.0% of the study population. The majority had low-grade (67.3%), early-stage (85.1% stage I/II), endometrial cancer of endometrioid histological phenotype (74.7%). The primary treatment was surgery in 87.8% of women, 45% of whom received adjuvant therapy. LVSI and deep myometrial invasion were present in 28.9% and 36.0% respectively. During the study period, 78 women (14.7%) relapsed, 110 (20.6%) died, and the remainder were alive as at 30th April 2021 (**Table 1**).

Associations Between T2DM Status and Endometrial Cancer Clinico-Pathological Parameters

Women with T2DM were more obese (median BMI 36kg/m^2) than those without (median BMI 31kg/m^2, p<0.001). There was an association between T2DM status and socioeconomic quintile, with those from the more deprived neighbourhoods being more likely to have T2DM than those from affluent areas (p=0.045). Women with T2DM were less likely to receive hysterectomy (81.3%) compared to those without (89.4%), although the difference was not statistically significant (p=0.071). There was no evidence of an association between T2DM status and the receipt of adjuvant chemo-radiotherapy (22.5% vs 17.8%, p=0.136) or radiotherapy only (24.6% vs 18.7%, p=0.136). There was a significant correlation between T2DM status and elevated baseline CRP (p=0.013). There was no evidence of an association between T2DM status and age (p=0.141), FIGO stage (p=0.501), histological subtype (p=0.980), disease grade (p=0.654), LVSI (p=0.979) or depth of myometrial invasion (p=0.425) (**Table 2**).

Diabetic Status and Overall Survival

Women were followed up for a median duration of 40 months (range 1-165 months). The overall survival rates for the study cohort were 94% (95%CI 92-96%) at 12 months, 84% (95%CI 81-87%) at 36 months and 75% (95% CI 70-80%) at 60 months. Age at diagnosis, FIGO stage, disease grade, histology, LVSI and depth of myometrial invasion were consistent in demonstrating the expected prognostic associations. There was a 7% increase in overall mortality risk per unit increase in age (HR 1.07, 95% CI 1.05-1.09), p<0.001), but no evidence of an effect of BMI (HR 0.99, 955 ci 0.98-1.01, P=0.629). The risk of overall mortality was higher in women diagnosed with advanced-stage (FIGO III/IV) (HR 3.06, 95% CI 2.03-4.61, p<0.001), high-grade (HR 3.01, 95% CI 2.06-4.40, p<0.001), non-endometrioid (HR 2.98, 95% CI 2.04-4.34, p<0.001) endometrial cancers. LVSI and deep myometrial invasion also correlated with higher risks of death (HR 2.26, 95% CI 1.55-3.28, p<0.001 and 1.78 95%CI 1.22-2.59, P=0.003), respectively. There was a 75% increase in overall mortality for women with CRP>5.5mg/dl compared to those with CRP <5.5mg/dl (HR 1.75, 95% CI 1.09-2.80), p=0.020).

Women with T2DM had a 97% increase in overall mortality compared to those without, in univariable analysis (HR 1.97, 95%CI 1.32-2.94, p=0.001) (**Table 3** and **Figure 1**). Following adjustment for age, BMI, FIGO stage, disease grade, histology, LVSI, depth of myometrial invasion, socioeconomic quintile and baseline CRP, women with T2DM had a two-fold increase in overall mortality compared to those without (adjusted HR 2.07, 95%CI 1.21-3.55, p=0.008).

T2DM Status and Cancer-Specific Survival

In total, there were 110 recorded deaths, 76 (69.1%) of which were due to endometrial cancer while the remaining 34 (30.9%) were non-cancer deaths. The cancer-specific survival for the study cohort was 96% (95%CI 94-97%) at 12 months, 89% (95% CI 85-91%) at 36 months and 81% (76-85%) at 60 months. Cancer specific mortality was worse with increasing age (HR 1.06, 955 CI 1.04-1.09, p<0.001), advanced FIGO stage (HR 5.01, 95% CI 3.16-7.94, p<0.001), high-grade disease (HR 5.76, 95%CI 3.48-9.53, p<0.001), non-endometrioid histology (HR 4.84, 95%CI 3.05-7.69, p<0.001), presence of LVSI (HR 3.46, 95%CI 2.20-5.45, p<0.001), deep myometrial invasion (HR 2.23, 95%CI 1.42-3.50, p=0.001) and higher baseline CRP (HR 2.09, 95%CI 1.15-3.81, p=0.016). There was no evidence of an effect of BMI on cancer-specific deaths (HR 0.98, 95%CI 0.95-1.00, p=0.059).

Women with pre-existing T2DM had a 73% increase in cancer specific mortality compared to those without (HR 1.73, 95%CI 1.05-2.85, p=0.030) (**Table 3**). Following adjustment for age, BMI, FIGO stage, disease grade, histology, LVSI, depth of myometrial invasion and baseline CRP, those with T2DM had a

TABLE 1 | Socio-demographic characteristics of the study population.

Variable	n (% total)
Age at diagnosis	Median age 66 years (IQR 56 73)
<65 years	243 (45.6%)
≥65 years	290 (54.4%)
Body Mass Index (kg/m^2)	Median BMI 32kg/m^2 (IQR 26, 39)
Underweight	6 (1.1%)
Normal weight	82 (15.4%)
Overweight	127 (23.8%)
Obese	318 (59.7%)
Tumour grade	
1	239 (44.8%)
2	120 (22.5%)
3	174 (32.7%)
Tumour stage	
I	397 (74.6%)
II	56 (10.5%)
III	70 (13.2%)
IV	9 (1.7%)
Histology	
Endometrioid	398 (74.7%)
Non-endometrioid	135 (25.3%)
LVSI (n=530)	
No	377 (71.1%)
Yes	153 (28.9%)
Depth of myometrial invasion	
<50%	341 (64.0%)
≥50%	192 (36.0%)
Social deprivation quintile	
Quintile I (Most deprived)	197 (37.0%)
Quintile II	125 (23.5%)
Quintile III	60 (11.3%)
Quintile IV	94 (17.6%)
Quintile V (Least deprived)	57 (10.7%)
History of type 2 diabetes mellitus (n=535)	
Yes	107(20.1%)
No	426(79.9%)
Primary treatment	
Surgery	468 (87.8%)
Hormonal (Fertility sparing reasons)	23 (4.3%)
Hormonal (Not fit for surgery)	39 (7.3%)
Radiotherapy	3 (0.7%)
Adjuvant treatment	
Yes	240 (45.0%)
No	293 (55.0%)
Recurrence	
Yes	78 (14.7%)
No	454 (85.3%)
Survival status at end of follow up	
Alive	423 (79.4%)
Cancer-specific mortality	76 (14.3%)
Non-cancer related mortality	34 (6.4%)
Total	**533 (100%)**

Bold: p < 0.05.

two-fold increase in the risk of death from endometrial cancer compared to those without (adjusted HR 2.15, 95% CI 1.05-4.39), p=0.035).

T2DM Status and Recurrence-Free Survival

Over the study period, there were 78 recurrences (14.7%) with a median time to recurrence 13.5 months (IQR 8-25 months). The recurrence-free survival for the study cohort was 93% (95% CI 90-95%) at 12 months, 83% (79-86%) at 36 months and 80% (75-84%) at 60 months. There was evidence of an association between recurrence free survival and age (HR 1.05, 95%CI 1.02-1.07, p<0.001), FIGO stage (HR 4.89, 95% CI 3.1-7.7, p<0.001), disease grade (HR 4.72, 95% CI 2.95-7.56, p<0.001), histology (HR 3.67, 95% CI 2.35-5.71, p<0.001), LVSI (HR 4.00, 95% CI 2.55-6.28, p<0.001), and depth of myometrial invasion (HR 2.39, 95% CI 1.53-3.73, p<0.001). There was no evidence of an effect of BMI on recurrence free survival (HR 0.99, 95%CI 0.96-1.01, p=0.255).

Women with T2DM had a 70% increase in the risk of recurrence compared to those without in univariable analysis (HR 1.71, 95% CI 1.04-2.80, p=0.034). Following adjustment for age, BMI, FIGO stage, disease grade, histology, LVSI, depth of myometrial invasion and baseline CRP, those with T2DM had a two-fold increase in the risk of disease recurrence compared to those without (adjusted HR 2.22, 95% CI 1.08-4.56, p=0.030).

DISCUSSION

Main Findings

This was a prospective cohort study of 533 women with histologically confirmed endometrial cancer followed up for a median duration of 40 months. In this study, we found T2DM status to be an independent predictor of endometrial cancer outcomes. T2DM status was associated with BMI, baseline CRP and socioeconomic quintile but not FIGO stage, disease grade, histology, LVSI or depth of myometrial invasion. When these sociodemographic and clinico-pathological factors were controlled for, women with T2DM had a two-fold increase in overall mortality, cancer-specific mortality and disease recurrence. If validated in an independent cohort, T2DM status may help refine endometrial cancer risk assessment and when considered alongside other clinico-pathological parameters, may guide decisions about adjuvant therapy in endometrial cancer.

Strengths and Limitations

This study benefits from a large sample size of women with endometrial cancer recruited to several population-based studies that posed few restrictions according to clinico-pathological parameters, alleviating concerns about the possibility of selection bias. The availability of data on socio-demographic and clinico-pathological characteristics allowed for a robust adjustment for confounding factors and effect modifications. To our knowledge, this is the first study to adjust for baseline CRP, a parameter that is known to be associated with T2DM status and which has been reported to independently predict outcomes in endometrial cancer (6). The established endometrial cancer prognostic factors, including FIGO stage, disease grade, histological subtype, LVSI and depth of myometrial invasion, were consistent in demonstrating the expected associations. We did not collect comorbidity or medication use data, and neither did we have information regarding endometrial cancer molecular subgroup for our study cohort, and this may have led to an over- or under-estimation of endometrial cancer outcomes. The

TABLE 2 | Baseline socio-demographic characteristics stratified by T2DM status.

Parameters	Categories	Frequency	No T2DM (n=426)	T2DM (n=107)	P value
Age (years)	<65	243	201 (47.2%)	42 (39.3%)	0.141
	≥65	290	225 (52.8%)	65 (60.7%)	
BMI (kg/m^2)	Underweight	6	6 (1.4%)	0 (0.0%)	**0.005**
	Normal	82	74 (17.4%)	8 (7.5%)	
	Overweight	127	107 (25.1%)	20 (18.7%)	
	Obese	318	239 (56.1%)	79 (73.8%)	
FIGO stage	I	397	318 (74.6%)	79 (73.8%)	0.501
	II	56	44 (10.3%)	12 (11.2%)	
	III	70	54 (12.7%)	16 (15.0%)	
	IV	9	9 (2.1%)	0 (0.0%)	
Histology	Endometrioid	398	318 (74.6%)	80 (74.8%)	0.980
	Others	135	108 (25.4%)	27 (25.2%)	
Grade	I	239	189 (44.4%)	50 (46.7%)	0.654
	II	120	94 (22.1%)	26 (24.3%)	
	III	174	143 (33.5%)	31 (29.0%)	
LVSI (n=530)	No	377	301 (71.2%)	76 (71.0%)	0.979
	Yes	153	122 (28.8%)	31 (29.0%)	
Myometrial invasion	<50%	341	269 (63.1%)	72 (67.3%)	0.425
	≥50%	192	157 (36.9%)	35 (32.7%)	
CRP (n=355)	<5mg/dl	199	169 (59.3%)	30 (42.0%)	**0.013**
	≥5mg/dl	156	116 (40.7%)	40 (57.1%)	
Social quintile	I	197	149 (35.0%)	48 (44.9%)	**0.045**
	II	125	95 (22.3%)	30 (28.0%)	
	III	60	54 (12.7%)	6 (5.6%)	
	IV	94	81 (19.0%)	13 (12.1%)	
	V	57	47 (11.0%)	10 (9.3%)	
Primary Treatment	Surgery	468	381 (89.4%)	87 (90.7%)	0.071
	Hormonal	62	43 (10.1%)	19 (17.8%)	
	Radiotherapy	3	2 (0.5%)	1 (0.9%)	
Adjuvant therapy	None	293	225 (52.8%)	68 (63.6%)	0.136
	Chemoradiotherapy	115	96 (22.5%)	19 (17.8%)	
	Radiotherapy only	125	105 (24.6%)	20 (18.7%)	
Recurrence	No	454	370 (86.9%)	85 (79.4%)	0.054
	Yes	78	56 (13.1%)	22 (20.6%)	
Alive status	No	110	75 (17.6%)	35 (32.7%)	**0.001**
	Yes	423	351 (82.4%)	72 (67.3%)	

Bold: $p < 0.05$.

generally favourable prognosis of endometrial cancer and consequent low event rate affects the reliability of our conclusions. The relatively small number of women with T2DM reduces the precision of our estimates. Finally, as this was a prospective study of mostly White British women managed at a specialist cancer centre, we cannot necessarily generalise our study findings to women from other treatment centres, nationalities or ethnicities.

Interpretation

Large epidemiological and mechanistic studies have been consistent in suggesting an association between T2DM and endometrial carcinogenesis (13, 16, 17, 19, 20). Women with T2DM are at a 62% increased risk of endometrial cancer, independent of obesity, compared to those without (13). Insulin resistance, hormonal imbalance and systemic inflammation are the three main biological pathways implicated in endometrial cancer development (14). Insulin resistance results in hyperinsulinemia and hyperglycaemia which alongside chronic inflammation promotes endometrial tumorigenesis and metastasis by the direct pro-proliferative and anti-apoptotic effect of insulin and insulin growth factor (IGF-1) on endometrial cells (15). However, whether T2DM independently impacts on endometrial cancer outcomes is unclear. The systematic review of relevant cohort studies by Liao and colleagues concluded that the evidence for an association between T2DM and endometrial-cancer specific mortality was low quality (17). Of the six included studies, only two reported relative risk ratios and were quantitatively synthesized (summary estimate RR 1.32 [1.10-1.60]) (17). One study reported a hazard ratio of 1.64 that was not statistically significant (21) while the remaining three studies (22–24) reported standardised mortality ratios that could not be pooled together. In our study, we show evidence that T2DM impacts on endometrial cancer overall, cancer-specific and recurrence free survival, following robust adjustment for important clinico-pathological confounders. T2DM status was associated with BMI, socioeconomic quintile and baseline CRP, consistent with previous work (25–27). Our findings are consistent with the recent report by Nagle and colleagues of a two-fold increase in cancer-specific mortality in endometrial cancer patients with T2DM compared to those without (28). If validated in a larger independent cohort, our findings have important clinical and therapeutic implications. Pre-existing T2DM was recorded for 20% of patients, for whom personalised care and careful follow-up is justified.

TABLE 3 | Cox regression analyses of T2DM status and endometrial cancer survival outcomes with crude and adjusted hazard ratios and 95% confidence intervals.

T2DM Categories	One year survival % (95%CI)	3-year survival % (95%CI)	5-year survival % (95%CI)	Unadjusted HR (95%CI)	p-value	Adjusted HR (95%CI)	p-value
Overall Survival							
No T2DM	95% (92%-97%)	87% (83%-90%)	79% (74%-84%)	1.00		1.00	
T2DM	92% (85%-96%)	73% (63%-81%)	60% (47%-70%)	1.96 (1.32-2.94)	**0.001**	2.07 (1.21-3.55)	**0.008**
Cancer-Specific Survival							
No T2DM	96% (94%-98%)	91% (87%-93%)	84% (78%-88%)	1.00		1.00	
T2DM	95% (88%-98%)	81% (70%-88%)	71% (58%-81%)	1.73 (1.05-2.85)	**0.030**	2.15 (1.05-4.39)	**0.035**
Recurrence free survival							
No T2DM	94% (915-96%)	85% (815-89%)	81% (76%-86%)	1.00		1.00	
T2DM	89% (81% -94%)	72% (60%-81%)	72% (60%-81%)	1.71 (1.04-2.80)	**0.034**	2.22 (1.08-4.56)	**0.030**

Adjusted model includes age, BMI, disease histology, grade, FIGO stage, LVSI, depth of myometrial invasion, primary treatment and baseline CRP. Bold: p < 0.05.

The impact of T2DM on endometrial cancer outcomes may be related to tumour (cancer stage, disease grade, and tumour biology), patient (age, obesity, and other comorbidities) or health care factors (variation in type of care offered) (29). T2DM can impact on the FIGO stage at endometrial cancer diagnosis. It is indeed plausible that having a comorbidity like T2DM can result in increased contact with the National Health Service, thus creating opportunities for the early diagnosis of endometrial cancer. Conversely, pre-existing T2DM may distract either, or both, the patient and health care providers, resulting in delayed cancer diagnosis and poor outcomes (29). In our study, we found no evidence of an association between T2DM status and FIGO stage, and there was minimal evidence of confounding by FIGO stage; correction for FIGO stage did not considerably affect the T2DM hazard ratios. Comorbid diabetes may also influence disease grade and tumour biology. Mechanistically, the pro-proliferative and anti-apoptotic effect of insulin and IGF on endometrial cells, induced by insulin resistance in T2DM may be expected to lead to more aggressive endometrial cancer phenotypes (15, 30). In our study, however, there was no evidence of an association between T2DM status and disease grade or histological subtype; and neither mediated the link between T2DM and endometrial cancer outcomes, as the hazard ratios remained significant after adjusting for these variables. T2DM may also affect endometrial cancer outcomes through patient related factors such as age, BMI and the presence of related comorbidities. However, both age and BMI were adjusted for in the multivariable analyses, suggesting that they could not have underpinned our study findings.

Healthcare and treatment related factors may also explain the association between T2DM and endometrial cancer outcomes (29). There is evidence to suggest that cancer patients with a comorbidity are less likely to be offered curative treatment than those with no comorbidity (31). Indeed, women with T2DM are more likely to have other comorbid conditions such as hypertension and heart disease and thus may be less likely to be offered surgery, compared to those without (31, 32). Furthermore, women with T2DM who undergo surgery may be at an increased risk of peri-and post-operative complications that contribute to poor outcomes (31, 33). Women with comorbidities like T2DM may also be less likely to receive adjuvant chemotherapy, be more liable to receive a reduced

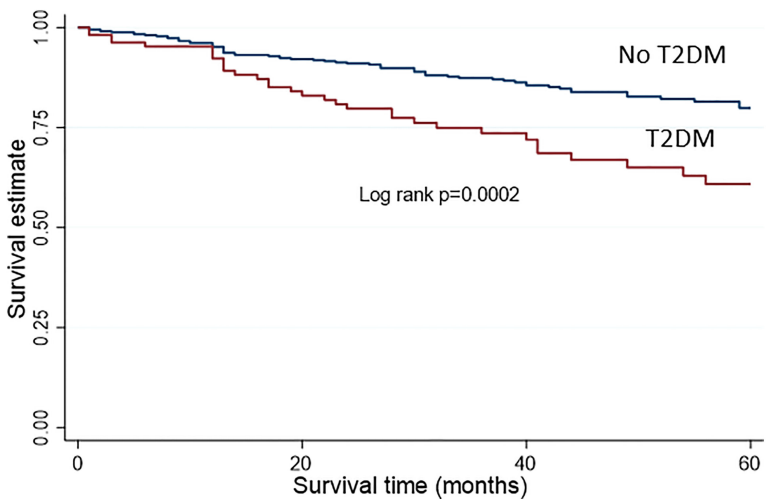

FIGURE 1 | Kaplan Meier survival analysis for overall survival.

dose and more likely to not complete treatment (34–38). In our study, there was no evidence of a significant difference in treatment allocation by T2DM status in either the primary or adjuvant settings. However, treatment-related factors relating to dosing and completion of treatment cannot be ruled out. Furthermore, limited data suggest that metformin is associated with improved overall and progression-free survival outcomes in endometrial cancer (39, 40). Two meta-analyses, involving 1,594 and 3,923 women with endometrial cancer respectively, concluded that metformin reduces the risk of recurrence and death in endometrial cancer survivors (39, 40). However, we were unable to include this in our multivariable model due to lack of data on medication use in our cohort.

In conclusion, we found that T2DM confers an increased risk of death from endometrial cancer. Well-designed longitudinal studies with large sample sizes are now needed to confirm these findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The primary research studies were: Metformin (North West Research Ethics Committee, NW REC, 11/NW/0442, approved 19 August 2011), Weight loss (NW REC, 12/NW/0050, approved 23 January 2012), PREMIUM (NW REC, 14/NW/1236, approved 23 September 2014), PETALS (NRES Committee North West, Lancaster, 15/NW/0733, approved 18 September 2015) and DETECT (NW REC, Greater Manchester, 16/NW/0660, approved 16 September 2016). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Supervision and funding acquisition, EJC; Conceptualization, KN and EJC; study design, KN and EJC; statistical analysis, KN; writing, original draft preparation, KN, Review and editing KN, HJA, and EJC; all authors provided critical comments, read, and approved the final version for publication.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2021) 71:209–49. doi: 10.3322/caac.21660
2. Crosbie E, Morrison J. The Emerging Epidemic of Endometrial Cancer: Time to Take Action. *Cochrane Database Syst Rev* (2014) 12:1465–858. doi: 10.1002/14651858.ED000095
3. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M. Estimating the Global Cancer Incidence and Mortality in 2018: GLOBOCAN Sources and Methods. *Int J Cancer* (2019) 144:1941–53. doi: 10.1002/ijc.31937
4. Crosbie EJ, Kitson SJ, McAlpine JN, Mukhopadhyay A, Powell ME, Singh N. Endometrial Cancer. *Lancet* (2022) 399(10333):1412–28. doi: 10.1016/S0140-6736(22)00323-3
5. Njoku K, Barr CE, Crosbie EJ. Current and Emerging Prognostic Biomarkers in Endometrial Cancer. *Front Oncol* (2022) 12:890908. doi: 10.3389/fonc.2022.890908
6. Njoku K, Ramchander NC, Wan YL, Barr E, Crosbie EJ. Pre-Treatment Inflammatory Parameters Predict Survival From Endometrial Cancer: A Prospective Database Analysis. *Gynecol Oncol* (2021) 164:146–53. doi: 10.1016/j.ygyno.2021.11.009
7. Levine DA, Network CGAR. Integrated Genomic Characterization of Endometrial Carcinoma. *Nature* (2013) 497:67–73. doi: 10.1038/nature12113
8. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J. Final Validation of the ProMisE Molecular Classifier for Endometrial Carcinoma in a Large Population-Based Case Series. *Ann Oncol* (2018) 29:1180–8. doi: 10.1093/annonc/mdy058
9. Morrison J, Balega J, Buckley L, Clamp A, Crosbie EJ, Drew Y, et al. British Gynaecological Cancer Society (BGCS) Uterine Cancer Guidelines: Recommendations for Practice. *Eur J Obstet Gynecol Reprod Biol* (2022) 270:50–89. doi: 10.1016/j.ejogrb.2021.11.423
10. Robbins JR, Gayar OH, Zaki M, Mahan M, Buekers T, Elshaikh MA, et al. Impact of Age-Adjusted Charlson Comorbidity Score on Outcomes for Patients With Early-Stage Endometrial Cancer. *Gynecol Oncol* (2013) 131:593–7. doi: 10.1016/j.ygyno.2013.10.007
11. Ward KK, Shah NR, Saenz CC, McHale MT, Alvarez EA, Plaxe SC, et al. Cardiovascular Disease is the Leading Cause of Death Among Endometrial Cancer Patients. *Gynecol Oncol* (2012) 126:176–9. doi: 10.1016/j.ygyno.2012.04.013
12. Njoku K, Abiola J, Russell J, Crosbie EJ. Endometrial Cancer Prevention in High-Risk Women. *Best Pract Res Clin Obstet Gynaecol* (2020) 65:66–78. doi: 10.1016/j.bpobgyn.2019.12.005
13. Saed L, Varse F, Baradaran HR, Moradi Y, Khateri S, Friberg E, et al. The Effect of Diabetes on the Risk of Endometrial Cancer: An Updated a Systematic Review and Meta-Analysis. *BMC Cancer* (2019) 19:527. doi: 10.1186/s12885-019-5748-4
14. Shikata K, Ninomiya T, Kiyohara Y. Diabetes Mellitus and Cancer Risk: Review of the Epidemiological Evidence. *Cancer Sci* (2013) 104:9–14. doi: 10.1111/cas.12043
15. Tian W, Teng F, Zhao J, Gao J, Sun D, Liu G, et al. Estrogen and Insulin Synergistically Promote Type 1 Endometrial Cancer Progression. *Cancer Biol Ther* (2017) 18:1000–10. doi: 10.1080/15384047.2017.1394547
16. Zhang Z-H, Su P-Y, Hao J-H, Sun Y-H. The Role of Preexisting Diabetes Mellitus on Incidence and Mortality of Endometrial Cancer: A Meta-Analysis of Prospective Cohort Studies. *Int J Gynecol Cancer* (2013) 23(2):294–303. doi: 10.1097/IGC.0b013e31827b8430

17. Liao C, Zhang D, Mungo C, Tompkins DA, Zeidan AM. Is Diabetes Mellitus Associated With Increased Incidence and Disease-Specific Mortality in Endometrial Cancer? A Systematic Review and Meta-Analysis of Cohort Studies. *Gynecol Oncol* (2014) 135:163–71. doi: 10.1016/j.ygyno.2014.07.095
18. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP Guidelines for the Management of Patients With Endometrial Carcinoma. *Int J Gynecol Cancer* (2021) 31(1):12–39. doi: 10.1136/ijgc-2020-002230
19. Friberg E, Mantzoros CS, Wolk A. Diabetes and Risk of Endometrial Cancer: A Population-Based Prospective Cohort Study. *Cancer Epidemiol Prev Biomarkers* (2007) 16:276–80. doi: 10.1158/1055-9965.EPI-06-0751
20. Friberg E, Orsini N, Mantzoros CS, Wolk A. Diabetes Mellitus and Risk of Endometrial Cancer: A Meta-Analysis. *Diabetologia* (2007) 50:1365–74. doi: 10.1007/s00125-007-0681-5
21. Khan MMH, Mori M, Sakauchi F, Aklimunnessa K, Kubo T, Fujino Y, et al. Risk of Endometrial Cancer Mortality by Ever-Use of Sex Hormones and Other Factors in Japan. *Asian Pacific J Cancer Prev* (2006) 7:260–6.
22. Swerdlow AJ, Laing SP, Qiao Z, Slater SD, Burden AC, Botha JL, et al. Cancer Incidence and Mortality in Patients With Insulin-Treated Diabetes: A UK Cohort Study. *Br J Cancer* (2005) 92:2070–5. doi: 10.1038/sj.bjc.6602611
23. Kessler II. Cancer Mortality Among Diabetics. *J Natl Cancer Inst* (1970) 44:673–86. doi: 10.1093/jnci/44.3.673
24. Verlato G, Zoppini G, Bonora E, Muggeo M. Mortality From Site-Specific Malignancies in Type 2 Diabetic Patients From Verona. *Diabetes Care* (2003) 26:1047–51. doi: 10.2337/diacare.26.4.1047
25. Ebrahimi M, Heidari-Bakavoli AR, Shoeibi S, Mirhafez SR, Moohebati M, Esmaily H, et al. Association of Serum Hs-CRP Levels With the Presence of Obesity, Diabetes Mellitus, and Other Cardiovascular Risk Factors. *J Clin Lab Anal* (2016) 30:672–6. doi: 10.1002/jcla.21920
26. Verma S, Hussain ME. Obesity and Diabetes: An Update. *Diabetes Metab Syndr Clin Res Rev* (2017) 11:73–9. doi: 10.1016/j.dsx.2016.06.017
27. Connolly V, Unwin N, Sherriff P, Bilous R, Kelly W. Diabetes Prevalence and Socioeconomic Status: A Population Based Study Showing Increased Prevalence of Type 2 Diabetes Mellitus in Deprived Areas. *J Epidemiol Community Heal* (2000) 54:173–7. doi: 10.1136/jech.54.3.173
28. Nagle CM, Crosbie EJ, Brand A, Obermair A, Oehler MK, Quinn M, et al. The Association Between Diabetes, Comorbidities, Body Mass Index and All-Cause and Cause-Specific Mortality Among Women With Endometrial Cancer. *Gynecol Oncol* (2018) 150:99–105. doi: 10.1016/j.ygyno.2018.04.006
29. Sarfati D, Koczwara B, Jackson C. The Impact of Comorbidity on Cancer and its Treatment. *CA Cancer J Clin* (2016) 66:337–50. doi: 10.3322/caac.21342
30. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and Cancer: A Consensus Report. *Diabetes Care* (2010) 33:1674–85. doi: 10.2337/dc10-0666
31. Søgaard M, Thomsen RW, Bossen KS, Sørensen HT, Nørgaard M. The Impact of Comorbidity on Cancer Survival: A Review. *Clin Epidemiol* (2013) 5:3. doi: 10.2147/CLEP.S47150
32. Zhang W, Ayanian JZ, Zaslavsky AM. Patient Characteristics and Hospital Quality for Colorectal Cancer Surgery. *Int J Qual Heal Care* (2007) 19:11–20. doi: 10.1093/intqhc/mzl047
33. Kennedy GD, Rajamanickam V, O'Connor ES, Loconte NK, Foley EF, Leverson G, et al. Optimizing Surgical Care of Colon Cancer in the Older Adult Population. *Ann Surg* (2011) 253:508. doi: 10.1097/SLA.0b013e3181f19518
34. Lee L, Cheung WY, Atkinson E, Krzyzanowska MK. Impact of Comorbidity on Chemotherapy Use and Outcomes in Solid Tumors: A Systematic Review. *J Clin Oncol* (2011) 29:106–17. doi: 10.1200/JCO.2010.31.3049
35. Koppie TM, Serio AM, Vickers AJ, Vora K, Dalbagni G, Donat SM, et al. Age-Adjusted Charlson Comorbidity Score is Associated With Treatment Decisions and Clinical Outcomes for Patients Undergoing Radical Cystectomy for Bladder Cancer. *Cancer Interdiscip Int J Am Cancer Soc* (2008) 112:2384–92. doi: 10.1002/cncr.23462
36. Van Leeuwen BL, Rosenkranz KM, Feng LL, Bedrosian I, Hartmann K, Hunt KK, et al. The Effect of Under-Treatment of Breast Cancer in Women 80 Years of Age and Older. *Crit Rev Oncol Hematol* (2011) 79:315–20. doi: 10.1016/j.critrevonc.2010.05.010
37. O'Connor TL, Edge SB, Kossoff EB, Groman A, Wilding GE, Ademuyiwa FO, et al. Factors Affecting the Delivery of Adjuvant/Neoadjuvant Chemotherapy in Older Women With Breast Cancer. *J Geriatr Oncol* (2012) 3:320–8. doi: 10.1016/j.jgo.2012.06.006
38. Booth CM, Shepherd FA, Peng Y, Darling G, Li G, Kong W, et al. Adjuvant Chemotherapy for non-Small Cell Lung Cancer: Practice Patterns and Outcomes in the General Population of Ontario, Canada. *J Thorac Oncol* (2012) 7:559–66. doi: 10.1097/JTO.0b013e31823f43af
39. Xie W, Li T, Yang J, Shang M, Xiao Y, Li Q, et al. Metformin Use and Survival Outcomes in Endometrial Cancer: A Systematic Review and Meta-Analysis. *Oncotarget* (2017) 8:73079. doi: 10.18632/oncotarget.20388
40. Chu D, Wu J, Wang K, Zhao M, Wang C, Li L, et al. Effect of Metformin Use on the Risk and Prognosis of Endometrial Cancer: A Systematic Review and Meta-Analysis. *BMC Cancer* (2018) 18:1–11. doi: 10.1186/s12885-018-4334-5

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Multimodal MRI-Based Radiomics-Clinical Model for Preoperatively Differentiating Concurrent Endometrial Carcinoma From Atypical Endometrial Hyperplasia

Jieying Zhang[1], Qi Zhang[1], Tingting Wang[2], Yan Song[3], Xiaoduo Yu[1], Lizhi Xie[4], Yan Chen[1] and Han Ouyang[1]*

[1] Department of Diagnostic Radiology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [2] Department of Gynecologic Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [3] Department of Pathology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [4] MR Research China, GE Healthcare, Beijing, China

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Tea Lanisnik Rizner, University of Ljubljana, Slovenia

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Maria Picchio, San Raffaele Hospital (IRCCS), Italy

***Correspondence:**
Xiaoduo Yu
yxd136@139.com

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Objectives: To develop and validate a radiomics model based on multimodal MRI combining clinical information for preoperative distinguishing concurrent endometrial carcinoma (CEC) from atypical endometrial hyperplasia (AEH).

Materials and Methods: A total of 122 patients (78 AEH and 44 CEC) who underwent preoperative MRI were enrolled in this retrospective study. Radiomics features were extracted based on T2-weighted imaging (T2WI), diffusion-weighted imaging (DWI), and apparent diffusion coefficient (ADC) maps. After feature reduction by minimum redundancy maximum relevance and least absolute shrinkage and selection operator algorithm, single-modal and multimodal radiomics signatures, clinical model, and radiomics-clinical model were constructed using logistic regression. Receiver operating characteristic (ROC) analysis, calibration curves, and decision curve analysis were used to assess the models.

Results: The combined radiomics signature of T2WI, DWI, and ADC maps showed better discrimination ability than either alone. The radiomics-clinical model consisting of multimodal radiomics features, endometrial thickness >11mm, and nulliparity status achieved the highest area under the ROC curve (AUC) of 0.932 (95% confidential interval [CI]: 0.880-0.984), bootstrap corrected AUC of 0.922 in the training set, and AUC of 0.942 (95% CI: 0.852-1.000) in the validation set. Subgroup analysis further revealed that this model performed well for patients with preoperative endometrial biopsy consistent and inconsistent with postoperative pathologic data (consistent group, F1-score = 0.865; inconsistent group, F1-score = 0.900).

Conclusions: The radiomics model, which incorporates multimodal MRI and clinical information, might be used to preoperatively differentiate CEC from AEH, especially for patients with under- or over-estimated preoperative endometrial biopsy.

Keywords: radiomics, magnetic resonance imaging, endometrial hyperplasia, endometrial neoplasms, texture analysis

1 INTRODUCTION

Atypical endometrial hyperplasia (AEH), also known as endometrial intraepithelial neoplasia, is considered a direct precursor of endometrial carcinoma (EC). Approximately 40% of AEH will proceed to EC within 12 months of onset (1, 2). In addition, previous studies have found that 37%-43% of AEH patients who undergo hysterectomy are diagnosed with concurrent endometrial carcinoma (CEC) on final pathology (3, 4).

Given the high risk of progression and CEC, the recommended treatment of AEH is total hysterectomy (with bilateral salpingo-oophorectomy when possible) in women who do not desire pregnancy. In contrast, non-surgical management may be appropriate for patients who plan on becoming pregnant in the future or those with comorbidities precluding surgical management (5). Previous studies have suggested that up to 12% of CEC patients suffer from high-grade tumors with deep myometrial invasion and have a 3-7% risk of lymph node involvement (6–9). Therefore, besides hysterectomy with bilateral salpingo-oophorectomy, a proportion of CEC patients may benefit from lymph node assessment as a guide to adjuvant therapy (10, 11). However, it is impossible to perform sentinel lymph node (SLN) mapping after hysterectomy due to disruption of the lymphatic channels originating from the uterine corpus and cervix during operation. Hence, an accurate preoperative diagnosis of AEH or CEC is crucial for selecting candidates for proper surgery or conservative treatment.

A primary diagnosis of AEH is usually made using dilation and curettage, hysteroscopy-guided biopsy, or hysteroscopic endometrial resection. Yet, these methods may fail to provide adequate tissue and lead to an improper diagnosis (12). Recent evidence suggested that non-invasive imaging tools may promote an accurate pre-treatment assessment of endometrial changes and optimize treatment planning (13). Magnetic resonance imaging (MRI) is a routine imaging modality used for the high-resolution evaluation of endometrial pathologies. Compared to conventional MRI, which has a relatively weak predictive value of CEC in patients with AEH (14, 15), the apparent diffusion coefficient (ADC) can be used to distinguish benign from malignant endometrial lesions (16). Still, so far, no studies have reported on the value of ADC in differentiating CEC from AEH.

Radiomics is a quantitative approach that extracts features from medical images using data-characterization algorithms and has been widely applied for differential diagnosis of cancers, evaluating therapeutic effects, and predicting the recurrence, metastasis, and survival time (17–19). A previous study used ^{18}F-FDG PET/CT (positron emission tomography (PET) with 2-deoxy-2-[fluorine-18] fluoro-D-glucose (18F-FDG)) quantitative parameters and texture analysis to distinguish CEC from AEH effectively (20). However, to the best of our knowledge, no research has determined whether an MRI-based radiomics study can detect CEC in AEH patients.

Thus, this study aimed to develop and validate a multimodal MRI-based radiomics-clinical model for detecting CEC from AEH before operation noninvasively. Also, we investigated the model performance in patients with preoperative endometrial biopsy consistent or inconsistent with postoperative pathological data.

2 MATERIAL AND METHODS

2.1 Patients

Our institutional ethics committee approved this study and waived the informed consent from patients. We retrospectively reviewed data of patients from our hospital database.

In total, 321 patients who underwent gynecological surgery between January 2011 and December 2019 were pathologically confirmed with AEH or stage IA CEC. Inclusion criteria were: 1) AEH or stage IA CEC confirmed surgically and pathologically; 2) pelvic MRI performed within 20 days prior to gynecological surgery; 3) no tumor-related therapy received before MR examination. Exclusion criteria were the following: 1) lacking one of the following MRI sequences: sagittal T2-weighted imaging (T2WI), axial diffusion-weighted imaging (DWI), or the corresponding ADC map (n=3); 2) endometrium too thin (maximum thickness less than 4mm) to be assessed on MRI (n=10); 3) Poor image quality or obvious image artifacts affecting the visualization of tumor (n=6); 4) incomplete clinical data (n=5). Ultimately, MRI results of 122 patients (78 AEH and 44 CEC) were included in the study. The patients were divided into a training set (87 patients) and an independent validation set (35 patients) according to the time of treatment. A pathologist (Y.S.) with 20 years' experience in gynecologic pathology reviewed the pathological data. **Figure 1** shows the flowchart of patient enrollment.

Abbreviations: AEH, Atypical endometrial hyperplasia; ADC, Apparent diffusion coefficient; AUC, Area under the receiver operating characteristic curve; CEC, Concurrent endometrial carcinoma; DWI, Diffusion-weighted imaging; EC, Endometrial carcinoma; ET, Endometrial thickness; GLDM, Gray level dependence matrix; GLSZM, Gray level size zone matrix; ICC, Intraclass correlation coefficients; LASSO, Least absolute shrinkage and selection operator; MRI, Magnetic resonance imaging; mRMR, Minimum redundancy maximum relevance; NPV, Negative predictive value; PPV, Positive predictive value; SLN, Sentinel lymph node; ROC, Receiver operating characteristic curve; T2WI, T2-weighted imaging; VOI, Volume of interest.

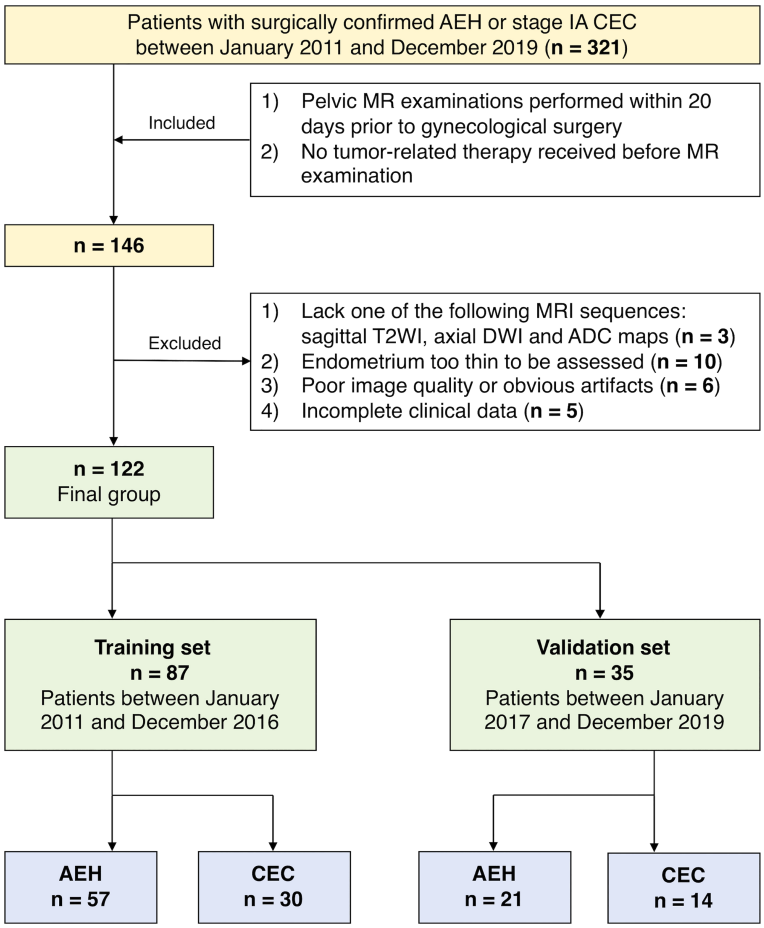

FIGURE 1 | Flowchart of patient enrollment in this study.

2.2 MRI Acquisition

All patients underwent conventional MR examination using 3.0T MR scanners (Signa HDxt and Discovery HD750, GE Medical System, Milwaukee, WI) with an eight-element phased-array wrap-around surface coil. Patients received an intramuscular injection of 10 mg raceanisodamine hydrochloride approximately 10 minutes before MRI to reduce bowel movement, excluding those with contraindications. The following sequences were included: sagittal T2WI and axial DWI. Diffusion gradients were applied in three orthogonal directions with b values of 0 and 800 s/mm^2, and DWI with b value of 800 s/mm^2 was involved in the analysis. ADC maps were manually generated from DWI on the post-processing workstation (Advantage Workstation 4.6; GE Medical System). Detailed sequence scanning parameters are shown in **Table 1**.

2.3 Clinical and Conventional MR Assessment

The following clinical data were collected from medical records: age, body mass index, menopausal status, childbearing history, history of metabolic syndrome or polycystic ovary syndrome, history of endocrine therapy for breast cancer, blood serum cancer antigen 125 and cancer antigen 19-9 level, and preoperative pathological data.

Two radiologists (J.Z. and X.Y., with 6- and 18-years' experience in gynecologic imaging, as Reader 1 and 2), who were blinded to the medical records and pathological data, independently measured endometrial stripe thickness on sagittal T2-weighted images. The average values were taken. Myometrial invasion [identified as interruption of the junction zone (21)] using all MR images was also assessed. The consistency between the two radiologists was evaluated by calculating Cohen's kappa coefficients. Discrepancies were resolved by discussion until consensus was achieved.

2.4 Data Analysis

2.4.1 Tumor Segmentation and Feature Extraction

Segmentation of images of the volume of interest (VOI) covering the whole tumor was performed using ITK-SNAP software (version 3.8.0, www.itksnap.org). AEH lesions would typically be

TABLE 1 | Detailed Sequences Scanning Parameters in Two MR Scanners.

Parameters	Axial T1WI	Axial T2WI	SagittalT2WI	Axial oblique T2WI	AxialDWI	Axial T1WI postcontrast
GE signa excite HD 3.0T						
Technique	FSE	FS FSE	FSE	FSE	SS-EPI	3D LAVA-XV
TR (ms)/TE (ms)	620/8.2	5900/121	4920/139.1	4900/131.5	4400/64.3	4.1/1.8
FOV (cm)	38	34	30	22	34	35
Matrix (phase × frequency)	320×224	320×256	320×256	320×256	256×256	350×350
Slice thickness (mm)	5	5	4	3	5	1
Slice gap	1	1	0.4	0	1	0
Average (NEX)	2	2	2	4	2	1
b-value (s/mm^2) *	–	–	–	–	0, 800	–
GE Discovery HD750 3.0T						
Technique	LAVA-Flex	FS FSE	FSE	FSE	SS-EPI	3D LAVA-XV
TR (ms)/TE (ms)	4.2/1.3	4650/85.0	4220/125.4	5500/102.0	4000/56.1	7.9/4.1
FOV (cm)	38	34	30	22	34	35
Matrix (phase × frequency)	320×224	320×256	320×256	320×256	128×128	350×350
Slice thickness (mm)	3	5	4	3	5	1
Slice gap	0	1	0.4	0	1	0
Average (NEX)	1	2	2	4	2	1
b-value (s/mm^2) *	–	–	–	–	0, 800	–

**ADC maps were calculated voxel by voxel with the monoexponential model using the formula: ADC = ln (S0/S800)/(b800−b0)*
where S800 and S0 are the signal intensities with and without a diffusion gradient, respectively.
T1WI, T1-weighted imaging; T2WI, T2-weighted imaging; FS, fat suppression; FSE, fast-recovery fast spin-echo; DWI, diffusion-weighted imaging; SS-EPI, single-shot echo-planar imaging; LAVA-Flex, liver acquisition with volume acceleration; LAVA-XV, liver acquisition with volume acceleration-extended volume; TR, repetition time; TE, echo time; FOV, field of view; NEX, number of excitations.

presented with intermediate signal intensity on T2WI, DWI, and the ADC map compared with normal endometrium. Some endometrial lesions would be detected as CEC if the lesion was presented with isointense or slightly lower signal intensity on T2WI, higher signal intensity on DWI, and a lower value on ADC map compared with adjacent endometrium. In contrast, the remaining CEC lesions could not be delineated. Representative cases are presented in **Figure S1**. Each VOI was manually drawn along the contour of the entire endometrium or tumor (with visible tumor) slice-by-slice by Reader 1 on T2WI, DWI, and ADC map. Hemorrhagic, necrotic, cystic areas, and adjacent normal tissues were avoided using T1-weighted images and dynamic contrast-enhanced images as references. With a 1-month interval, the above procedure was repeated by Reader 1 and 2 independently. Each extracted feature's inter- and intra-observer agreements were determined by calculating the intraclass correlation coefficients (ICC). Every case was then reviewed by another radiologist (H.O., with 30-years' experience in gynecologic imaging) to ensure high-quality final segmentation results.

The feature extraction was realized using an open-source Python package called Pyradiomics (22). Before feature extraction, we applied image normalization in T2WI and DWI sequences using the Pyradiomics normalization method by centering it at the mean with standard deviation based on all gray values in the image (not just those inside the segmentation), thereby reducing the potential effects introduced by scanners, scanning parameters, and protocols. Then we applied Z score normalization to ensure that the radiomics features were measured on the same scale. The radiomics features were classified into three categories according to the feature calculation method: (1) 14 shape-based features; (2) 18 first-order statistical features; (3) 68 texture features, including gray level co-occurrence matrix, gray level size zone matrix (GLSZM), gray level run length matrix, and gray level dependence matrix (GLDM). Detailed radiomics features are listed in **Table S1**.

2.4.2 Radiomic Feature Selection and Analysis

Stability analysis of radiomic features between inter-/intra-observer segmentations was first performed by removing the radiomic features with low reproducibility (ICC < 0.75). The remaining significant features were ranked using the minimum redundancy maximum relevance (mRMR) algorithm. Consequently, the top 10 features with low redundancy and high relevance were obtained for the following analyses.

The least absolute shrinkage and selection operator (LASSO) algorithm was applied to avoid overfitting. The 1-standard error of the minimum criteria (the 1-SE criteria) was used to tune the regularization parameter (λ) and for feature selection using 10-fold cross-validation. T2WI, DWI, and ADC radiomics scores (T2WI-score, DWI-score, and ADC-score) were calculated for each patient using a weighted linear combination of selected features. Finally, a combined radiomics signature (Radscore) was generated using logistic regression based on T2WI, DWI, and ADC features. **Figure 2** shows the workflow of radiomic analysis.

2.4.3 Clinical and Radiomics-Clinical Model Building, Discrimination, and Calibration

To select the optimal clinical parameters, the likelihood ratio test with Akaike's information criterion was applied as the stopping rule for stepwise logistic regression analysis. The model with the lowest Akaike's information criterion score was selected as a clinical model. Then, we developed a radiomics-clinical model based on Radscore and the optimal clinical parameters using multivariate logistic regression.

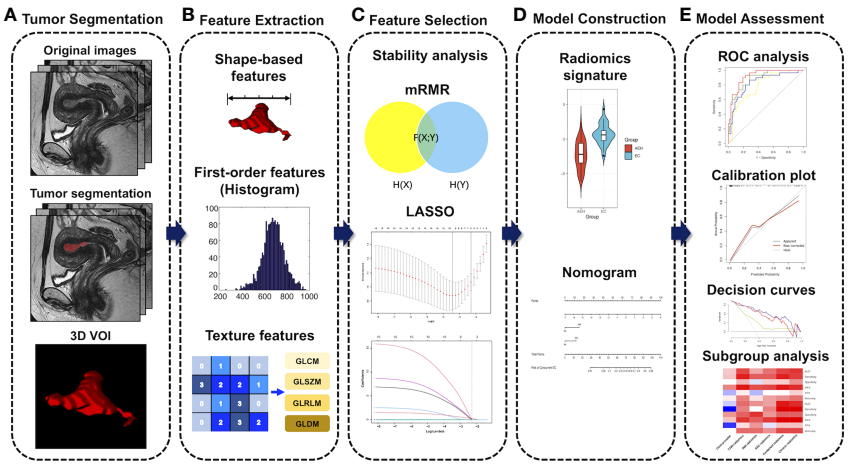

FIGURE 2 | Workflow of radiomic analysis. **(A)** MR imaging segmentation. Three-dimensional (3D) segmentation of tumors in MR images. **(B)** Radiomic feature extraction. Radiomic features, including shape, intensity, and texture, were extracted from the tumor volume. **(C)** Feature selection process. The stability analysis, the minimum redundancy maximum relevance (mRMR), and the least absolute shrinkage and selection operator (LASSO) algorithm were used for the radiomic feature selection. **(D)** Model construction. Radiomics signatures were constructed using a binary logistic regression model. Finally, a nomogram for the optimal model was developed. **(E)** Model assessment. The performances of our models were evaluated by discrimination, calibration, and clinical utility, as well as subgroup analysis. VOI, volume of interest; GLCM, gray level co-occurrence matrix; GLSZM, gray level size zone matrix; GLRLM, gray level run length matrix; GLDM, gray level dependence matrix.

2.5 Statistical Analysis

Statistical analyses were performed using R software (version 4.0.3; http://www.Rproject.org). Differences between groups were assessed using t-tests or Mann-Whitney U tests for continuous variables; Chi-square test or Fisher's exact test were applied for categorical variables. Receiver operating characteristic (ROC) curves were used to display and evaluate model performance. The area under the ROC curves (AUC), sensitivity, specificity, accuracy, and F1-score were used for evaluating the model performance. F1-score assumes that recall [equivalently, sensitivity, TP/(TP+FN)] and precision [equivalently, positive predictive value (PPV), TP/(TP+FP)] are of equal importance, where TP, FN, and FP represent true positive, false negative, and false positive, respectively. The higher F1-score synthetically reflects higher sensitivity and higher PPV. The formula for F1-score is as follows:

$$\text{F1} - \text{score} = \frac{2Precison \times Recall}{Precison + Recall}$$

DeLong's test was used to compare the AUC of each model. Calibration curves and the Hosmer-Lemeshow test were used to assess the goodness of fit of the models. Decision curve analysis (DCA) was conducted to estimate the clinical usefulness of the models by calculating the net benefits at different values of threshold probability. Model internal validation in the training set was performed using the enhanced bootstrap resampling method (n=1000), which obtained the estimates of optimism in the regression models to provide a bias-corrected AUC value through a Somers' D rank correlation metric whereby AUC = (1 + Somers' D)/2. A $p < 0.05$ was considered statistically significant.

3 RESULTS

3.1 Patient Characteristics

One hundred and twenty-two patients were enrolled in our study, including 78 AEH and 44 CEC patients. Baseline patients' characteristics and preoperative biopsy results in the training and validation sets are summarized in **Table 2**.

Based on the median, endometrial thickness (ET) was divided into ≤11mm and <11mm groups. Detailed information on ET in the subgroups (according to menopausal status and parity) of AEH and CEC patients is shown in **Table S2**. The consistency between the two radiologists was good to excellent in the evaluation of myometrial invasion (Kappa value=0.781) and measurement of ET (ICC = 0.908). In total, 29 (23.7%) patients (18 AEH and 11 CEC) had conflicting results between preoperative biopsy and postoperative pathology. Three (6.8%) patients in the CEC group had intermediate-risk EC (2 with non-endometrioid EC and 1 with high-grade tumor), and the remaining had low-risk EC, according to the 2021 ESGO/ESTRO/ESP guidelines for EC management (23).

3.2 Radiomics Signature Analysis

We extracted 300 features from the T2WI, DWI, and ADC maps of each VOI and reduced them to 283 by stability analysis. For T2WI, DWI, and ADC radiomics signature, the 7, 3, and 1 most relevant features were selected using the variable selection algorithm, respectively. Then, we determined the 5 top features consisting of 3 from T2WI, 1 from DWI, and 1 from ADC maps to build the combined radiomics signature (**Table 3**).

T2WI-score, DWI-score, ADC-score, and Radscore, calculated as the linear combination of these features with

TABLE 2 | Baseline Characteristics of Patients in the Training and Validation sets.

Characteristics	Training Set (n=87)			Validation Set (n=35)			$p^{\#}$ value
	AEH (n=57)	CEC (n=30)	*p* value	AEH (n=21)	CEC (n=14)	*p* value	
Age, years, mean ± SD	46.7 ± 4.9	46.7 ± 7.1	0.982	47.1 ± 5.2	48.2 ± 5.5	0.564	0.427
BMI, kg/m2†			0.610			0.697	0.752
≤24.9	26 (45.6)	11 (36.7)		12 (57.1)	7 (50.0)		
25~29.9	22 (38.6)	12 (40.0)		6 (28.6)	6 (42.9)		
≥30	9 (15.8)	7 (23.3)		3 (14.3)	1 (7.1)		
Menopausal Status†			0.377			0.721	0.148
Premenopausal	45 (78.9)	26 (86.7)		15 (71.4)	9 (64.3)		
Postmenopausal	12 (21.1)	4 (13.3)		6 (21.1)	4 (35.7)		
Nulliparity†	2 (3.5)	5 (16.7)	0.045*	1(4.8)	3 (21.4)	0.279	0.727
CA125 (+)	5 (8.8)	5 (16.7)	0.303	0 (0.0)	1 (7.1)	0.400	0.175
CA19-9 (+)	2 (3.5)	3 (10.0)	0.335	0 (0.0)	1 (7.1)	0.400	0.672
Diabetes	3 (5.3)	1 (3.3)	1.000	1 (4.8)	0 (0.0)	1.000	1.000
PCOS	0 (0.0)	1 (3.3)	0.345	0 (0.0)	1 (7.1)	0.400	0.493
History of endocrine therapy†	1 (1.8)	1 (3.3)	1.000	0 (0.0)	2 (14.3)	0.153	0.578
Endometrial Thickness†			0.005*			0.296	0.842
≤11mm	37 (64.9)	10 (33.3)		14 (66.7)	6 (42.9)		
>11mm	20 (35.1)	20 (66.7)		7 (33.3)	8 (57.1)		
Myometrial invasion†			0.126			0.685	0.295
No	51 (89.5)	23 (76.7)		17 (81.0%)	10 (71.4%)		
Yes	6 (10.5)	7 (23.3)		4(19.0%)	4(28.6%)		
Preoperative Endometrial biopsy†			<0.001*			0.002*	0.360
Hyperplasia without atypia	8 (14.0)	0 (0.0)		1 (4.8)	0 (0.0)		
Atypical hyperplasia	42 (73.7)	6 (20.0)		18 (85.7)	5 (35.7)		
Cancer	7 (12.3)	24 (80.0)		2 (9.5)	9 (64.3)		

†Data in parentheses are percentages.

**p < 0.05.*

$p^{\#}$ value represents the comparison between training and validation sets.

AEH, atypical endometrial hyperplasia; CEC, concurrent endometrial carcinoma; BMI, body mass index; CA125, cancer antigen 125; CA19-9, cancer antigen 19-9; PCOS, polycystic ovary syndrome.

coefficients of the logistic regression model, were all significantly higher in the CEC group than the AEH group in the training set (all $p < 0.001$; **Figure S2**). The combined radiomics signature achieved the highest AUC of 0.920 and bootstrap corrected AUC of 0.892 in the training set and was then confirmed in the validation set with an AUC of 0.942 (**Table 4**). As shown in **Figures 3A, B**, Delong's test demonstrated statistical differences in AUC values between the combined and DWI radiomics signature ($p = 0.030$) in the validation set. The mathematical formula used to calculate radiomics scores is shown in **Method S1**.

TABLE 3 | Features of T2WI, DWI, ADC, and Combined Radiomics Signatures.

Feature Name	Coefficients
T2WI Radiomics Signature	
Intercept	-1.252
glszm_SizeZoneNonUniformityNormalized	-0.850
glszm_SmallAreaLowGrayLevelEmphasis	0.397
firstorder_10Percentile	0.054
shape_Maximum2DDiameterSlice	-0.871
shape_Flatness	0.769
firstorder_Skewness	1.100
gldm_LargeDependenceLowGrayLevelEmphasis	0.604
DWI Radiomics Signature	
Intercept	-0.777
shape_Maximum2DDiameterRow	-0.444
firstorder_Kurtosis	-0.740
shape_Flatness	0.678
ADC Radiomics Signature	
Intercept	-0.920
firstorder_10Percentile	-1.595
Combined Radiomics Signature	
Intercept	-1.235
T2WI_shape_Maximum2DDiameterSlice	-0.773
T2WI_gldm_LargeDependenceLowGrayLevelEmphasis	0.750
DWI_shape_Flatness	0.585
T2WI_firstorder_Skewness	-1.472
ADC_firstorder_10Percentile	0.529

3.3 Clinical and Radiomics-Clinical Model Construction and Performance Assessment

In the clinical model, two parameters were independently associated with CEC in AEH patients, including the status of nulliparity (odds ratio [OR]: 7.082; 95% confidence interval [CI]: 1.159-43.288; p = 0.034) and ET>11mm (OR: 4.148, 95%CI: 1.553-11.073; p = 0.005). These two parameters, along with Radscore, were used to build the radiomics-clinical model. Nomogram (**Figure 3E**) was established for this model. The auto- and cross-correlations of selected features in the radiomics-clinical model derived from the training set are shown in **Figure S3**.

The clinical model showed moderate performance with AUC of 0.695 and 0.641, which was significantly improved by the radiomics-clinical model to 0.932 and 0.942 in the training and validation sets, respectively (Delong's test, $p < 0.001$; **Figures 3C, D**). There was no significant difference between

TABLE 4 | Performances of Different Models in the Training and Validation Sets.

Model	Data sets	AUC	95%CI	Bootstrap Corrected AUC	Sensitivity	Specificity	Accuracy	F1-score
T2WI Radiomics	Training Set	0.887	0.818-0.956	0.838	0.930	0.720	0.790	0.843
	Validation Set	0.895	0.778-1.000	NA	0.929	0.857	0.886	0.897
DWI Radiomics	Training Set	0.785	0.688-0.883	0.752	0.900	0.600	0.700	0.781
	Validation Set	0.735	0.566-0.903	NA	0.500	0.904	0.743	0.627
ADC Radiomics	Training Set	0.833	0.741-0.925	0.832	0.870	0.720	0.770	0.807
	Validation Set	0.854	0.729-0.979	NA	0.643	0.905	0.800	0.739
Combined Radiomics	Training Set	0.920	0.865-0.974	0.892	0.900	0.810	0.840	0.860
	Validation Set	0.942	0.857-1.000	NA	0.857	0.952	0.914	0.900
Clinical Model	Training Set	0.708	0.588-0.827	0.687	0.730	0.670	0.690	0.692
	Validation Set	0.641	0.448-0.834	NA	0.571	0.667	0.629	0.600
Clinical-Radiomics Model	Training Set	0.932	0.880-0.984	0.922	0.870	0.880	0.870	0.871
	Validation Set	0.942	0.852-1.000	NA	0.857	1.000	0.943	0.923

ACC, accuracy; AUC, area under the receiver operating characteristic curve; CI, confidence interval; NA, not applicable.

the AUC of the combined radiomics signature and radiomics-clinical model in the training and validation sets (Delong's test, $p<0.05$). Calibration curves showed good fitness for the radiomics-clinical model (Hosmer-Lemeshow test, $p = 0.933$ in the training set, 0.400 in the validation sets) (**Figures 4A, B**). The patients' risk scores, indicating the models' high classification ability, are shown in **Figures 4C, D**. DCA of the models is shown in **Figure 5**.

As shown in **Figure 6A**, in the subgroup of patients with preoperative endometrial biopsy inconsistent (under- or over-estimated) with postoperative pathology, the combined radiomics signature and radiomics-clinical model achieved the highest sensitivity and NPV of 1.000, with an AUC of 0.955 and 0.934, respectively. The F1-score of the two subgroups is shown in **Figure 6B**. The radiomics-clinical model showed good potency among the six classification models (consistent group, F1-score = 0.865; inconsistent group, F1-score = 0.900), while the combined radiomics signature performed even better in patients with inconsistent biopsy results (F1-score = 0.923).

4 DISCUSSION

In this study, we developed a multimodal MRI-based radiomics-clinical model for preoperative differentiation of CEC from AEH. The model consisting of radiomics features and clinical data (ET >11mm and nulliparity status) demonstrated the best discrimination ability and goodness of fit. Moreover, in patients with under- or over-estimated preoperative biopsy results, the sensitivity and NPV were greatly improved after applying the model with relatively high PPV. Furthermore, despite differences in the MR scanners among various subjects, the radiomics-clinical model revealed an excellent capacity for detecting CEC from AEH in the internal validation, with a bootstrap corrected AUC of 0.922 in the training set and AUC of 0.942 in the validation set, thus surpassing other models.

Previous studies (24–27) showed that AEH and EC shared common predisposing risk factors, such as age, postmenopausal status, nulliparity, obesity, diabetes, PCOS, and long-term tamoxifen therapy. Liakou et al. (14) found that myometrial invasion on conventional MRI was associated with increased CEC risk for AEH patients; nevertheless, the sensitivity and specificity of MRI in identifying cancer were poor (37% and 89%, respectively). In the current study, we adopted the aforementioned clinical parameters into our clinical predictive model. Nulliparity and ET >11mm observed on conventional MRI were found to be independently associated with the differentiation of CEC from AEH. Nulliparity is an established risk factor for endometrial cancer, and each pregnancy provides an additional risk reduction (28). The study of ET as a predictive factor for endometrial pathology with abnormal uterine bleeding is a debated topic with conflicting results, especially in premenopausal patients, since its predictive performance is affected by menstrual cycle phases. Vetter et al. (29) demonstrated that ET >2cm on preoperative transvaginal ultrasound was associated with increased odds of CEC in AEH patients while controlling for age. Wise et al. (30) proved a strong association between ET > 11 mm and AEH/EC in premenopausal women. Based on the median, we found that the same ET cut-off value (>11 mm) on MRI was associated with CEC in AEH patients in the current study. Moreover, our study produced consistent results that a higher proportion of CEC than AEH patients had an ET >11mm in different subgroups based on menopausal status and parity. However, the clinical model's performance was unsatisfactory, especially for patients with inconsistent preoperative biopsy results.

Next, we constructed radiomics signatures based on different MRI images (T2WI, DWI, and ADC maps). T2WI radiomics signature performed better for categorizing CEC and AEH than DWI. A reasonable explanation could be that T2WI is the critical conventional sequence of non-enhanced MRI in diagnosing endometrial diseases, providing detailed anatomical characteristics with high contrast and spatial resolution. On T2WI, AEH usually has a similar signal intensity with that of the normal endometrium, while EC shows intermediate-low signal intensity relative to hyperintense normal endometrium (21, 31). In this study, multiple T2WI radiomic features were selected in the T2WI radiomics signature, such as tumor shape, intensity, and gray level texture features (from GLSZM and GLDM), reflecting different aspects of intratumor heterogeneity and thus improving the discriminative ability of CEC and AEH.

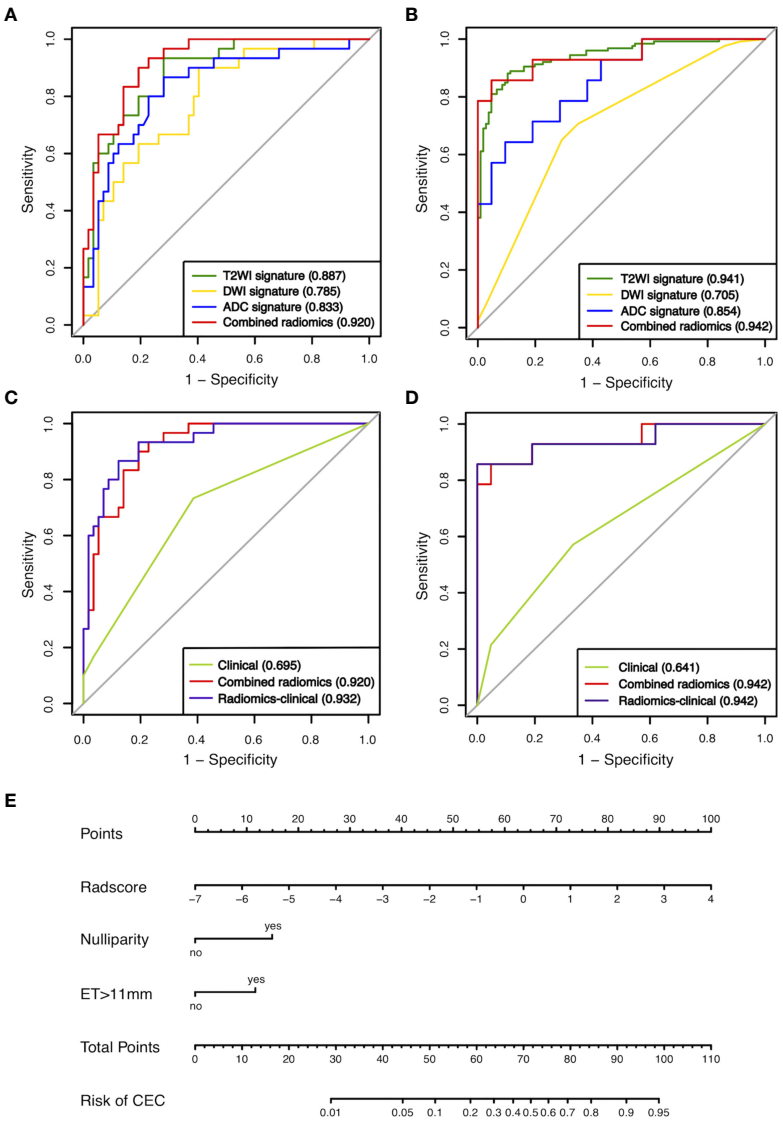

FIGURE 3 | ROCs of the four radiomics signatures in the training **(A)** and validation **(B)** sets. ROCs of the clinical model, radiomics signature, and radiomics-clinical model in the training **(C)** and validation sets **(D)**. **(E)** Preoperative nomogram of the radiomics-clinical model. ET, endometrial thickness.

CEC patients tend to have small tumors that may not be associated with endometrial thickening or have a signal intensity similar to that of the normal endometrium. In those cases, functional sequences, such as DWI, can be beneficial. A high b-value makes images more sensitive to water diffusion, thus increasing contrast enhancement between normal and cancerous tissue (32). Therefore, the presence of restricted diffusion on DWI within thickened endometrium will raise suspicion for the existence of EC. This study included only first-order statistics (Kurtosis) and shape-based features (Maximum 2D Diameter Row, Flatness) in the DWI radiomics signature. No other texture features were highly correlated to the classification task, probably due to its relatively poor spatial resolution. Flatness was included in both T2WI and DWI radiomics signatures, disillusioning largest from smallest principal components in the VOI shape, with a value range between 1 (non-flat, sphere-like) and 0 (a flat object, or single-slice segmentation). Flatness may provide information as complementation for ET in detecting small-size CEC from AEH.

Numerous studies have reported that ADC measurements (without confounding T1 or T2 effects of DWI signal) could be used as additional tools for differentiating between benign and malignant conditions (19, 33, 34). Moharamzad et al. (35) performed a meta-analysis and concluded that the combined sensitivity and specificity of mean ADC values for differentiating EC from benign lesions were 93% and 94%, respectively. Chen et al. (36) developed an MRI-based radiomics model including ADC_10Percentile for distinguishing EC from its benign mimics.

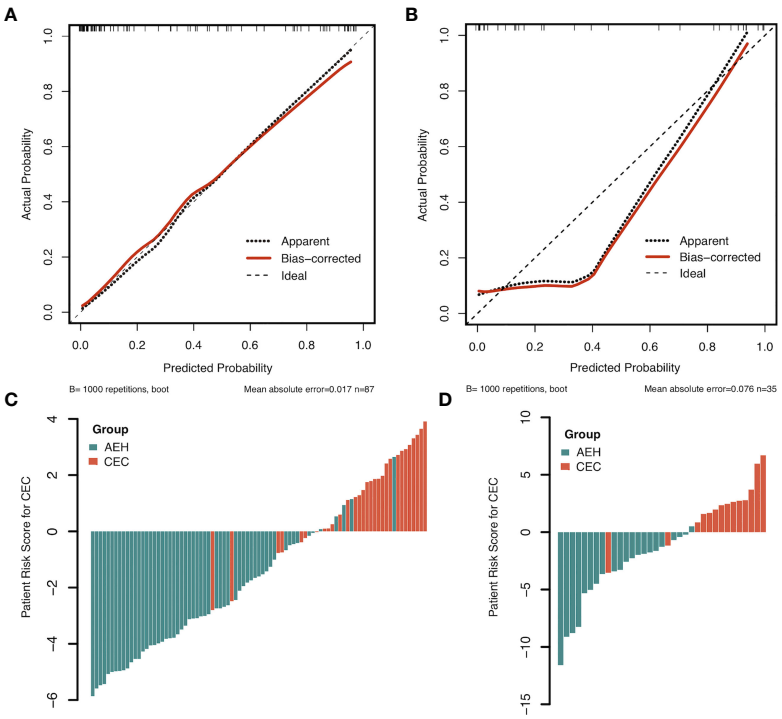

FIGURE 4 | The calibration plots of the radiomics-clinical model in the training **(A)** and validation sets **(B)**. Patient risk scores output by the radiomics-clinical model in the training **(C)** and validation sets **(D)**, while orange bars show scores for those who have concurrent endometrial carcinoma.

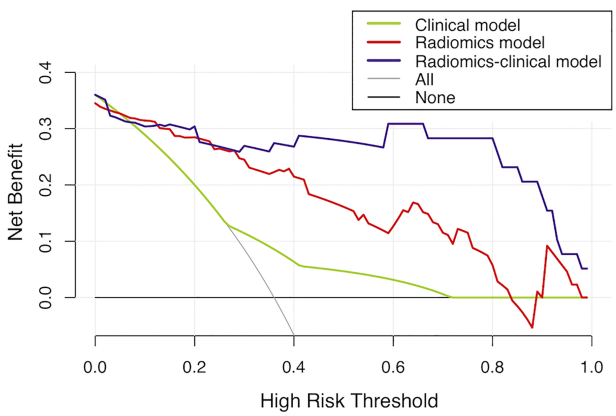

FIGURE 5 | Decision curve analysis for the models in the validation set. It can be concluded that when the threshold probability is over 30% approximately, the radiomics-clinical model could provide extra profits over the "treat-all" or "treat-none" scheme, the combined radiomics signature, and the clinical model.

Furthermore, Yan and colleagues (37) selected ADC_10Percentile as the only component of ADC radiomics signature in developing a radiomic nomogram predicting high-risk EC preoperatively. Similarly, we found that ADC_10Percentile may further promote the differentiation of CEC from AEH, compared to mean ADC values. A possible explanation is that lower percentiles of ADC may better represent aggressive solid components within CEC (38).

Finally, we discovered that a combined radiomics signature and radiomics-clinical model obtained more precise and comprehensive information about the tumors and yielded better diagnostic performance in the classification tasks than single-modal signatures. In clinical practice, it commonly happens that endometrial sampling is not possible (usually due to cervical stenosis) or the histopathology results are inconclusive or inconsistent with the clinical suspicion. Our study proved that

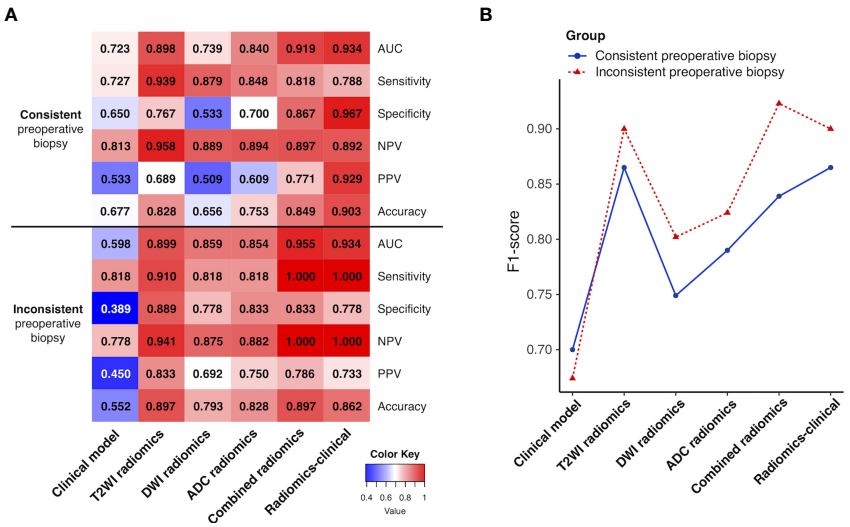

FIGURE 6 | **(A)** Heatmap showing the models' performance in the subgroups of patients with preoperative endometrial biopsy consistent and inconsistent with postoperative pathologic data. A deeper red indicates a larger value. **(B)** Line chart of the F1-score of the models in two subgroups. NPV, negative predictive value; PPV, positive predictive value.

the combined radiomics signature and radiomics-clinical models performed fairly well, especially for patients with preoperative endometrial biopsy inconsistent with postoperative pathologic data, thus indicating the supplementary value of MRI-based radiomics to preoperative endometrial biopsy.

Accurate preoperative prediction of the presence of CEC in AEH patients is vital for making proper personalized treatment decisions and assessing the prognosis of patients. Previous studies exploring the risk of CEC in patients with AEH have mainly focused on examining clinical factors such as sampling methods and histologic characteristics of AEH (39, 40). For the first time, we have developed and validated a multimodal MRI-based radiomics-clinical model for evaluating tumor heterogeneity and thus detecting CEC from AEH preoperatively. Strengths of this study include final pathology review at a single institution and the inclusion of clinical data as well as objective quantitative parameter (Radscore) to better predict the risk of underlying cancer at the time of hysterectomy for AEH. Knowledge of lymph node status in EC patients would allow a more tailored recommendation for postoperative therapy or surveillance. More recently, SLN mapping has been introduced into the surgical management of EC to obtain adequate nodal status information with a reduction in lymphadenectomy-related morbidity (such as lymphedema and lymphocele) (41). It is essential to know that the ability to perform SLN mapping in EC depends on intact lymphatic channels, and it cannot be performed after hysterectomy (29). AEH patients diagnosed with high-risk EC at the time of hysterectomy alone would then subsequently require a full lymphadenectomy. Therefore, for AEH patients with a high risk of CEC evaluated by our preoperative radiomics-clinical model, SLN mapping during hysterectomy should be considered.

The present study has some limitations. First, this was a retrospective study conducted at a single center and with a relatively small sample size. We have to acknowledge that despite the results of this study being promising, further investigation with larger study cohorts is necessary to validate our preliminary study. Second, AEH could not be accurately contoured with similar signal intensity to normal endometrium, while some CEC lesions could be detected on multimodal MR images (we contoured the visible tumor as VOIs in these cases). The bias introduced by inconsistency in VOI drawing was inevitable; however, it reflected the "real world" of routine diagnostic work. It was minimized by consulting another experienced radiologist in our study. Third, although we excluded patients with ET<4mm in this study because of the limitation of visual evaluation, the risk of developing CEC was relatively low in both pre- and post-menopausal women (42, 43). Finally, genomic information was not yet obtained and incorporated into our models. A combination of gene marker panels and radiomic features could have an extraordinary impact on the management of AEH in future studies.

To sum up, this new diagnostic model incorporating multimodal MRI-based radiomics and clinical information may be used to distinguish CEC from AEH noninvasively and effectively before the operation, especially for patients with under- or over-estimated preoperative endometrial biopsy. Nevertheless, a multicenter study with a larger dataset is needed to further validate our models' reproducibility and generalizability.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of National Cancer Center/ National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JZ: conceptualization, formal analysis, validation, visualization, funding acquisition, writing - original draft, writing - review & editing. QZ: investigation, data curation, software. TW: conceptualization, investigation, data curation. YS: investigation, resources. XY: conceptualization, methodology, formal analysis, writing - review & editing. LX: resources, software. YC: project administration. HO: project administration, supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.887546/full#supplementary-material

REFERENCES

1. Mutter GL, Kauderer J, Baak JP, Alberts D. Biopsy Histomorphometry Predicts Uterine Myoinvasion by Endometrial Carcinoma: A Gynecologic Oncology Group Study. *Hum Pathol* (2008) 39(6):866–74. doi: 10.1016/j.humpath.2007.09.023
2. Baak JP, Mutter GL, Robboy S, van Diest PJ, Uyterlinde AM, Orbo A, et al. The Molecular Genetics and Morphometry-Based Endometrial Intraepithelial Neoplasia Classification System Predicts Disease Progression in Endometrial Hyperplasia More Accurately Than the 1994 World Health Organization Classification System. *Cancer* (2005) 103(11):2304–12. doi: 10.1002/cncr.21058
3. Trimble CL, Kauderer J, Zaino R, Silverberg S, Lim PC, Burke JJ 2nd, et al. Concurrent Endometrial Carcinoma in Women With a Biopsy Diagnosis of Atypical Endometrial Hyperplasia: A Gynecologic Oncology Group Study. *Cancer* (2006) 106(4):812–9. doi: 10.1002/cncr.21650
4. Rakha E, Wong SC, Soomro I, Chaudry Z, Sharma A, Deen S, et al. Clinical Outcome of Atypical Endometrial Hyperplasia Diagnosed on an Endometrial Biopsy: Institutional Experience and Review of Literature. *Am J Surg Pathol* (2012) 36(11):1683–90. doi: 10.1097/PAS.0b013e31825dd4ff
5. Auclair MH, Yong PJ, Salvador S, Thurston J, Colgan TTJ, Sebastianelli A. Guideline No. 392-Classification and Management of Endometrial Hyperplasia. *J Obstet Gynaecol Can* (2019) 41(12):1789–800. doi: 10.1016/j.jogc.2019.03.025
6. Karamursel BS, Guven S, Tulunay G, Kucukali T, Ayhan A. Which Surgical Procedure for Patients With Atypical Endometrial Hyperplasia? *Int J Gynecol Cancer* (2005) 15(1):127–31. doi: 10.1111/j.1048-891X.2005.15013.x
7. Touhami O, Grégoire J, Renaud MC, Sebastianelli A, Grondin K, Plante M. The Utility of Sentinel Lymph Node Mapping in the Management of Endometrial Atypical Hyperplasia. *Gynecol Oncol* (2018) 148(3):485–90. doi: 10.1016/j.ygyno.2017.12.026
8. Whyte JS, Gurney EP, Curtin JP, Blank SV. Lymph Node Dissection in the Surgical Management of Atypical Endometrial Hyperplasia. *Am J Obstet Gynecol* (2010) 202(2):176.e1–.e4. doi: 10.1016/j.ajog.2009.10.855
9. Costales AB, Schmeler KM, Broaddus R, Soliman PT, Westin SN, Ramirez PT, et al. Clinically Significant Endometrial Cancer Risk Following a Diagnosis of Complex Atypical Hyperplasia. *Gynecol Oncol* (2014) 135 (3):451–4. doi: 10.1016/j.ygyno.2014.10.008
10. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: Diagnosis, Treatment and Follow-Up. *Int J Gynecol Cancer* (2016) 26(1):2–30. doi: 10.1097/igc.0000000000000609
11. Jang JW, Lee LJ. External Beam, Brachytherapy, or Chemotherapy? Defining Adjuvant Therapy for Early-Stage and High- and High-Intermediate-Risk Endometrial Cancer. *J Clin Oncol* (2019) 37(21):1778–84. doi: 10.1200/jco.19.00362
12. Clark TJ, Voit D, Gupta JK, Hyde C, Song F, Khan KS. Accuracy of Hysteroscopy in the Diagnosis of Endometrial Cancer and Hyperplasia: A Systematic Quantitative Review. *JAMA* (2002) 288(13):1610–21. doi: 10.1001/jama.288.13.1610
13. Haldorsen IS, Salvesen HB. What Is the Best Preoperative Imaging for Endometrial Cancer? *Curr Oncol Rep* (2016) 18(4):25. doi: 10.1007/s11912-016-0506-0
14. Liakou CG, La Russa MC, Akrivos N, Ames V, Scott-Barrett S, Duncan TJ, et al. The Role of Magnetic Resonance Imaging in the Pre-Operative Evaluation of Women Diagnosed With Atypical Endometrial Hyperplasia. *Anticancer Res* (2020) 40(5):2989–93. doi: 10.21873/anticanres.14279
15. Natarajan P, Vinturache A, Hutson R, Nugent D, Broadhead T. The Value of MRI in Management of Endometrial Hyperplasia With Atypia. *World J Surg Oncol* (2020) 18(1):34. doi: 10.1186/s12957-020-1811-5
16. Fujii S, Matsusue E, Kigawa J, Sato S, Kanasaki Y, Nakanishi J, et al. Diagnostic Accuracy of the Apparent Diffusion Coefficient in Differentiating Benign From Malignant Uterine Endometrial Cavity Lesions: Initial Results. *Eur Radiol* (2008) 18(2):384–9. doi: 10.1007/s00330-007-0769-9
17. Zhao L, Gong J, Xi Y, Xu M, Li C, Kang X, et al. MRI-Based Radiomics Nomogram May Predict the Response to Induction Chemotherapy and Survival in Locally Advanced Nasopharyngeal Carcinoma. *Eur Radiol* (2020) 30(1):537–46. doi: 10.1007/s00330-019-06211-x
18. Wormald BW, Doran SJ, Ind TE, D'Arcy J, Petts J, deSouza NM. Radiomic Features of Cervical Cancer on T2-And Diffusion-Weighted MRI: Prognostic Value in Low-Volume Tumors Suitable for Trachelectomy. *Gynecol Oncol* (2020) 156(1):107–14. doi: 10.1016/j.ygyno.2019.10.010
19. Xie H, Hu J, Zhang X, Ma S, Liu Y, Wang X. Preliminary Utilization of Radiomics in Differentiating Uterine Sarcoma From Atypical Leiomyoma: Comparison on Diagnostic Efficacy of MRI Features and Radiomic Features. *Eur J Radiol* (2019) 115:39–45. doi: 10.1016/j.ejrad.2019.04.004
20. Wang T, Sun H, Guo Y, Zou L. (18)F-FDG PET/CT Quantitative Parameters and Texture Analysis Effectively Differentiate Endometrial Precancerous Lesion and Early-Stage Carcinoma. *Mol Imaging* (2019) 18:1–10. doi: 10.1177/1536012119856965
21. Takeuchi M, Matsuzaki K, Uehara H, Yoshida S, Nishitani H, Shimazu H. Pathologies of the Uterine Endometrial Cavity: Usual and Unusual Manifestations and Pitfalls on Magnetic Resonance Imaging. *Eur Radiol* (2005) 15(11):2244–55. doi: 10.1007/s00330-005-2814-x
22. van Griethuysen JJM, Fedorov A, Parmar C, Hosny A, Aucoin N, Narayan V, et al. Computational Radiomics System to Decode the Radiographic Phenotype. *Cancer Res* (2017) 77(21):e104–e7. doi: 10.1158/0008-5472.CAN-17-0339

23. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP Guidelines for the Management of Patients With Endometrial Carcinoma. *Int J Gynecol Cancer* (2021) 31(1):12–39. doi: 10.1136/ijgc-2020-002230
24. Wise MR, Jordan V, Lagas A, Showell M, Wong N, Lensen S, et al. Obesity and Endometrial Hyperplasia and Cancer in Premenopausal Women: A Systematic Review. *Am J Obstet Gynecol* (2016) 214(6):689–97. doi: 10.1016/j.ajog.2016.01.175
25. Giannella L, Cerami LB, Setti T, Bergamini E, Boselli F. Prediction of Endometrial Hyperplasia and Cancer Among Premenopausal Women With Abnormal Uterine Bleeding. *BioMed Res Int* (2019) 2019:8598152. doi: 10.1155/2019/8598152
26. Weber AM, Belinson JL, Piedmonte MR. Risk Factors for Endometrial Hyperplasia and Cancer Among Women With Abnormal Bleeding. *Obstet Gynecol* (1999) 93(4):594–8. doi: 10.1016/s0029-7844(98)00469-4
27. Sanderson PA, Critchley HO, Williams AR, Arends MJ, Saunders PT. New Concepts for an Old Problem: The Diagnosis of Endometrial Hyperplasia. *Hum Reprod Update* (2017) 23(2):232–54. doi: 10.1093/humupd/dmw042
28. Troisi R, Bjorge T, Gissler M, Grotmol T, Kitahara CM, Myrtveit Saether SM, et al. The Role of Pregnancy, Perinatal Factors and Hormones in Maternal Cancer Risk: A Review of the Evidence. *J Intern Med* (2018) 283(5):430–45. doi: 10.1111/joim.12747
29. Vetter MH, Smith B, Benedict J, Hade EM, Bixel K, Copeland LJ, et al. Preoperative Predictors of Endometrial Cancer at Time of Hysterectomy for Endometrial Intraepithelial Neoplasia or Complex Atypical Hyperplasia. *Am J Obstet Gynecol* (2020) 222(1):60.e1–.e7. doi: 10.1016/j.ajog.2019.08.002
30. Wise MR, Gill P, Lensen S, Thompson JM, Farquhar CM. Body Mass Index Trumps Age in Decision for Endometrial Biopsy: Cohort Study of Symptomatic Premenopausal Women. *Am J Obstet Gynecol* (2016) 215(5):598.e1–e8. doi: 10.1016/j.ajog.2016.06.006
31. Pintican R, Bura V, Zerunian M, Smith J, Addley H, Freeman S, et al. MRI of the Endometrium - From Normal Appearances to Rare Pathology. *Br J Radiol* (2021) 94:20201347. doi: 10.1259/bjr.20201347
32. Levy A, Medjhoul A, Caramella C, Zareski E, Berges O, Chargari C, et al. Interest of Diffusion-Weighted Echo-Planar MR Imaging and Apparent Diffusion Coefficient Mapping in Gynecological Malignancies: A Review. *J Magn Reson Imaging* (2011) 33(5):1020–7. doi: 10.1002/jmri.22546
33. Zhang Q, Peng Y, Liu W, Bai J, Zheng J, Yang X, et al. Radiomics Based on Multimodal MRI for the Differential Diagnosis of Benign and Malignant Breast Lesions. *J Magn Reson Imaging* (2020) 52:596–607. doi: 10.1002/jmri.27098
34. Xu M, Fang M, Zou J, Yang S, Yu D, Zhong L, et al. Using Biparametric MRI Radiomics Signature to Differentiate Between Benign and Malignant Prostate Lesions. *Eur J Radiol* (2019) 114:38–44. doi: 10.1016/j.ejrad.2019.02.032
35. Moharamzad Y, Davarpanah AH, Yaghobi Joybari A, Shahbazi F, Esmaeilian Toosi L, Kooshkiforooshani M, et al. Diagnostic Performance of Apparent Diffusion Coefficient (ADC) for Differentiating Endometrial Carcinoma From Benign Lesions: A Systematic Review and Meta-Analysis. *Abdom Radiol (NY)* (2020) 46:1115–28. doi: 10.1007/s00261-020-02734-w
36. Chen X, Wang X, Gan M, Li L, Chen F, Pan J, et al. MRI-Based Radiomics Model for Distinguishing Endometrial Carcinoma From Benign Mimics: A Multicenter Study. *Eur J Radiol* (2021) 146:110072. doi: 10.1016/j.ejrad.2021.110072
37. Yan BC, Li Y, Ma FH, Feng F, Sun MH, Lin GW, et al. Preoperative Assessment for High-Risk Endometrial Cancer by Developing an MRI- and Clinical-Based Radiomics Nomogram: A Multicenter Study. *J Magn Reson Imaging* (2020) 52(6):1872–82. doi: 10.1002/jmri.27289
38. Kang Y, Choi SH, Kim YJ, Kim KG, Sohn CH, Kim JH, et al. Gliomas: Histogram Analysis of Apparent Diffusion Coefficient Maps With Standard- or High-B-Value Diffusion-Weighted MR Imaging–Correlation With Tumor Grade. *Radiology* (2011) 261(3):882–90. doi: 10.1148/radiol.11110686
39. Giannella L, Delli Carpini G, Sopracordevole F, Papiccio M, Serri M, Giorda G, et al. Atypical Endometrial Hyperplasia and Unexpected Cancers at Final Histology: A Study on Endometrial Sampling Methods and Risk Factors. *Diag (Basel)* (2020) 10(7):474. doi: 10.3390/diagnostics10070474
40. Leitao MMJr., Han G, Lee LX, Abu-Rustum NR, Brown CL, Chi DS, et al. Complex Atypical Hyperplasia of the Uterus: Characteristics and Prediction of Underlying Carcinoma Risk. *Am J Obstet Gynecol* (2010) 203(4):349.e1–6. doi: 10.1016/j.ajog.2010.05.004
41. Fanfani F, Monterossi G, Di Meo ML, La Fera E, Dell'Orto F, Gioe A, et al. Standard Ultra-Staging Compared to One-Step Nucleic Acid Amplification for the Detection of Sentinel Lymph Node Metastasis in Endometrial Cancer Patients: A Retrospective Cohort Comparison. *Int J Gynecol Cancer* (2020) 30(3):372–7. doi: 10.1136/ijgc-2019-000937
42. Visser NC, Sparidaens EM, van den Brink JW, Breijer MC, Boss EA, Veersema S, et al. Long-Term Risk of Endometrial Cancer Following Postmenopausal Bleeding and Reassuring Endometrial Biopsy. *Acta Obstet Gynecol Scand* (2016) 95(12):1418–24. doi: 10.1111/aogs.13022
43. Ozdemir S, Celik C, Gezginc K, Kiresi D, Esen H. Evaluation of Endometrial Thickness With Transvaginal Ultrasonography and Histopathology in Premenopausal Women With Abnormal Vaginal Bleeding. *Arch Gynecol Obstet* (2010) 282(4):395–9. doi: 10.1007/s00404-009-1290-y

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An Applicable Machine Learning Model Based on Preoperative Examinations Predicts Histology, Stage, and Grade for Endometrial Cancer

Ying Feng[1†], Zhixiang Wang[2†], Meizhu Xiao[3], Jinfeng Li[3], Yuan Su[1], Bert Delvoux[4], Zhen Zhang[2], Andre Dekker[2], Sofia Xanthoulea[4], Zhiqiang Zhang[3], Alberto Traverso[2], Andrea Romano[4], Zhenyu Zhang[3], Chongdong Liu[3], Huiqiao Gao[3‡], Shuzhen Wang[3*‡] and Linxue Qian[1]*

[1] Department of Ultrasound, Beijing Friendship Hospital, Capital Medical University, Beijing, China, [2] Department of Radiation Oncology (Maastro), GROW School for Oncology and Reproduction, Maastricht University Medical Centre+, Maastricht, Netherlands, [3] Department of Obstetrics and Gynecology, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China, [4] Department of Obstetrics and Gynecology, GROW-School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, Netherlands

Purpose: To build a machine learning model to predict histology (type I and type II), stage, and grade preoperatively for endometrial carcinoma to quickly give a diagnosis and assist in improving the accuracy of the diagnosis, which can help patients receive timely, appropriate, and effective treatment.

Materials and Methods: This study used a retrospective database of preoperative examinations (tumor markers, imaging, diagnostic curettage, etc.) in patients with endometrial carcinoma. Three algorithms (random forest, logistic regression, and deep neural network) were used to build models. The AUC and accuracy were calculated. Furthermore, the performance of machine learning models, doctors' prediction, and doctors with the assistance of models were compared.

Results: A total of 329 patients were included in this study with 16 features (age, BMI, stage, grade, histology, etc.). A random forest algorithm had the highest AUC and Accuracy. For histology prediction, AUC and accuracy was 0.69 (95% CI=0.67-0.70) and 0.81 (95%CI=0.79-0.82). For stage they were 0.66 (95% CI=0.64-0.69) and 0.63 (95% CI=0.61-0.65) and for differentiation grade 0.64 (95% CI=0.63-0.65) and 0.43 (95% CI=0.41-0.44). The average accuracy of doctors for histology, stage, and grade was 0.86 (with AI) and 0.79 (without AI), 0.64 and 0.53, 0.5 and 0.45, respectively. The accuracy of

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***Correspondence:**
Huiqiao Gao
hsmy1230@163.com
Shuzhen Wang
darrywang2003@163.com

†These authors have contributed equally to this work and share the first authorship

‡These authors have contributed equally to this work

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doctors' prediction with AI was higher than that of Random Forest alone and doctors' prediction without AI.

Conclusion: A random forest model can predict histology, stage, and grade of endometrial cancer preoperatively and can help doctors in obtaining a better diagnosis and predictive results.

Keywords: machine learning, endometrial carcinoma, diagnosis, prediction, random forest, preoperatively

1 INTRODUCTION

Endometrial carcinoma (EC) represents the sixth most common malignant tumor worldwide (1). In 2020, the number of new cases of endometrial cancer was 417,367, and the number of new deaths was 97,370 (1). This may be due to increased obesity, aging, and physical inactivity (2, 3). Endometrial carcinoma occurs most commonly in postmenopausal women (4). The first symptom is often abnormal vaginal bleeding. Transvaginal ultrasound is an effective examination to evaluate the presence of endometrial carcinoma, besides pelvic and physical examination (2, 5). A histopathology diagnosis is commonly assessed by dilation and curettage (D&C) or endometrial biopsy before surgery. However, the preoperative endometrial biopsy and final diagnosis are not completely consistent with only a moderate agreement rate on grade, especially for grade 2 tumors (2). In addition, other serological and imaging tests are routine tests for the diagnosis of endometrial carcinoma (2, 3).

With the development of computer science, clinical decision support systems (CDSSs) are being developed. A CDSS is defined as a system that enhances clinical information and medical knowledge to help doctors and nurses with clinical decisions for better health care (6). CDSS is a major subject of medical artificial intelligence (AI). CDSS can be used pre-diagnosis (prepare diagnoses), during diagnosis (review and filter diagnoses), and post-diagnosis (predict future events).

However, there are no studies that use an AI model to predict histology, stage, and grade for endometrial carcinoma based on the preoperative examinations. Such an AI model can be a part of an endometrial cancer CDSS to improve the efficiency of doctors, reduce the rate of misdiagnosis, and improve the quality of health care.

Machine learning (ML), a type of AI (7), is widely used in medical fields, such as anatomy, medical diagnoses, and brain-machine interfaces (8). In 2022 Otani et al. proposed an ML-based classifier to predict the EC risk from the multiparametric magnetic resonance images (MRI) (9). And, in 2021, Nakajo et al. proved that an 18F-FDG PET-based radiomic analysis using a machine learning approach may be useful for predicting tumor progression and prognosis in patients with endometrial cancers (10).

In this study, we used ML to build three models to predict histology (type I and type II), stage, and grade for endometrial carcinoma to quickly give a diagnosis and assist in improving the accuracy of the diagnosis, which can help patients receive timely, appropriate, and effective treatment.

2 METHODS

2.1 Study Subject

This study used a retrospective database of preoperative examinations in patients with endometrial carcinoma who were first treated in the Department of Obstetrics and Gynecology at Beijing Chaoyang Hospital, Capital Medical University, from January 2000 to April 2014. Inclusion criteria were as follows: (1) undergoing surgical treatment at Beijing Chaoyang Hospital, (2) confirmation of endometrial carcinoma by postoperative pathology, (3) without neoadjuvant chemotherapy and hormone therapy, (4) all treatments have been completed, (5) complete clinical-pathological data. The case exclusion criteria were: (1) presence of primary malignant tumors of other organs, (2) metastatic cancer caused by malignant tumors of other organs, (3) not the first-time surgical treatment at Beijing Chaoyang Hospital, (4) with neoadjuvant chemotherapy and hormone therapy, (5) incomplete clinical-pathological data. The obtained data included age, BMI, childbirth history, preoperative serum tumor markers, imaging results, histopathology diagnosis after D&C, hypertension, diabetes, menopause, symptoms, postoperative histology, stage based on the 2014 International Federation of Gynecology and Obstetrics (FIGO) staging system (11), and grade. Ethics approval for this research was given by the Beijing Chaoyang Hospital, Capital Medical University.

2.2 Data and Machine Learning Algorithms

A total of 16 features mentioned above were used for the development of the classification models.

For data preprocessing, first, we transformed semi-structured and unstructured features such as preoperative serum tumor markers and imaging results into structured features. Then, we normalized the continuous variables such as age and BMI into 0 to 1.

In this study, we trained and compared three classifiers, including logic regression(LR) (12), random forest (RF) (13), and a deep neural network(DNN) (14). The DNN is based on the extension of the perceptron: a neural network with many hidden layers. Random forest is an ensemble algorithm (Ensemble Learning), which belongs to the Bagging algorithms. By combining multiple weak classifiers, the result is voted or averaged, so that the result of the overall model has higher accuracy and generalization performance.

The DNN model was composed of two fully connected layers which have a Rectified Linear Unit (ReLU) activation function to increase the nonlinearity of the neural network model and dropout layers with the rate of 0.5 to avoid over-fitting and one fully connected layer without activation function. The cross-entropy loss was used to guide the training process by using a stochastic gradient descent (SGD) optimizer with a 0.0002 learning rate. The random forest included 100 decision trees.

The classification models were trained and tested with the selected features to predict the histology, stage, and grade of endometrial cancer. For model training, we trained and validated the model 100 times (RF, LR) and 10 times (DNN) repeating random sampling verification. We split the dataset into training and testing datasets with a ratio of 7:3 in each validation. Then we used the Synthetic Minority Oversampling Technique (SMOTE) method in the training set for over-sampling, which adds artificially simulated new samples to the data set to decrease the influence of imbalanced data.

To evaluate the performance of the classification models, we calculated the Area Under the Curve (AUC) and the accuracy.

In addition, we also investigated whether the AI algorithms can play a role in the accuracy and speed of the doctor's diagnosis. We generated four test sets for doctors with 40 patients, half of the patients with an AI prediction class and its possibility, and the other half of the patients without any assistance. Then we sent the test sets to obstetric oncologists to measure the AUC, accuracy, and the time consumption for predicting the disease category with and without AI assistance. The function of accuracy is shown below.

$$accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

TP, True Positive; TN, True Negative; FP, False Positive; FN, False Negative.

Data pre-processing and machine learning models were implemented within Python 3.8, and scikit-learn 0.24 and PyTorch 1.10 packages.

Comparison of Different Models

The comparison of accuracy between models was performed by using the two-way ANOVA test in GraphPad Prism.

3 RESULTS

3.1 Clinical Information of Cases

A total of 344 endometrium cancer cases were reviewed and collected. Of these, 14 cases were excluded because of 70% or more of missing clinical data. As there was only one undifferentiated case, this category could not be tested because the test sample would be 0. Therefore, 329 cases were enrolled into the train and test. The mean age was 56 (range 28-83) years old (**Table 1**). The mean BMI was 26.87±4.43. Among these cases, 86.3% of the patients were type I EC. Most (75.7%) of the cases were FIGO stage I and 31 cases were grade (G) 1, 114 cases were G2, 38 cases were G3, and 17 cases were unknown.

TABLE 1 | Clinicopathological data of patients with endometrial cancer.

Features	Frequency (%) N=329
Age, mean (range)	56 (28-83)
BMI, mean±SD	26.87 ± 4.43
Hypertension	
+	144 (43.8)
–	184 (55.9)
Unknown	1 (0.3)
Diabetes	
+	71 (21.6)
–	256 (77.8)
Unknown	2 (0.6)
Gestation	
+	312 (94.8)
–	17 (5.2)
Parturition	
+	301 (91.8)
–	28 (8.5)
Menopause	
+	192 (58.3)
–	13 (4.0)
Unknown	124 (37.7)
Histology	
type I	284 (86.3)
type II	45 (13.7)
FIGO Stage (2009)	
I	249 (75.7)
II	28 (8.5)
III	42 (12.8)
IV	10 (3.0)
Differentiation	
G1	31 (37.7)
G2	114 (45.6)
G3	38 (11.6)
Unknown	17 (5.2)

G, grade; SD, standard deviation; FIGO, the international federation of obstetrics and gynecology.

3.2 Comparison of the Models for the Prediction

3.2.1 Histology

The AUC and accuracy score of the LR were 0.69 (95% CI=0.67-0.70) and 0.74 (95%CI=0.72-0.75). The AUC and accuracy score of RF were 0.69 (95% CI=0.67,0.70) and 0.81 (95%CI=0.79-0.82). The AUC and accuracy score of DNN were 0.60 (95% CI=0.54-0.65) and 0.83 (95% CI=0.75-0.90). The LR and RF algorithms had a similar score which was significantly better ($p<0.05$) than DNN.

3.2.2 Stage

The AUC and accuracy score of the logistic regression were 0.56 (95% CI=0.54-0.59) and 0.42 (95% CI=0.41-0.44). The AUC and accuracy score of the random forest were 0.66 (95% CI=0.64-0.69) and 0.63 (95% CI=0.61-0.65). The AUC and accuracy score of DNN was 0.48 (95% CI=0.46-0.51) and 0.78 (95% CI=0.71,0.84). The RF was significantly better than LR and DNN.

3.2.3 Grade

The AUC and accuracy score of the LR were 0.61 (95% CI=0.60-0.62) and 0.36 (95% CI=0.35-0.38). The AUC and accuracy score

of RF was 0.64 (95% CI=0.63-0.65) and 0.43 (95% CI=0.41-0.44). The AUC and accuracy score of DNN were 0.47 (95% CI=0.45-0.50) and 0.43 (95% CI=0.40-0.45). The LR and RF algorithms have a similar score significantly better than DNN.

3.3 Performance Comparison Between ML Model, Doctors' Prediction, and Doctors With the Assistance of AI

The result of the doctors'prediction is shown in **Table 2**. The average accuracy for histology was 86% (with AI) and 79% (without AI), respectively. The average accuracy for the stage was 64% and 53%, respectively. The average accuracy for differentiation was 50% and 45%, respectively. The time consumption for each patient to make a decision was 29.25 s (with AI) and 28.75 s (without AI), respectively. For type and stage diagnosis, the AI model can improve 6% and 10% of a doctor's accuracy. But the accuracy decreases 7% for the differentiation diagnosis. The average time consumption with AI was 10 s longer than that without AI, though the AI model only cost 3 ms to predict one patient.

The comparison of a doctor's prediction with and without AI assistance is shown in **Figure 2**. Compared to LR (**Figure 2A**), the accuracy of doctors' prediction with AI is higher than that of LR and doctors' prediction without AI among histology, stage, and grade. The comparison with RF (**Figure 2B**) also showed similar results. However, the accuracy of the DNN's prediction of the stage was significantly higher than that of doctors' prediction with and without AI assist (**Figure 2C**). But the accuracy of the combination of doctor and AI was relatively better as a whole.

TABLE 2 | Comparison of doctors' predictions with and without AI assistance.

Project	Without AI (accuracy %)	With AI (accuracy %)
Histology	79	86
Stage	53	64
Differentiation	45	50

AI, artificial intelligence.

4 DISCUSSIONS

Endometrial cancer is a relatively common gynecological tumor. The development and application of AI in the medical field has gradually generated significance and value. This study built AI models to predict histology, stage, and grade of EC. Besides the prediction of AI models, we also compared the AI models, doctors' predictions, and doctors' predictions assisted by the AI model.

From the point of AUC alone, LR and RF models perform better in the prediction of histology and grade. RF is better in the prediction of the stage (**Figure 1**). If only accuracy is considered, DNN and RF models work well in the prediction of histology and grade (**Table 3**). In the real world, not every patient can complete all examinations. In this way, the patients with missing values were also included in the dataset. However, compared with RF and DNN, the LR is sensitive to missing values, which means the missing values will significantly influence the performance of LR (15). On the other hand, DNN with hidden layers has more capability to learn from nonlinear and complex relationships. But it has higher requirements for the sample size of training data than LR and RF (16).

Taking into account the above reasons, the RF model was relatively better than other models, so RF was used to assist doctors.

The doctor's clinical experience combined with the assistance of AI increases the accuracy of histology, stage, and grade (**Table 2**). The main reason is that doctors analyze the highly relevant features of the disease (such as BMI, D&C, imaging, etc.) based on their clinical experience and draw conclusions, while the algorithm learns the influence weights of different features according to the distribution of training data, and more accurate judgments can be obtained for some patients who are not obvious in the preoperative features. Overall, the accuracy of

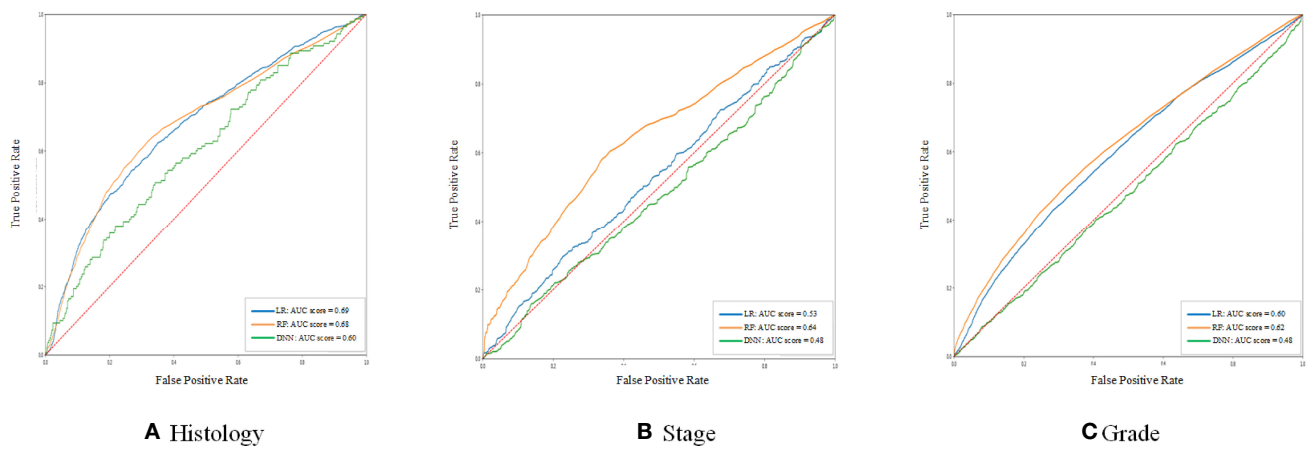

FIGURE 1 | The ROC curve of the histology stage and grade between different models. **(A–C)** shows the ROC curve and AUC score of three different models for histology, stage, and grade prediction, respectively.

TABLE 3 | The AUC score and accuracy of three ML models for histology, stage, and grade prediction.

Model	Histology		Stage		Grade	
	AUC	Accuracy	AUC	Accuracy	AUC	Accuracy
LR	0.69 (0.67-0.70)	0.74 (0.72-0.75)	0.56 (0.54-0.59)	0.42 (0.41-0.44)	0.61 (0.60-0.62)	0.36 (0.35-0.38)
RF	0.69 (0.67-0.70)	0.81 (0.79-0.82)	0.66 (0.64-0.69)	0.63 (0.61-0.65)	0.64 (0.64-0.65)	0.43 (0.41-0.44)
DNN	0.60 (0.54-0.65)	0.83 (0.75-0.90)	0.48 (0.46-0.51)	0.78 (0.71-0.84)	0.47 (0.45-0.50)	0.43 (0.40-0.45)

LR, Logic Regression; RF, Random Forest; DNN, Deep Neural Network; AUC, Area Under the Curve.

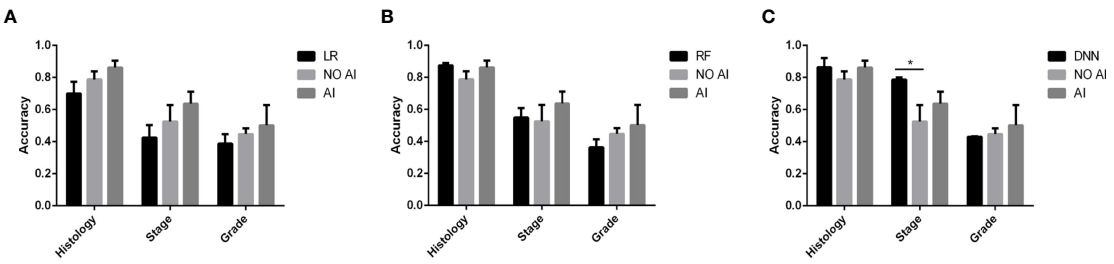

FIGURE 2 | The accuracy comparison between doctors with and without AI assistance and AI in predicting stage and grade. **(A–C)** shows the different AI assistance models. * Indicates $P < 0.05$

doctors with AI assistance is relatively the best choice among the histology, stage, and grade whether compared to AI alone or doctor alone (**Figure 2**). Therefore, the judgment of the doctor with the RF assistance is the best choice.

The accuracy of grade and stage is not that high, and the AUC is also relatively low. The reasons can be: 1. The pathological results of preoperative curettage are not completely accurate, and there are false negatives (3); 2. The staging of endometrial cancer is the clinicopathological stage, the determination of staging requires a combination of preoperative conditions, staged surgery and postoperative pathology, as well as grade, but the aim of this study is the preoperative diagnosis, so only preoperative features are given to AI models and doctors, and the intraoperative and postoperative characteristics were not included. Despite this, the AUC of RF is greater than 0.6 among histology, stage, and grade, so it has predictive value, especially given that it is only based on preoperative features.

Furthermore, in the past years, there is general agreement that AI may assist physicians in making better clinical decisions. This technology can provide additional information to help doctors make proper diagnoses (17). In the classification of grade, the outcome of AI alone and doctor alone is not very good, but doctors' prediction including the AI results improved the accuracy. In the classification of histology, both doctors and AI had high accuracy, but the accuracy of doctors combined with AI was improved. The same is true for staging. The accuracy of staging is not high, but doctors combined with AI improved the accuracy. Comparing with and without AI assistance, the time consumption for doctors with AI assistance is only 10 s longer, only 0.5 s per patient, 1.7% longer than before, which can be seen as almost no additional time cost. The extension of time consumption is not because of the speed predicted by AI, but because doctors need to analyze the information from AI.

Therefore, the AI model we built can effectively assist doctors in preoperative diagnosis and prediction of histology, stage, and grade.

There are several limitations to this study. Some multi-category classifications, such as staging and differentiation, have small sample sizes, resulting in poor overall performance. This was a single-center (country) study and an independent validation set from another country can make the results more convincing. Prospective, multi-center, large sample size research will help improve the performance of this AI model. In addition, the features of the database are mainly derived from text information, and the dimension of information should be improved. In the future, more dimensional information can be directly extracted from the images and examinations, so that intuitive information can be extracted.

5 CONCLUSION

This study demonstrated that a random forest model can predict histology, stage, and grade of endometrial cancer preoperatively and help doctors in obtaining a better diagnosis and predictive results with minimal additional time, which can help patients receive timely, appropriate, and effective treatment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

Conception and design: YF, ZW, HG, and SW. Development of methodology: YF, ZW, HG, SW, ZYZ, MX, JL, AT, and AD. Acquisition of data: YF, ZW, HG, SW, ZYZ, MX, and JL. Analysis and interpretation of data: YF, ZW, HG, SW, ZYZ, AT, and AD. Writing, review, and/or revision of the manuscript: YF, ZW, MX, JL, HG, BD, ZQZ, SW, YS, ZZ, AT, AR, CL, SX, AD, ZYZ, and LQ. Administrative, technical, or material support: YF, ZW, MX, HG, JL, ZZ, ZQZ, SW, BD, AR, CL, ZYZ,SX, YS, AD, AT, and LQ. Study supervision: YF, ZW, HG, SW, ZYZ, AT, AD, and LQ. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Hyuna Sung JF, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020 GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2021) 71:209–49. doi: 10.3322/caac.21660
2. Martin K, Amant F, Mirza MR, Creutzberg CL. Cancer of the Corpus Uteri: 2021 Update. *Int J Gynaecol Obstet* (2021) 155:45–60. doi: 10.1002/ijgo.13866
3. National Comprehensive Cancer Network (NCCN). *Uterine Neoplasms NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)* (2022) Version 1. Available at: https://www.nccn.org/professionals/physician_gls/pdf/uterine.pdf
4. Kong A, Johnson N, Kitchener HC, Lawrie TA. Adjuvant Radiotherapy for Stage I Endometrial Cancer. *Cochrane Database System Rev* (2012) 4: CD003916. doi: 10.1002/14651858.CD003916.pub3
5. Karlsson B, Granberg S, Wikland M, Ylöstalo P, Torvid K, Marsal K, et al. Transvaginal Ultrasonography of the Endometrium in Women With Postmenopausal Bleeding–a Nordic Multicenter Study. *Am J Obstet Gynecol* (1995) 172:1488–94. doi: 10.1016/0002-9378(95)90483-2
6. Osheroff JA, Teich JM, Levick D, Saldana L, Velasco FT, Sittig DF, et al. *Improving Outcomes with Clinical Decision Support: An Implementer's Guide (2nd ed.)*. New York: Himss Publishing (2012):348. doi: 10.4324/9780367806125
7. Rajkomar A, Dean J, Kohane I. Machine Learning in Medicine. Reply. *N Engl J Med* (2019) 380:2589–90. doi: 10.1056/NEJMc1906060
8. Hu J, Niu H, Carrasco J, Lennox B, Arvin F. Voronoi-Based Multi-Robot Autonomous Exploration in Unknown Environments *via* Deep Reinforcement Learning. *IEEE Trans Vehicul Technol* (2020) 69:14413–23. doi: 10.1109/TVT.2020.3034800
9. Otani S, Himoto Y, Nishio M, Fujimoto K, Moribata Y, Yakami M, et al. Radiomic Machine Learning for Pretreatment Assessment of Prognostic Risk Factors for Endometrial Cancer and its Effects on Radiologists' Decisions of Deep Myometrial Invasion. *Magn Reson Imaging* (2022) 85:161–7. doi: 10.1016/j.mri.2021.10.024
10. Nakajo M, Jinguji M, Tani A, Kikuno H, Hirahara D, Togami S, et al. Application of a Machine Learning Approach for the Analysis of Clinical and Radiomic Features of Pretreatment [18 F]-FDG PET/CT to Predict Prognosis of Patients With Endometrial Cancer. *J Mol Imaging Biol* (2021) 23(5):756–65. doi: 10.1007/s11307-021-01599-9
11. Berek JS, Kehoe ST, Kumar L, Friedlander M. Cancer of the Ovary, Fallopian Tube, and Peritoneum. *Int J Gynecol Obstet* (2018) 143:59–78. doi: 10.1002/ijgo.12614
12. Wright RE. Logistic Regression. In L. G. Grimm, & P. R. Yarnold (Eds.), Reading and Understanding Multivariate Statistics. Washington DC: American Psychological Association (1995) (pp. 217–244).
13. Breiman L. Random Forests. *Machine Learning* (2001) 45:5–32. doi: 10.1023/A:1010933404324
14. LeCun Y, Bengio Y, Hinton G. Deep Learning. *Nature* (2015) 521:436–44. doi: 10.1038/nature14539
15. Amini P, Maroufizadeh S, Hamidi O, Samani RO, Sepidarkish M. Factors Associated With Macrosomia Among Singleton Live-Birth: A Comparison Between Logistic Regression, Random Forest and Artificial Neural Network Methods. *Epidemiol Biostat and Public Health* (2016) 13:e11985-1-9. doi: 10.2427/11985
16. Yoo W, Ference BA, Cote ML, Schwartz A. A Comparison of Logistic Regression, Logic Regression, Classification Tree, and Random Forests to Identify Effective Gene-Gene and Gene-Environmental Interactions. *Int J Appl Sci Technol* (2012) 2:268.
17. Haleem A, Javaid M, Khan IH. Current Status and Applications of Artificial Intelligence (AI) in Medical Field: An Overview. *Curr Med Res Pract* (2019) 9:231–7. doi: 10.1016/j.cmrp.2019.11.005

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REVIEW
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Machine Learning for Endometrial Cancer Prediction and Prognostication

Vipul Bhardwaj[1†‡], Arundhiti Sharma[1‡], Snijesh Valiya Parambath[2], Ijaz Gul[3], Xi Zhang[4], Peter E. Lobie[1,3,4], Peiwu Qin[1,3] and Vijay Pandey[1,3]*

[1] Tsinghua Berkeley Shenzhen Institute, Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen, China, [2] Division of Molecular Medicine, St. John's Research Institute, Bangalore, India, [3] Institute of Biopharmaceutical and Health Engineering, Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen, China, [4] Shenzhen Bay Laboratory, Shenzhen, China

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***Correspondence:**
Vijay Pandey
vijay.pandey@sz.tsinghua.edu.cn

†ORCID:
Vipul Bhardwaj
orcid.org/0000-0001-5509-8840

‡These authors have contributed equally to this work

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Endometrial cancer (EC) is a prevalent uterine cancer that remains a major contributor to cancer-associated morbidity and mortality. EC diagnosed at advanced stages shows a poor therapeutic response. The clinically utilized EC diagnostic approaches are costly, time-consuming, and are not readily available to all patients. The rapid growth in computational biology has enticed substantial research attention from both data scientists and oncologists, leading to the development of rapid and cost-effective computer-aided cancer surveillance systems. Machine learning (ML), a subcategory of artificial intelligence, provides opportunities for drug discovery, early cancer diagnosis, effective treatment, and choice of treatment modalities. The application of ML approaches in EC diagnosis, therapies, and prognosis may be particularly relevant. Considering the significance of customized treatment and the growing trend of using ML approaches in cancer prediction and monitoring, a critical survey of ML utility in EC may provide impetus research in EC and assist oncologists, molecular biologists, biomedical engineers, and bioinformaticians to further collaborative research in EC. In this review, an overview of EC along with risk factors and diagnostic methods is discussed, followed by a comprehensive analysis of the potential ML modalities for prevention, screening, detection, and prognosis of EC patients.

Keywords: machine learning, endometrial cancer, artificial intelligence, deep learning, histopathology, prediction model, algorithm

INTRODUCTION

Rising endometrial cancer (EC) incidence and disease mortality represent a serious concern for women, particularly in countries with rapid socioeconomic transitions where the incidence rate of this cancer is the highest (1–3). The International Federation of Gynecology and Obstetrics (FIGO) staging method is used to determine the surgico-pathological staging of EC (4). The majority of EC patients are diagnosed at an early stage (80% in stage I), with a 5-year survival rate exceeding 95%, the highest of all gynecological cancers (5). Individuals with early detection or who have EC with a reduced risk show a favorable prognosis. Individuals detected with higher stage EC who have

developed recurrence exhibit a worse 5-year survival ranging between 47% and 58% for stage III EC patients and 15% and 17% for stage IV EC patients; and possess a limited number of accessible prognostic or therapeutic alternatives (6, 7). Expensive screening, and a high rate of misdiagnosis majorly contribute to high disease mortality (8–10). Although endometrial biopsy with dilation and curettage is the standard diagnostic approach for EC, there exist no clinically validated EC screening approaches (11). Progestin treatment is appropriate for women experiencing atypical endometrial hyperplasia (AEH), a precancerous type of endometrial lesion, or stage 1A EC lacking muscle penetration (12). Most women diagnosed with EC exhibit a good prognosis after surgery alone; however, poorer outcomes are associated with high-grade, recurrent, and metastatic EC (13). Therefore, routine screening, early detection, and precise prediction of recurrence or survival after oncotherapeutic regimens may improve the survival of EC patients, rather than a simple presentation of symptomology. In this review, machine learning (ML)–based strategies and techniques that could improve the prediction and prognostication of EC are discussed.

ML approaches (algorithms) have evolved in oncology to raise the reliability of predication of cancer susceptibility, recurrence, and survival (14, 15). ML is a subfield of artificial intelligence (AI) that combines a range of statistical, probabilistic, and optimization techniques to enable computers to "learn" from previous examples and detect complicated patterns in vast, noisy, or complex datasets (16, 17). AI allows computers to execute "cognitive" tasks for humans, such as language comprehension, reasoning, and problem solving. The use of an appropriate AI system enables computers to discover patterns in available datasets and derive inferences using the data without requiring explicit commands (18). Currently, AI has mostly been utilized for image identification tasks in healthcare (19). Several articles have reported the high accuracy of AI in diagnosing conditions such as skin cancer, and diabetic retinopathy (20–23). ML algorithms have been effectively employed in the treatment of cancer, such as breast cancer (24), oesophageal cancer (25), head and neck cancer (26), osteosarcoma (27), prostate cancer (28), and thoracic cancer (29). ML offers the opportunity to "systematically evaluate every variable, present, and future, to locate groupings of cancer cases with similar outcomes" as cancer prediction and prognostication systems become more complicated with rising variables. Implementation of ML approaches for EC prediction and prognostication should be of utility as patients with diverse outcomes may be subcategorized into specific clinical stages.

CLINICO-PATHOLOGICAL FEATURES OF EC

EC has typically been classified into two types: type I and type II (30). These two classifications differ in terms of epidemiology, histology, prognosis, and treatment (30, 31). Type I EC is the most common form and accounts for most diagnosed cases (80%), has an overall 5-year survival rate of 81.3%, and usually has less than 20% chance of recurrence (31, 32). Type I EC offers a good prognosis in the majority of patients because they are low grade and limited to the uterus at the point of diagnosis (33). Type I EC is predominantly associated with obesity-related complications and with excessive endometrial cell proliferation (34). Type I EC is also observed to be susceptible to excessive exposure to estrogen, *via* both endogenous and exogenous routes and mainly affects younger women (premenopausal or perimenopausal) (33). As a result, hyperestrogenism, hyperlipidemia, diabetes, and anovulatory uterine bleeding are common in individuals with type I EC. Hence, pathological conditions that are associated with metabolic deregulation have been recognized as an autonomous risk component for the early onset of the EC (35). Endometrial intraepithelial neoplasia (often termed complex AEH) is a type I EC precursor lesion that is frequently associated with a thicker endometrium and shares the same estrogen exposure risk factors as EC (36, 37). Hyperplasia contributes to a 1%–3% risk of the development of cancer. Low-grade endometrioid adenocarcinomas [International Federation of Gynecology and Obstetrics (FIGO) grades 1 and 2] are the most common type I cancer in women (38). Grade 1 cancers are well differentiated, resemble normal tissue, and often show favorable prognosis (39). Grade 2 malignancies contain a solid component that ranges from 6% to 50% and are classified as differentiated. Grade 1–2 ECs are also classified as type I; grade 3 tumors that contain a solid component ranging >50% are high grade and poorly differentiated, and they do not appear as normal endometrial tissue, are aggressive, and associated with poor prognosis. Grade 3 EC is classified as type II EC, often affects old age women (postmenopausal), and is not associated with endocrine disorders (40). Type II cancers are high-grade, non-endometrioid histology and are mostly composed of serous carcinomas and clear cell carcinomas (41). Type II ECs are frequently diagnosed at a late stage, associated with intermediate-to-poor prognosis, and a high rate of recurrence with a decreased 5-year overall survival rate (55%) which contributes disproportionally to disease mortality (31). Although type II ECs contribute to only 20% of all ECs, they are associated with ~40% of all EC patients with poor overall survival (42). Around 20% of endometrioid cancers are subcategorized as high grade (FIGO grade 3) and type II EC (31). Dedifferentiated, undifferentiated, mixed cell, neuroendocrine, and carcinosarcomas (also known as malignant mixed mullerian tumors) are similar to serous and clear cell carcinoma histology. Type II EC is more prevalent in elderly women and is especially frequently observed in African-American women with thin and atrophic endometrium (13, 21).

Increased risk of developing type I EC, is related to unopposed exposure of the endometrium to estrogen (E2) (43). Hormone replacement therapy represents an example of exogenous estrogen exposure (44). Premature menarche, late

menopause, tamoxifen treatment, nulliparity, infertility or inability to ovulate, and a polycystic ovarian disease can all increase uterine estrogenic exposure (45). Family history, age (over 50), hypertension, diabetes mellitus, obesity, and thyroid disease are all independent risk factors through which the risk of EC increases (33, 42, 46, 47). Being obese is the most significant risk factor for hyperplasia progression to malignant EC (48). Obesity affects more than 70% of people with initial stage EC (49). Obesity is also hypothesized to increase the risk of EC by increasing the peripheral conversion of androgens to estrone in adipose cells (39). Obese premenopausal women are also highly susceptible to prolonged anovulation, which is an additional risk factor for EC (48). Genetic conditions like Cowden syndrome, Lynch syndrome, and polymerase proofreading-associated polyposis are associated with an increased risk of developing EC among women (50, 51). Lynch syndrome is a cancer risk disease characterized by a monoallelic germline mutation in a mismatch repair (MMR) gene, particularly MLH1, MSH2/6, or PMS2, or by a germline deletion inside epithelial cell adhesion molecule (EpCAM) that contributes to epigenetic silencing of the neighboring MSH2 gene (52). Carriers of these mutations are more likely to develop ECs (53).

Early detection of EC can improve the chances of a good prognosis. Abnormal uterine bleeding and postmenopausal vaginal bleeding (PMB) are categorized as the most prevalent symptom of EC. Despite the fact that PMB is present in 90% of women with EC (regardless of tumor stage), it is not a reliable indicator of the disease. Only 9% of PMB patients are diagnosed with EC (54). Internal pelvic examination with a Pap (Papanicolaou) smear test is generally regarded as the initial investigation when symptoms, signs, and/or family history imply the existence of gynecologic pathology (55). However, the Pap smear is not a useful predictor of EC and is predominantly utilized for cervical cancer screening and detection (56). Transvaginal ultrasonography (TVUS) is the most helpful tool to use in gynecologic practice to monitor the female reproductive organs as it can help to determine the thickness and features of the endometrial lining, as well as the size of the uterus, the adnexa, and the presence of excess pelvic fluid (57). The TVUS probe (which acts similarly to an ultrasound transducer) is inserted into the vagina for the transvaginal scan (TVS). Images from the TVS are then utilized to determine if there is a mass (tumor) in the uterus or if the endometrium is thicker than normal, which could indicate EC. It is also used to determine if cancer has spread to the uterine muscular layer (myometrium) (58). As a triage tool, TVS-based endometrial thickness screening lacks sufficient specificity because it cannot distinguish benign lesions, such as polyps, from their malignant counterparts exposing a large proportion of women to further testing (59–61). The endometrial histological information provided by endometrial biopsy is a gold standard for diagnostic evaluation and sufficient for preoperative assessment (62, 63). In combination with EC biopsies, dilation and curettage (D&C) are often recommended to confirm the EC diagnosis; however, the D&C method is painful, expensive, requires general anesthesia and has a high rate of misdiagnosis in up to 31% of women and demands multiple repeats for confirmation (8–10, 64). Another technique used to investigate EC is hysteroscopy, which allows for direct viewing of the endometrial cavity, which is often used to examine abnormal uterine bleeding (42). Hysteroscopy can be combined with a focused biopsy or curettage. In the detection of EC, hysteroscopy yields higher accuracy than does blind D&C and had a sensitivity of 99.2% and a specificity of 86.4% (65). Thus, except for histology of endometrial biopsies, there is no clinically accepted method for screening, detection, prediction, and diagnosis of EC.

To determine local extension and any metastatic disease, imaging studies such as magnetic resonance imaging (MRI), computerized tomography (CT), or positron emission testing/CT may be used. However, imaging studies are limited in the detection of lymph node dissemination, which is observed in at least 90% of the cases using microscopic-based approaches (66). However, one of the more interesting and difficult challenges for clinicians is accurately predicting the outcome of an illness. As a result, research is increasingly employing ML-based approaches that are capable of discovering disease-associated patterns and links in the large datasets and may accurately predict potential disease risks and outcomes for individual patients.

MACHINE LEARNING: METHODS AND ALGORITHMS

In 1959, Arthur Samuel first coined the expression "machine learning". ML determines a machine's capability to understand and simulate upcoming scenarios and potential effects predicated upon massive datasets. Hence, the science of having a machine function learning from the data, recognizing patterns, and giving the outputs with minimal human input is ML. It has brought to society autodriven vehicles, functional comprehension of voice, robust online search, and a dramatically enhanced understanding of the human genome. ML is a bustling field of medicine with tools being used to integrate medical challenges with computer science and statistics. ML may lead to more detailed diagnostic algorithms in medicine and personalized patient care. ML is a data mining software for creating analytical models that is fully automated and is a subset of AI-based on the notion that machines could extrapolate data, interpret trends, and generate results with little human input. Data samples contain the basic constituents that are required to develop a strategy for the application of the ML algorithm. Sample representation contains multiple features and each function consists of numerous classification values. Understanding the particular form of data that is used in advance facilitates the proper collection of methods and algorithms that could perhaps be employed for the evaluation. Compared with conventional biostatistical approaches, the strengths of ML comprise versatility and scalability, which make it possible for multiple functions such as stratifying threats, diagnosing and classifying, and predicting survival. A further value of ML algorithms is to integrate different forms of data (e.g., population

data, experimental outcomes, and data from imaging) to identify patterns that could effectively classify the data into respective categories. Amid such benefits, the use of ML in health care poses specific difficulties that include preprocessing of data, experimental design, and algorithm refinement related to the specific clinical issue (67). A comprehensive overview of AI and its subfields [ML and deep learning (DL)] is summarized in **Figure 1**.

ML plays an instrumental role to speed digital transformation and is ushering in an age of automation. ML has become so prevalent that it has now become one of the preferred methods for researchers to handle a wide range of biological problems. The emergence of computer-aided systems that have been instructed to perform complex tasks in medical imaging, bioinformatics, and medical robotics has stemmed from the accessibility of advancing computational power, strongly enhanced pattern recognition algorithms, and enhanced image processing (IP) software operating at incredibly fast acceleration. A "cognitive" computer having exposure to "big data" may scan billions of bits of unstructured data, retrieve user data, and detect complicated patterns with growing confidence. Several ML algorithms are mathematical models that transfer a collection of observable variables through a data point or sample, referred to as "features" or "predictors", into a set of outcome variables, referred to as "labels" or "targets" (68, 69). Widely used ML algorithms with their advantage and limitations are shown in **Table 1**. The algorithms are trained to be competent to anticipate labels by analyzing specific information in a phase termed "training". Presently, three prominent methods are used to train ML algorithms: supervised, unsupervised, and reinforcement learning (**Figure 2**).

IMBALANCED DATA IN ML

ML algorithms are powered by the volume of data in datasets. A balanced dataset is one in which the distribution of labels is approximately equal. Labels, in this case, indicate the class related to each data point. The class label is projected by evaluating the input data or predictor in a classification issue when the target or output variable is a categorical variable. A class imbalance is common in most classification issues. In certain cases although, when the dominant class is much bigger as compared to the minority class, the disparity is quite pronounced. They occur when one of the target class labels has a significantly lower number of observations than the other class labels. An imbalanced class dataset is a type of dataset that is particularly common in real classification scenarios (76). Any conventional strategy to solve this type of machine learning challenge frequently produces ineffective results. In unusual situations like fraud detection or disease prediction, it is vital to correctly determine the minority classes. As a result, the model should not be biased toward recognizing just the majority and therefore must assign the minority class the same relevance or value as the majority. Most machine learning algorithms, on the other hand, struggle with imbalanced datasets (77). When dealing with imbalanced datasets, there is no one-stop solution to increase the predictive accuracy of

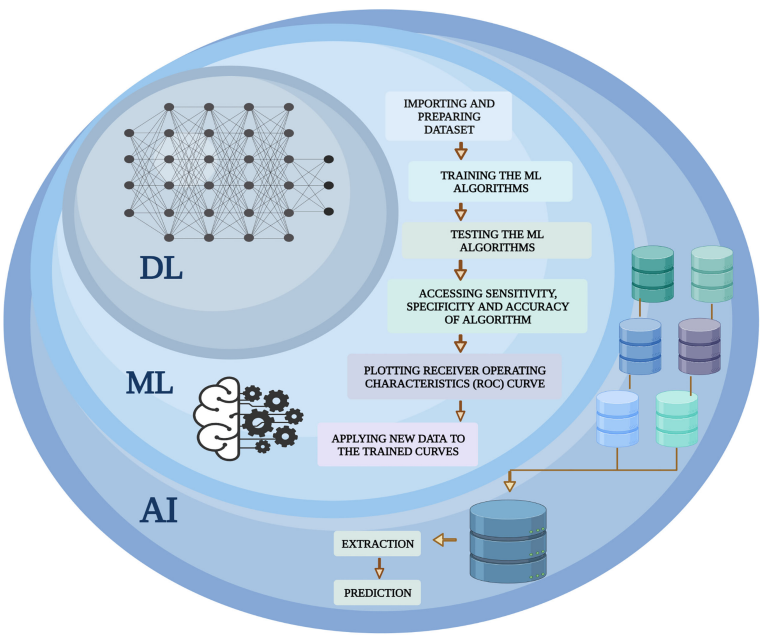

FIGURE 1 | An overview of the machine learning (ML) integration with artificial intelligence (AI) and deep learning (DL). A computer science branch that uses machines and programs to mimic human intelligence is known as AI, whereas DL is a subgroup of AI that employs models from statistics and mathematics. ML covers a variety of algorithms and statistical methods, including logistic regression, random forest, and DL-based approaches. The ML algorithm is continually integrated with a new dataset to test the validity and utility of algorithms.

TABLE 1 | List of algorithms used in ML with the advantages and limitations.

ML Algorithms	Advantages	Limitations
Decision Tree (70)	- Training method that is simple to comprehend and efficient - Training is unaffected by the sequence of training occurrences. - Pruning reduces the complexity of the classifier and improves predictive accuracy by the reduction of overfitting	- Classifications must be mutually exclusive - The final decision tree is determined by the order in which the algorithm parameters are selected - Inaccuracies in the training set might lead to excessively complicated decision trees - When attribute's values are missing, it is uncertain which branch to choose when that attribute is checked.
Naïve Bayes (71)	- Statistical modeling–based basis -Training method that is simple to comprehend and efficient - Training is unaffected by the sequence of training occurrences - Useful in a variety of accuracy areas.	- Presupposes that those characteristics are statistically independent - Expects that numeric characteristics have a normal distribution - Classifications must be mutually exclusive - Redundant characteristics classification error - Attribute and class frequencies have an impact
k-Nearest neighbor (72)	- Cases are quickly classified. - Beneficial in non-linear classification situations - Resilient in the face of irrelevant or new features - Capable of withstanding noisy instances or instances with missing attribute values - May be used for regression as well as categorization	- Implies that instances with identical characteristics will be classified similarly - Believes that all characteristics are equally important - When the number of characteristics grows, it becomes too computationally complex
Neural Network (73)	- It has the potential to be utilized for classification or regression. - Capable of representing Boolean functions (AND, OR, NOT) - Tolerable to loud input - More than one output can be used to classify instances	- The algorithm's structure is tough to grasp - Overfitting can occur when there are too many characteristics - Experimentation is the only way to discover the best network structure
Support Vector Machine (74)	- Nonlinear class boundaries are modeled - It is unlikely that overfitting will occur - Decreased computational complexity to a quadratic optimization issue - It is simple to adjust the complexity of the decision rule and the frequency of mistake	- Compared to Bayes and decision machine trees, training takes longer - Finding optimum settings is difficult when training data are not linearly separable - The algorithm's structure is difficult to understand
Genetic Algorithm (75)	- Simple algorithm that is simple to implement - Can be utilized for feature categorization and selection - Utilized mostly in optimization - Always comes up with a "decent" answer (not always the best solution)	- It is difficult to compute or create a scoring function - It is not the more productive approach for locating some optima since it prefers to discover local optima instead of global selection - Complications involved in the representation of training/output data

a model. It may be required to use several processes to determine the best sampling methodology for the dataset. The most successful strategies will differ depending on the peculiarities of the imbalanced data set (). Resampling strategies, ensemble learning techniques (78–80), use of right evaluation metrics, boosting, cost-sensitive learning (81), one-class learning (82), and active learning have all been considered as solutions to the class imbalance problem. In resampling strategies, the most commonly employed methodologies to assess the class imbalanced issues are by using either random oversampling or random undersampling.

ML APPLICATION IN EC PREDICTION, DIAGNOSIS, AND PROGNOSIS

The advanced stage of diagnosis and limited therapy options seriously hinders the prognosis of EC patients. Several investigations have suggested that screening, early detection, monitoring, and prediction of EC could significantly improve the prognosis of patients. Advances in ML techniques offer unique and promising perspectives for the detection and prediction of several cancers such as breast, colorectal, and prostate cancer. Lately, ML has had a significant impact on the development of potential computational tools for stratifying, scoring, and prognosticating cancer patients to improve patient survival (12). Recent studies have reported that ML algorithms have been utilized to identify lymph node metastases, scoring KI67 positivity in breast cancer, scoring tumor-infiltrating lymphocytes in melanoma, and Gleason grading in prostate cancer (83). ML has also successfully attempted to predict tumor recurrence with high accuracy using pathological images (84). Furthermore, Pariss et al. used a novel ML-based algorithm to demonstrate an improved prognostic prediction for patients with EC (85). Thus, ML-based approaches can be employed to maximize the sensitivity and specificity of EC diagnosis and prognosis.

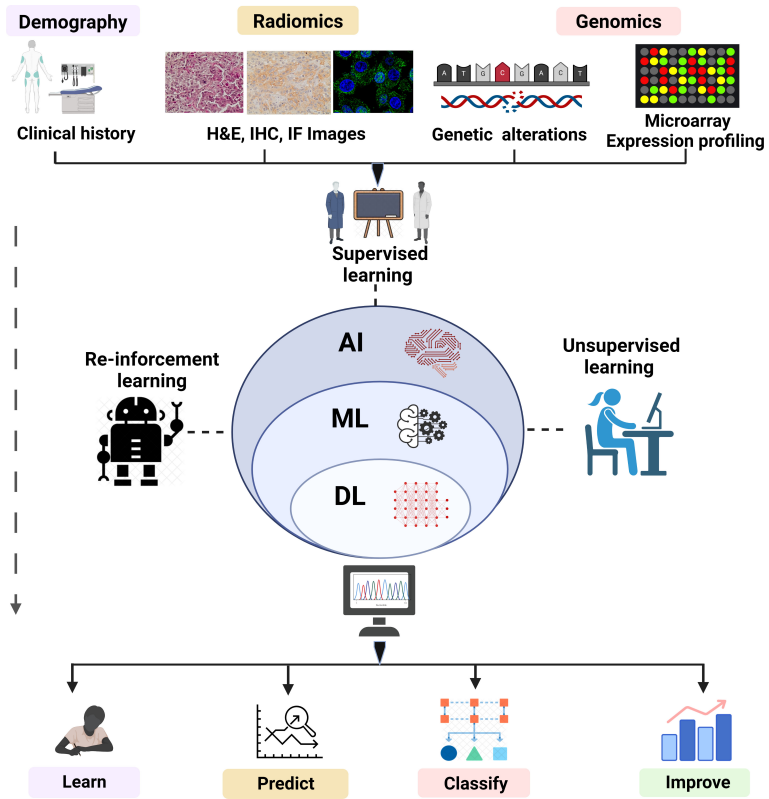

FIGURE 2 | Overview of ML (supervised, unsupervised and reinforcement learning). The overview of ML depicts the analysis and testing of statistical models and algorithms that computational approaches used to perform a specific task without being explicitly programmed. The figure represents subdisciplines of AI (ML and DL) and their subtypes including supervised, unsupervised, and reinforced algorithms employed in fields such as pattern recognition, object detection, text interpretation, and genomics. The algorithms of ML learn, improve, predict, and classify the data.

ML Approaches in Image and Pattern Recognition

Pattern recognition is the process of automated distinguishing, recognizing, and segmenting patterns and regularities in data using ML algorithms. It categorizes data using statistics, mathematical models, or knowledge derived from patterns and their representation. In supervised pattern recognition, the data are trained using specific labels. A label is assigned to each input value that is utilized to generate a pattern-based output. On the other hand, in unsupervised pattern recognition, various computer algorithms like clustering or principal components analysis are used to detect unknown patterns in the absence of labeled data. IP is a type of computer technology that allows us to process, analyze, and extract data from pictures. Some studies applying machine learning patterns and image recognition algorithms in EC have been summarized below.

There has been substantial progress in the application of pattern recognition and IP in the detection, classification, and identification of EC. Attempts have primarily been made in medical imaging to incorporate AI in preoperative diagnostic tools such as endoscopy, CT, MRI, ultrasound imaging, and pathological imaging. MRI is an essential medical imaging tool assisting in the identification and preoperative assessment of EC patients, and there have been some reports on its use in conjunction with DL approaches (86–88)]. Hodneland et al. demonstrated a fully automated approach for tumor segmentation in EC using a 3D convolutional neural network (CNN) named UNet3D (89) applied to a cohort of 139 EC patients with preoperative pelvic MR images (86). The whole value of tumor texture features and tumor volume estimations along with the tumor segmentation accuracy was obtained. The study showed that the available ML algorithms may offer accurate tumor segmentation at the level of a human expert in EC. The model generates tumor volume, tumor borders, and volumetric tumor maps. Hence, the self-generated approach for primary EC tumor segmentation seems to exhibit the prospect to seek near-real-time whole-volume radiomic tumor profiling, including tumor volume and texture properties, which could be useful for risk stratification and developing more personalized treatment strategies. Deep muscle invasion is an important determinant of uterine cancer prognosis. A study by Dong et al. created a DL model to predict deep muscle invasion based on 4896 MR images from 72 EC patients and compared it to radiologist's readings and achieved (90) an accuracy rate of

75%; nevertheless, the difference was not statistically significant. In a similar study, Chen et al. performed an analysis on 530 MR images and generated 84%, 66.7%, and 87.5% accuracy, sensitivity, and specificity, respectively (91). Lymph node metastasis (LNM) is one of the strong predictive factors for EC (88)]. Xu et al. developed a prediction model for LNM of normal size using MR images and CA125 values from 200 specimens of EC patients. The result showed approximately 85% accuracy (92). Recently, endometrial cytology has been reported as a viable diagnostic tool for detecting EC with good sensitivity and specificity (93, 94)]. A study was performed by Markis et al. in which they aimed to develop an automatic diagnostic system to analyze liquid endometrial cytology images of 416 patients using DL and found 90% accuracy for this model (95). Collectively, these studies suggest that ML has made remarkable progress in EC care.

ML Model for Classifying Endometrial Lesions

Hysteroscopy for endometrial lesions is one of the gold standard procedures in an examination of the endometrium. Hysteroscopy is used to differentiate uterine body tumors, such as endometrial polyps and EC, which however depends on the hysteroscopic expertise. It has certain limitations such as it is dependent upon the comprehensive knowledge and understanding of the target pathology, lesion size, penetration depth of the lesion, skills, and expertise of the physician, availability of equipment, and assessment of patient comorbidities (65). ML-aided approaches to examine EC not only increase accuracy but also provide a minimally invasive and less expensive tool to correctly diagnose EC. A study conducted by Zhang et al. developed a CNN-based computer-aided diagnosis system using the VGGNet-16 model for diagnostic hysteroscopy image classification (96). Using 1,851 hysteroscopic images of uterine patients as input, Zhang et al. also investigated the VGGNet-16 CNN model efficiency for the classification of endometrial lesions. The result showed 80.8% overall accuracy suggesting that the CNN model could be used as a tool for EC diagnosis.

ML Model for Classifying DNA Mismatch Repair–Deficient ECs

Approximately 3% of ECs are caused by germline mutations in the MMR genes such as MLH1/2, MSH6, and PMS2 and are termed MMR-deficient (MMR-D) tumors (97, 98). Recently, a study by Veeraraghvan et al. used contrast-enhanced CT to identify DNA MMR-D and/or tumor mutational burden-high (TMB-H) subtypes in ECs (99). This study built two ML models using generalized linear regression (GLMNet) and recursive feature elimination random forest (RF) classifiers to effectively differentiate between low copy number or high copy number MMR-D in ECs and also increasing rate of TMB-H in ECs. The authors analyzed data from a cohort of 422 patients and prefiltering was performed using GLMNet. Their findings indicated that radiomic models using ML algorithms can be utilized as a reproducible complementary or companion diagnostics for clinical trial enrollment and standard-of-care treatment.

ML ALGORITHMS IN EC PROGNOSIS

Lately, ML algorithms have been utilized in cancer care with an aim to better understand cancer prognostication. The ability of ML-based algorithms to detect, predict, and identify cancer using complex datasets indicates their importance. Over the past decade, several ML algorithms have been widely applied to EC prediction and prognostication. In a study, Praiss et al. (85) adapted an unsupervised ML algorithm named Ensemble Algorithm for Clustering Cancer Data (EACCD) and used it to classify patients based on TNM [tumor (T), nodes (N), metastases (M)] staging, grade, and age. EACCD is a combination of clustering method that derives dissimilarity among two combinations by continuously applying criteria-based clustering, followed by combining the learned dissimilarity estimate with a hierarchical clustering approach to find ultimate clusters of combinations. This innovative ML method improved the prognostic prediction for EC (85, 100). In another study, Chen and colleagues developed a tool ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumors) that uses gene expression data to predict tumor content and the degree of infiltrating stromal/immune cells from tumor tissues (101). ESTIMATE is a reliable algorithm that is widely accepted and has been used to determine the immune and stromal scores in various cancers such as breast cancer (102), glioblastoma (103), prostate cancer, colon cancer (104), and cutaneous melanoma (105). ESTIMATE total scores were found to be substantially closely associated with tumor purity in clinical tumor samples and tumor cell line samples, and they offered a simple and effective method for determining the number of tumor cells in a sample. ESTIMATE could further be improvised by the inclusion of endothelial cell signatures and tumor type–specific normal epithelial cells.

Most women with early stages of EC show a good prognosis. However, among them, 15% of patients with stage I and II EC develop recurrence (106). A study done by Akazawa et al. utilized EC patients and applied five ML algorithms: RF (107), logistic regression (LR) (108), decision tree (DT) (109), support vector machine (SVM) (110), and boosted tree (111), for the prediction of recurrence based on multiple clinical parameters such as age, body mass index, stage, histological type, grade, surgical content, and adjuvant chemotherapy. To verify the effectiveness of these classifications, accuracy and area under the curve (AUC) were analyzed. The maximum accuracy was reported by the SVM followed by LR and the least by boosted trees. The best AUC was observed in LR and the least in RF. Hence, they reported LR as the best predictive model for the study (108). They demonstrated the feasibility of AI prediction in patients with EC through the current investigation and concluded that, in the early stage of EC, the

application of an ML algorithm made it possible to predict recurrence (111, 112). This finding can help to improve the efficiency and accuracy to predict recurrence and treatment response.

Lymph node involvement (LNI) is a significant prognostic indicator for several cancers including EC. However, at present, there is no validated method to predict LNI accurately in EC. Recently, a study by Günakan et al. investigated the use of the Naïve Bayes (NB) algorithm (113) for LNI prediction in EC patients. This study used various histopathological factors such as final histology, presence of lymphovascular space invasion (LVSI), grade, tumor diameter, depth of myometrial invasion, cervical glandular stromal invasion, tubal or ovarian involvement, and pelvic LNI. The study reported that the algorithm predicted the LNI using histopathological factors with high accuracy and concluded that ML could occupy a position in decision-making for managing EC. Subsequent studies using sentinel lymph nodes (SLNs), biochemical data, or imaging combined with ML algorithms might assist in EC management (114). Another study by Reijnen et al. aimed at developing and validating externally a preoperative Bayesian network (BN) to predict LNM and disease-specific survival (DSS) in EC patients (115). The study included 763 patients, who had been treated surgically for EC. Using score-based ML, an externally validated ENDORISK- BN (116) was developed for EC patients involving the various molecular, histological, and clinical biomarkers. Both outcomes showed high discriminative performance and good calibration. With a marginal rate of false negative 1.6%, ENDORISK was able to identify more than 55% of the patients at 5% risk for LNM. This approach guides both the patient and the clinician regarding personalized risk assessments evaluating the needs of patients and also the scope of the surgical solution. The work also demonstrated how, by adding easily available and multimodal biomarkers, BN may be utilized to personalize therapeutic decision-making in oncology (115, 117).

Endometrioid endometrial adenocarcinoma (EEA) is the most common type of EC among all types. A poor prognosis for disease dissemination has been associated with a high tumor grade, late surgical stage, and LVSI (118). All of these characteristics suggest that traditional clinical features are insufficient to accurately predict EEA prognosis. Therefore, developing a predictive prognostic model for EEA would be of great clinical value. A study by Yin et al. developed a prognostic model for EEA that combines gene expression and traditional features using RF. Three models were derived using (a) 11 genes alone, (b) stage and grade, and (c) both 11 genes and stage and grade. The study reported that the RF model derived from the "11 genes and grade" performed better than RF models derived from the 11 genes or grade alone, indicating that the RF model derived from the "genes and clinical features" had a stronger predictive ability for the prognosis of EEA. Thus, a combined RF model and clinical criteria may serve better for the stratification of patients in the clinic (119–122).

ML Models for EC Screening, Risk Prediction, and Classification

Hart et al. used ML algorithms to categorize patients as low, medium, or high risk (123). They evaluated the model's performance on the three-tier risk classification to that of physicians' judgment and found encouraging results. These models offer a non-invasive and a cost-effective method of identifying high-risk subpopulations which might gain from early EC screening ahead of disease development. They discovered a unique and successful technique for premature cancer diagnosis and preventative measures for individual patients by performing a statistical biopsy of personal health data. Predicated on publicly available personal health data, seven alternative models, namely LR, neural network (NN), SVM, DT, RF, linear discriminant analysis, and NB (124) were developed to estimate the likelihood of an individual woman having EC in 5 years. The RF model outperformed the other six models regarding AUC with the NN coming in second. Both models were employed in dividing the population into three risk groups *viz.* low-, medium-, and high-risk groups. It does not aid in choosing the most effective preventative approaches (e.g., diet and exercise, progestin or antiestrogen therapy, and insulin-lowering therapy). Nonetheless, the ML approach holds great promise for assisting in the early detection of EC, as it produces high-accuracy predictions based primarily on personal health information before disease onset without the need for any invasive or expensive procedures such as endometrial biopsy or TVUS (123). Establishing personalized cancer preventive measures for each patient might benefit from a risk prediction model which classifies the population between low-, medium-, and high-risk categories (125). A model like this can assist doctors to identify high-risk groups for whom they can recommend measures to reduce the risk of EC, including dietary and activity modifications, progestin or antiestrogen medication, insulin-lowering therapy, and scheduled endometrial biopsies.

Prediction of EC Risk

According to a current analysis, establishing personalized cancer preventive measures for each patient might benefit from a risk prediction model which classifies the population between low-, medium-, and high-risk categories (125). A model like this can assist doctors to identify high-risk groups for whom they can recommend measures to reduce the risk of EC, including dietary and activity modifications, progestin or antiestrogen medication, insulin-lowering therapy, and scheduled endometrial biopsies.

EC Risk Factors: A Statistical Meta-Analysis and the Use of Artificial Neural Network to Develop a Risk Prediction Model

Using a statistical meta-analysis technique, Hutt et al. aimed at establishing the rank order of risk factors for EC and

generating a collective risk and per centum risk for each component, followed by creating a NN computer model that could predict whether a patient's overall cancer risk would increase or decrease. The objective was to determine whether a patient should be tested, to predict diagnosis, and to advise preventative actions to patients. To quantify relative risk, a meta-analysis of available data was performed, followed by the design and implementation of a risk prediction computer model based on a NN algorithm. Data for the meta-analysis of EC patients with the risk factors were taken from National Cancer Institute (NCI). Using a statistical meta-analysis technique, they were able to identify the rank order of risk variables for EC which was used to generate a pooled risk and risk percentage for each factor. Furthermore, using a computer NN model system, they developed a model that could predict an overall increase or decrease in cancer risk and cancer diagnosis for specific patients with 98.6% accuracy. The findings of the study effectively reduce the amount of unnecessary invasive testing of EC patients. This might be a valuable tool for physicians to utilize in concert with additional indicators to determine whether individuals require enhanced preventative measures before the potential development of EC (126).

LIMITATIONS OF ML APPROACHES IN EC

The sensitivity and specificity of EC data are frequently necessary to train ML-based models. Data access should be carefully controlled to protect privacy without impeding innovation and technological development to enhance performance. Some of the major challenges associated with the implementation of ML in EC datasets are as follows:

i. Retrospective versus prospective studies: Recruitment of the bulk of patients in the presented studies has been retrospective using the past labeled data for the training and testing of the used algorithms. Through prospective studies only, may one infer the true utility of the built systems when utilized in the real world. The introduction of wearable technology is facilitating massive prospective trials of historical standards; for instance, a study to diagnose atrial fibrillation in 419,093 consenting Apple watch holders is currently taking place (127).
ii. Peer-reviewed randomized controlled trials as the gold standard: To develop trust and acceptance of ML amongst the medical community, peer-reviewed evidence plays a pivotal role. In addition to this, there are very few randomized controlled trials published to date. ML experiments require high-quality reporting. The probability of bias and the possible utility of prediction models can only be accurately measured if all facets of a diagnostic or prognosis model are fully reported (128).
iii. Metrics often do not reflect clinical applicability: Amid the widespread usage in ML research, the AUC of a receiver operating characteristic is usually not the strongest measure for representing clinical validity and is difficult for many clinicians to comprehend. Clinicians ought to be trained to determine how the proposed algorithms can enhance patient treatment in a real-world setting, but most articles do not attempt to do so; other methods have been proposed, such as decision curve review that attempts at measuring the total advantage by using a formula to direct future behavior (129–131).
iv. Difficulty in comparing different algorithms: Since each test's output is recorded using various tools and methods on various samples with distinct sample distributions as well as characteristics, quantitative comparison of algorithms through studies is difficult. Algorithms must be compared on a similar self-dependent test set, which is depictive of the target population utilizing the comparable effectivity measures to produce fair comparisons. Lacking this, physicians would struggle to determine which algorithm is most likely beneficial for the patients' (127).
v. Dataset shift: The clinical and operational practices evolve, and the data are generated in a non-stationary environment amidst the shifting population of patients. When a novel predictive algorithm is introduced, it may induce shifts in operation, leading to a different distribution from the one used to generate the results. As a result, detection systems drift and update models about the quality of the results (132).
vi. Algorithmic bias: Clinical assessment should be performed on a representative sample of the planned implementation population, and algorithms should be constructed keeping the global community in mind. Factors such as age, race, sex, sociodemographic stratum, and position should be considered when analyzing output by population subgroups. Researchers should be guided to make sure that the proper measures are taken to assess bias while adopting models as there is a better understanding of these problems, and clinicians are empowered to engage objectively in system design and growth. Algorithm bias may be divided into three inputs: (1) bias of the model (models chosen to best reflect the major, not exclusively underrepresented groups), (2) variance of the model and model ambiguity (owing to a lack of data from minorities), and (3) noise in the results (the impact of a collection of unknown parameters on model predictions, which can be avoided by selecting subpopulations in which to test supplementary variables) (127).
vii. Challenges in generalization to new populations and settings: External validation, which involves evaluating an AI system with accurately scaled datasets obtained from organizations except for those that supplied the statistical model training, is required for the accurate evaluation of real-world clinical output and generalizability. This will guarantee that any important

changes in target patient demographics and conditions of illness in real-world clinical settings are appropriately reflected in the system where it will be used. This technique is currently uncommon in the literature and is a major source of concern. A current systematic assessment of research that assessed AI algorithms for medical imaging diagnostic analysis discovered that only 6% of 516 relevant scientific publications completed external validation (133, 134).

viii. Logistical issues in implementing AI systems: The reality that almost all medical data are not widely accessible for ML is causing several of the existing difficulties in applying AI to clinical research. Data are often segmented in a variety of medical imaging archival programs, diagnostic systems, EHRs, automated monitoring software, and insurance databases, making it impossible to integrate (135, 136).

ix. Human obstacles to AI adoption in healthcare: A good understanding of human and computer interactions should be a focus as there are significant human obstacles to adoption. It will be critical to retain an emphasis on clinical applicability and medical outcomes to make sure that this technology reaches and benefits the individual (127).

Also, other key points to be addressed are that the input data should be of good quality, with few artifacts or noise levels. While ML models might handle noisy input to a certain measure, incorrect labels may significantly affect an ML model's performance. Similar to different statistical approaches, many ML models require a training set with no missing characteristics, so the training sets must be as thorough as feasible. While data augmentation approaches, ranging from random imputations to ever further complex ML-based algorithms, could be implemented to substitute the missing data, they typically do not produce the identical results as utilizing a complete dataset for training. Furthermore, bigger datasets are typically desired since they allow the ML model to understand the real variance in the data with a lower chance of small outliers negatively affecting the model.

FUTURE PERSPECTIVE

An electronic health record (EHR) includes data comprising the physician's notes and other information documenting a patient's clinical history (137). The use of clinical data in research is challenging due to several reasons: in that, raw clinical data are often variable and are not well annotated, the clinical data are accumulated in an unlinked manner making it complex for research application, and the multimodal (i.e., radiology images, physician notes, pathology images, and laboratory results) nature of the data makes it hard to be automatically analyzed by ML algorithms without prior data curation by human intervention (138). Therefore, introducing a novel data integration and decision support system intended to harness the potential of EHRs for EC management might be an attractive addition to the computer-guided EC research (139) (**Figure 3**). An information technology infrastructure should be established to ease the creation and testing of statistical learning models for EC. This development linked multiple organizational EHR database systems and continually gathers data in a safe, comprehensive, and extendable manner. MEDomics profiles of EC patients, which are at the heart of the conceptual methodology, are synthesized data structures that encompass a specific EC patient's full clinical service chronology. The longitudinal EC patient's profiles are then utilized for a variety of AI application designs, to communicate meaningful interventions back into the health system. EC patient's cohorts were identified and were used to examine institution-wide mortality outcomes among EC patients along with examining the efficiency of targeted therapy and overall survival.

Cancer genotype determination has garnered increased attention to take advantage of biomarker-based targeted therapies (140). Molecular diagnostics have shown promising results in determining genetic biomarkers that are determined by molecular assays but molecular assays are time-consuming and are not available to all patients (140). Developing a link between genotype and phenotype seems a promising approach. Recent studies have shown that AI-based histologic diagnosis links histology, molecular biomarkers, and prognosis in cancer (141, 142). In a recent development in EC diagnosis, a multiresolution deep CNN named "Panoptes" was utilized where pathological images were used to predict the gene mutations and histological and molecular subtypes in EC (**Figure 4**) (143). The model was able to read one slide in 4 min and could predict 18 common gene mutations without sequencing analysis, providing a cost-effective cancer detection method. It is anticipated that the multiplex diagnosis and prognosis of different cancer types could be developed where a model trained for one cancer type might apply to other relevant cancers.

Minimal invasive sampling procedures aid molecular studies to help in detection and prevention of EC. More frequent tests or more different tests would almost likely assist early detection approaches (among the high risk or symptomatic); however, the potential value of screening among the asymptomatic must also be evaluated. Molecular testing may aid in refining present diagnostic algorithms among symptomatic women by reducing the effectiveness and rejection frequency of histological diagnosis that now restricts the effectiveness of endometrial aspirate–based diagnosis (144). Various screening techniques are being developed to aid the early detection of EC; one such developed test is the PapSEEK test which recognized the majority of women having EC and one-third of women having ovarian cancer in the NCI-funded study that employed the Pap test (standard screening test) among women who had previously been confirmed with cancer (145). SLN operations are becoming increasingly common in the management of EC, and the performance of SLN biopsy and the positive effect of early-stage EC were evaluated in a clinical trial showing that the procedure was feasible and safe. SLN mapping is built upon the idea of LNM as a consequence of a systematic

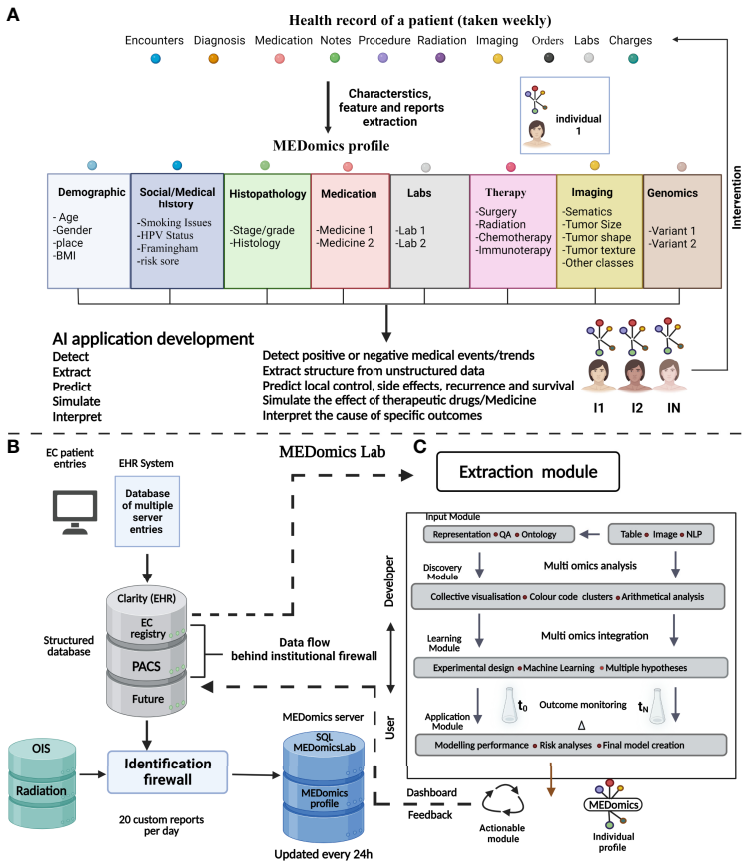

FIGURE 3 | The MEDomics approach for EC prediction and prognostication. An AI framework integrating EHRs with continuous learning infrastructure called "MEDomics" could use multimodal clinical data comprising thousands of cancer patients and millions of data points. The data are automatically extracted and integrated with analytical workflows. In addition, integration of natural language processing models is utilized for extraction of medical notes and classifying patients into different risk groups. The system has the potential to develop hypotheses based on the patients' data rather than laboratory data alone and can help to choose disease therapy and monitor disease prognosis. **(A)** Illustration of a patient's electronic health data records over a normal cancer care timeline. The MEDomics patterns are then used to construct AI and medical informatics software utilizing an "*in silico* randomized clinical trial" technique to discover, retrieve, anticipate, model, and evaluate important clinical endpoints. Management dashboards and other types of communication between health practitioners and data scientists are eventually used to transmit actionable solutions back into the health system. **(B)** Flow of medical data for the construction of AI and the building of MEDomics profiles. The EHRs and the Clarity relational database keep track of medical actions in real time. Clarity generates custom reports, which are then transmitted to the MEDomics server for deidentification, data structuring, and extraction/calculation of features. Data are eventually collected and refreshed every day on the MEDomics database installed behind the institutional firewall which is protected for access *via* a double identification. **(C)** MEDomics Lab as a system for medical research. Health records from institutional systems may be fed into a structured dataset, which is then fed into the MEDomics Lab system. The MEDomics Lab engine combines and processes these data, which is then employed in five separate computing modules: input, extraction, discovery, learning, and application. As a result, statistical models for precision oncology are developed, which may then be returned to hospital databases to aid clinical decision-making. QA, quality assurance; BMI, body mass index; HPV, human papillomavirus; PACS, picture archiving and communication system; OIS, oncology infrastructure system; *SQL*, Structured Query Language.

process. The lymph empties in a precise manner away from the tumor; hence, when the SLN, or initial node, is negative for metastasis, the nodes following the SLN would likewise be negative. While the disease needs to be properly staged to ensure an accurate prognosis and a selection of suitable treatment strategies, such techniques would assist patients to escape the adverse consequences involved in a total lymphadenectomy. Surgical expertise, commitment to an SLN methodology, and the utilization of pathological "ultra-staging" are all important variables in SLN mapping effectiveness (146, 147)

Although ML techniques can learn from both small and large datasets, the size and homogeneity of the datasets used for ML or DL models are equally important for the accuracy of the models. Most of the AI models are trained on small training datasets resulting in a compromised accuracy of the model. Generating larger datasets from real-world samples is a daunting task. Although challenging, the lab-on-a-chip and organ-on-a-chip technologies can be explored to simulate the tumor microenvironment and generate clinically relevant larger datasets required to develop powerful algorithms and models.

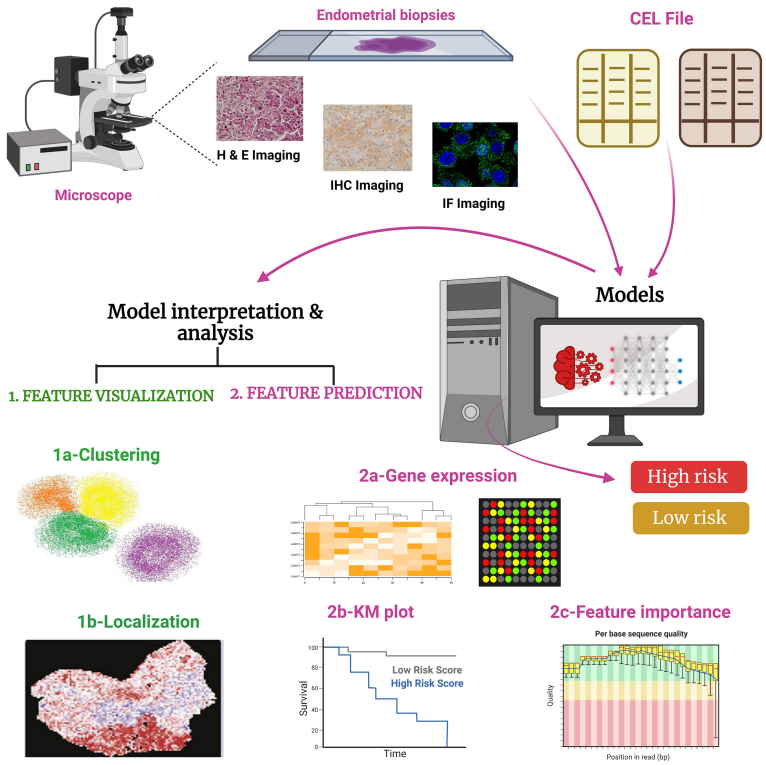

FIGURE 4 | Working of PANOPTES, a multiresolution deep convolutional neural network (CNN). In this trained model generated through the CNN algorithm, pathological images were used to predict the gene mutations and histological and molecular subtypes of EC. PANOPTES ML models using multiresolution architecture can classify histological subtypes of EC, molecular subtypes, and critical mutations (loss or gain of functions) with decent performance based on H&E (hematoxylin and eosin), IHC (immunohistochemistry), and IF (immunofluorescence) images. Also, some data can be accessed from the input CEL files. Predicated on the input, CNN models identify subtypes and mutations in EC. Multiresolution CNN models outperform single-resolution CNN models on the visual patterns. CNN models would incorporate human interpretable tumor characteristics according to feature extraction. Tumor grade identifies the molecular subtype and classifies into high-risk or low-risk cohorts from endometrioid histology samples to capture characteristics of varied sizes on the H&E, IHC, and IF slides, which is similar to a human operator pathological evaluation. Unlike traditional CNN architectures, Panoptes' input is a group of three tiles from the same region on the image slide rather than one tile. CEL file: differential expression profile generated by Affymetrix DNA microarray-based software analysis.

Since clinical data (the major source of cancer research) sharing is a pressing challenge due to ethical and legal issues, efforts can be made to develop a secured data sharing system. For instance, a learning system can be developed where the raw data remains with the source organization or institution, and a model is shared with the clinic to preprocess the raw data, and then the processed/curated data are to be shared with the research center (transfer learning making a web of information sharing in a protected way). In this regard, robust AI systems with smart strategies are needed. It is envisaged that, in near future, AI-based continuous learning could develop a smart decision-making system where both the physician and patient could be benefited.

AUTHOR CONTRIBUTIONS

VB, AS, and SP prepared the first draft of the manuscript and editing. IG and XZ, editing. PEL, PQ, and VP, conceptualization, writing, reviewing, and editing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2020. *CA Cancer J Clin* (2020) 70(1):7–30. doi: 10.3322/caac.21590
2. Anderson AS, Key TJ, Norat T, Scoccianti C, Cecchini M, Berrino F, et al. European Code Against Cancer 4th Edition: Obesity, Body Fatness and Cancer. *Cancer Epidemiol* (2015) 39(Suppl 1):S34–45. doi: 10.1016/j.canep.2015.01.017
3. Ginsburg O, Bray F, Coleman MP, Vanderpuye V, Eniu A, Kotha SR, et al. The Global Burden of Women's Cancers: A Grand Challenge in Global Health. *Lancet* (2017) 389(10071):847–60. doi: 10.1016/S0140-6736(16)31392-7
4. Lewin SN. Revised FIGO Staging System for Endometrial Cancer. *Clin Obstet Gynecol* (2011) 54(2):215–8. doi: 10.1097/GRF.0b013e3182185baa
5. Trojano G, Olivieri C, Tinelli R, Damiani GR, Pellegrino A, Cicinelli E. Conservative Treatment in Early Stage Endometrial Cancer: A Review. *Acta Biomed* (2019) 90(4):405–10. doi: 10.23750/abm.v90i4.7800
6. Lachance JA, Darus CJ, Rice LW. Surgical Management and Postoperative Treatment of Endometrial Carcinoma. *Rev Obstet Gynecol* (2008) 1(3):97–105.
7. *Key Statistics for Endometrial Cancer [Internet]* . Available at: https://www.cancer.org/cancer/endometrial-cancer/about/key-statistics.html.
8. Telner DE, Jakubovicz D. Approach to Diagnosis and Management of Abnormal Uterine Bleeding. *Can Fam Physician* (2007) 53(1):58–64.
9. Clark TJ, Mann CH, Shah N, Khan KS, Song F, Gupta JK. Accuracy of Outpatient Endometrial Biopsy in the Diagnosis of Endometrial Cancer: A Systematic Quantitative Review. *BJOG* (2002) 109(3):313–21. doi: 10.1111/j.1471-0528.2002.01088.x
10. Visser NCM, Reijnen C, Massuger LFAG, Nagtegaal ID, Bulten J, Pijnenborg JMA. Accuracy of Endometrial Sampling in Endometrial Carcinoma: A Systematic Review and Meta-Analysis. *Obstet Gynecol* (2017) 130(4):803–13. doi: 10.1097/AOG.0000000000002261
11. Yanoh K, Norimatsu Y, Hirai Y, Takeshima N, Kamimori A, Nakamura Y, et al. New Diagnostic Reporting Format for Endometrial Cytology Based on Cytoarchitectural Criteria. *Cytopathology* (2009) 20(6):388–94. doi: 10.1111/j.1365-2303.2008.00581.x
12. Chandra V, Kim JJ, Benbrook DM, Dwivedi A, Rai R. Therapeutic Options for Management of Endometrial Hyperplasia. *J Gynecol Oncol* (2016) 27(1):e8. doi: 10.3802/jgo.2016.27.e8
13. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2019. *CA Cancer J Clin* (2019) 69(1):7–34. doi: 10.3322/caac.21551
14. Hueman MT, Wang H, Yang CQ, Sheng L, Henson DE, Schwartz AM, et al. Creating Prognostic Systems for Cancer Patients: A Demonstration Using Breast Cancer. *Cancer Med* (2018) 7(8):3611–21. doi: 10.1002/cam4.1629
15. Yang CQ, Gardiner L, Wang H, Hueman MT, Chen D. Creating Prognostic Systems for Well-Differentiated Thyroid Cancer Using Machine Learning. *Front Endocrinol (Lausanne)* (2019) 10:288. doi: 10.3389/fendo.2019.00288
16. Deo RC. Machine Learning in Medicine. *Circulation* (2015) 132(20):1920–30. doi: 10.1161/CIRCULATIONAHA.115.001593
17. Handelman GS, Kok HK, Chandra RV, Razavi AH, Lee MJ, Asadi H. Edoctor: Machine Learning and the Future of Medicine. *J Intern Med* (2018) 284(6):603–19. doi: 10.1111/joim.12822
18. McCarthy JF, Marx KA, Hoffman PE, Gee AG, O'Neil P, Ujwal ML, et al. Applications of Machine Learning and High-Dimensional Visualization in Cancer Detection, Diagnosis, and Management. *Ann N Y Acad Sci* (2004) 1020:239–62. doi: 10.1196/annals.1310.020
19. Davenport T, Kalakota R. The Potential for Artificial Intelligence in Healthcare. *Future Healthc J* (2019) 6(2):94–8. doi: 10.7861/futurehosp.6-2-94
20. Gomez Rossi J, Rojas-Perilla N, Krois J, Schwendicke F. Cost-Effectiveness of Artificial Intelligence as a Decision-Support System Applied to the Detection and Grading of Melanoma, Dental Caries, and Diabetic Retinopathy. *JAMA Netw Open* (2022) 5(3):e220269. doi: 10.1001/jamanetworkopen.2022.0269
21. Chilamkurthy S, Ghosh R, Tanamala S, Biviji M, Campeau NG, Venugopal VK, et al. Deep Learning Algorithms for Detection of Critical Findings in Head CT Scans: A Retrospective Study. *Lancet* (2018) 392(10162):2388–96. doi: 10.1016/S0140-6736(18)31645-3
22. Esteva A, Kuprel B, Novoa RA, Ko J, Swetter SM, Blau HM, et al. Dermatologist-Level Classification of Skin Cancer With Deep Neural Networks. *Nature* (2017) 542(7639):115–8. doi: 10.1038/nature21056
23. Gulshan V, Peng L, Coram M, Stumpe MC, Wu D, Narayanaswamy A, et al. Development and Validation of a Deep Learning Algorithm for Detection of Diabetic Retinopathy in Retinal Fundus Photographs. *JAMA* (2016) 316(22):2402–10. doi: 10.1001/jama.2016.17216
24. Kesler SR, Rao A, Blayney DW, Oakley-Girvan IA, Karuturi M, Palesh O. Predicting Long-Term Cognitive Outcome Following Breast Cancer With Pre-Treatment Resting State fMRI and Random Forest Machine Learning. *Front Hum Neurosci* (2017) 11:555. doi: 10.3389/fnhum.2017.00555
25. Hayashida Y, Honda K, Osaka Y, Hara T, Umaki T, Tsuchida A, et al. Possible Prediction of Chemoradiosensitivity of Esophageal Cancer by Serum Protein Profiling. *Clin Cancer Res* (2005) 11(22):8042–7. doi: 10.1158/1078-0432.CCR-05-0656
26. Drago GP, Setti E, Licitra L, Liberati D. Forecasting the Performance Status of Head and Neck Cancer Patient Treatment by an Interval Arithmetic Pruned Perceptron. *IEEE Trans BioMed Eng* (2002) 49(8):782–7. doi: 10.1109/TBME.2002.800788
27. Man TK, Chintagumpala M, Visvanathan J, Shen J, Perlaky L, Hicks J, et al. Expression Profiles of Osteosarcoma That can Predict Response to Chemotherapy. *Cancer Res* (2005) 65(18):8142–50. doi: 10.1158/0008-5472.CAN-05-0985
28. Michael A, Ball G, Quatan N, Wushishi F, Russell N, Whelan J, et al. Delayed Disease Progression After Allogeneic Cell Vaccination in Hormone-Resistant Prostate Cancer and Correlation With Immunologic Variables. *Clin Cancer Res* (2005) 11(12):4469–78. doi: 10.1158/1078-0432.CCR-04-2337
29. Su M, Miften M, Whiddon C, Sun X, Light K, Marks L. An Artificial Neural Network for Predicting the Incidence of Radiation Pneumonitis. *Med Phys* (2005) 32(2):318–25. doi: 10.1118/1.1835611
30. Bokhman JV. Two Pathogenetic Types of Endometrial Carcinoma. *Gynecol Oncol* (1983) 15(1):10–7. doi: 10.1016/0090-8258(83)90111-7
31. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial Cancer. *Lancet* (2016) 387(10023):1094–108. doi: 10.1016/S0140-6736(15)00130-0
32. Suarez AA, Felix AS, Cohn DE. Bokhman Redux: Endometrial Cancer "Types" in the 21st Century. *Gynecol Oncol* (2017) 144(2):243–9. doi: 10.1016/j.ygyno.2016.12.010
33. Practice Bulletin No. 149: Endometrial Cancer. *Obstet Gynecol* (2015) 125(4):1006–26. doi: 10.1097/01.AOG.0000462977.61229.de
34. Onstad MA, Schmandt RE, Lu KH. Addressing the Role of Obesity in Endometrial Cancer Risk, Prevention, and Treatment. *J Clin Oncol* (2016) 34(35):4225–30. doi: 10.1200/JCO.2016.69.4638
35. Ni J, Zhu T, Zhao L, Che F, Chen Y, Shou H, et al. Metabolic Syndrome is an Independent Prognostic Factor for Endometrial Adenocarcinoma. *Clin Transl Oncol* (2015) 17(10):835–9. doi: 10.1007/s12094-015-1309-8
36. Prakash V, Fadare O, Tornos C, McCluggage WG. Committee Opinion No. 631, Endometrial Intraepithelial Neoplasia. *Obstet Gynecol* (2015) 126(4):897. doi: 10.1097/AOG.0000000000001071
37. Singh G, Puckett Y. Endometrial Hyperplasia. In: *StatPearls*. Treasure Island (FL: StatPearls Publishing (2021). Available at: http://www.ncbi.nlm.nih.gov/books/NBK560693/.
38. *Endometrial Cancer Treatment (PDQ®)–Health Professional Version*. Bethesda, MD: National Cancer Institute (2021). Available at: https://www.cancer.gov/types/uterine/hp/endometrial-treatment-pdq.
39. Passarello K, Kurian S, Villanueva V. Endometrial Cancer: An Overview of Pathophysiology, Management, and Care. *Semin Oncol Nurs* (2019) 35(2):157–65. doi: 10.1016/j.soncn.2019.02.002
40. Brooks RA, Fleming GF, Lastra RR, Lee NK, Moroney JW, Son CH, et al. Current Recommendations and Recent Progress in Endometrial Cancer. *CA Cancer J Clin* (2019) 69(4):258–79. doi: 10.3322/caac.21561
41. Hann LE, Giess CS, Bach AM, Tao Y, Baum HJ, Barakat RR. Endometrial Thickness in Tamoxifen-Treated Patients: Correlation With Clinical and Pathologic Findings. *AJR Am J Roentgenol* (1997) 168(3):657–61. doi: 10.2214/ajr.168.3.9057510
42. Sorosky JI. Endometrial Cancer. *Obstet Gynecol* (2012) 120(2 Pt 1):383–97. doi: 10.1097/AOG.0b013e3182605bf1
43. Wan J, Gao Y, Zeng K, Yin Y, Zhao M, Wei J, et al. The Levels of the Sex Hormones are Not Different Between Type 1 and Type 2 Endometrial Cancer. *Sci Rep* (2016) 6:39744. doi: 10.1038/srep39744

44. Chen WY. Exogenous and Endogenous Hormones and Breast Cancer. *Best Pract Res Clin Endocrinol Metab* (2008) 22(4):573–85. doi: 10.1016/j.beem.2008.08.001
45. Setiawan VW, Yang HP, Pike MC, McCann SE, Yu H, Xiang YB, et al. Type I and II Endometrial Cancers: Have They Different Risk Factors? *J Clin Oncol* (2013) 31(20):2607–18. doi: 10.1200/JCO.2012.48.2596
46. Saso S, Chatterjee J, Georgiou E, Ditri AM, Smith JR, Ghaem-Maghami S. Endometrial Cancer. *BMJ* (2011) 343:d3954. doi: 10.1136/bmj.d3954
47. Buchanan EM, Weinstein LC, Hillson C. Endometrial Cancer. *Am Fam Physician* (2009) 80(10):1075–80.
48. Schmandt RE, Iglesias DA, Co NN, Lu KH. Understanding Obesity and Endometrial Cancer Risk: Opportunities for Prevention. *Am J Obstet Gynecol* (2011) 205(6):518–25. doi: 10.1016/j.ajog.2011.05.042
49. Courneya KS, Karvinen KH, Campbell KL, Pearcey RG, Dundas G, Capstick V, et al. Associations Among Exercise, Body Weight, and Quality of Life in a Population-Based Sample of Endometrial Cancer Survivors. *Gynecol Oncol* (2005) 97(2):422–30. doi: 10.1016/j.ygyno.2005.01.007
50. Wong A, Ngeow J. Hereditary Syndromes Manifesting as Endometrial Carcinoma: How Can Pathological Features Aid Risk Assessment? *BioMed Res Int* (2015) 2015:219012. doi: 10.1155/2015/219012
51. Ngeow J, Stanuch K, Mester JL, Barnholtz-Sloan JS, Eng C. Second Malignant Neoplasms in Patients With Cowden Syndrome With Underlying Germline PTEN Mutations. *J Clin Oncol* (2014) 32(17):1818–24. doi: 10.1200/JCO.2013.53.6656
52. Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP. Milestones of Lynch Syndrome: 1895-2015. *Nat Rev Cancer* (2015) 15(3):181–94. doi: 10.1038/nrc3878
53. Meyer LA, Broaddus RR, Lu KH. Endometrial Cancer and Lynch Syndrome: Clinical and Pathologic Considerations. *Cancer Control* (2009) 16(1):14–22. doi: 10.1177/107327480901600103
54. Clarke MA, Long BJ, Del Mar Morillo A, Arbyn M, Bakkum-Gamez JN, Wentzensen N. Association of Endometrial Cancer Risk With Postmenopausal Bleeding in Women: A Systematic Review and Meta-Analysis. *JAMA Intern Med* (2018) 178(9):1210–22. doi: 10.1001/jamainternmed.2018.2820
55. Sachan PL, Singh M, Patel ML, Sachan R. A Study on Cervical Cancer Screening Using Pap Smear Test and Clinical Correlation. *Asia Pac J Oncol Nurs* (2018) 5(3):337–41. doi: 10.4103/apjon.apjon_15_18
56. ACOG Committee on Practice Bulletins–Gynecology. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin: Management of Anovulatory Bleeding. *Int J Gynaecol Obstet* (2001) 72(3):263–71. doi: 10.1016/s0020-7292(01)00357-5
57. Bennett GL, Andreotti RF, Lee SI, Dejesus Allison SO, Brown DL, Dubinsky T, et al. ACR Appropriateness Criteria(®) on Abnormal Vaginal Bleeding. *J Am Coll Radiol* (2011) 8(7):460–8. doi: 10.1016/j.jacr.2011.03.011
58. Smith P, Bakos O, Heimer G, Ulmsten U. Transvaginal Ultrasound for Identifying Endometrial Abnormality. *Acta Obstet Gynecol Scand* (1991) 70 (7–8):591–4. doi: 10.3109/00016349109007922
59. Smith-Bindman R, Kerlikowske K, Feldstein VA, Subak L, Scheidler J, Segal M, et al. Endovaginal Ultrasound to Exclude Endometrial Cancer and Other Endometrial Abnormalities. *JAMA* (1998) 280(17):1510–7. doi: 10.1001/jama.280.17.1510
60. O'Flynn H, Ryan NAJ, Narine N, Shelton D, Rana D, Crosbie EJ. Diagnostic Accuracy of Cytology for the Detection of Endometrial Cancer in Urine and Vaginal Samples. *Nat Commun* (2021) 12(1):952. doi: 10.1038/s41467-021-21257-6
61. Chan FY, Chau MT, Pun TC, Lam C, Ngan HY, Leong L, et al. Limitations of Transvaginal Sonography and Color Doppler Imaging in the Differentiation of Endometrial Carcinoma From Benign Lesions. *J Ultrasound Med* (1994) 13(8):623–8. doi: 10.7863/jum.1994.13.8.623
62. PDQ Screening and Prevention Editorial Board. Endometrial Cancer Screening (PDQ®): Patient Version. In: *PDQ Cancer Information Summaries*. Bethesda (MD: National Cancer Institute (US (2002). Available at: http://www.ncbi.nlm.nih.gov/books/NBK66051/.
63. Rs G, KS A, Mc R, Wa C. Pipelle Endometrial Sampling. Sensitivity in the Detection of Endometrial Cancer. *J Reprod Med* (1995) 40(8):553–5.
64. Du J, Li Y, Lv S, Wang Q, Sun C, Dong X, et al. Endometrial Sampling Devices for Early Diagnosis of Endometrial Lesions. *J Cancer Res Clin Oncol* (2016) 142(12):2515–22. doi: 10.1007/s00432-016-2215-3
65. Clark TJ, Voit D, Gupta JK, Hyde C, Song F, Khan KS. Accuracy of Hysteroscopy in the Diagnosis of Endometrial Cancer and Hyperplasia: A Systematic Quantitative Review. *JAMA* (2002) 288(13):1610–21. doi: 10.1001/jama.288.13.1610
66. Zerbe MJ, Bristow R, Grumbine FC, Montz FJ. Inability of Preoperative Computed Tomography Scans to Accurately Predict the Extent of Myometrial Invasion and Extracorporal Spread in Endometrial Cancer. *Gynecol Oncol* (2000) 78(1):67–70. doi: 10.1006/gyno.2000.5820
67. Ngiam KY, Khor IW. Big Data and Machine Learning Algorithms for Health-Care Delivery. *Lancet Oncol* (2019) 20(5):e262–73. doi: 10.1016/S1470-2045(19)30149-4
68. Bishop CM, Nasrabadi NM. Pattern Recognition and Machine Learning. New York: Springer (2006).
69. Duda RO, Hart PE, Stork DG. *Pattern Classification*. New York: John Wiley (2001).
70. Quinlan JR. Induction of Decision Trees. *Mach Learn* (1986) 1(1):81–106. doi: 10.1007/BF00116251
71. Langley P, Iba W, Thompson K. An Analysis of Bayesian Classifiers. San Jose: AAAI Press (1992), 223–8 p.
72. Patrick EA, Fischer FPIII. A Generalized K-Nearest Neighbor Rule. *Info Control* (1970) 16(2):128–52. doi: 10.1016/S0019-9958(70)90081-1
73. Rumelhart DE, Hinton GE, Williams RJ. Learning Representations by Back-Propagating Errors. *Nature* (1986) 323(6088):533–6. doi: 10.1038/323533a0
74. Vapnik V. *Estimation of Dependences Based on Empirical Data*. Germany: Springer Science & Business Media New York (2006). 515 p.
75. Holland JH. *Adaptation in Natural and Artificial Systems: An Introductory Analysis With Applications to Biology, Control, and Artificial Intelligence*. United Kingdom: MIT Press (1992). 236 p.
76. He H, Garcia EA. Learning From Imbalanced Data. *IEEE Trans Knowledge Data Engineering* (2009) 21(9):1263–84. doi: 10.1109/TKDE.2008.239
77. Kotsiantis S, Kanellopoulos D, Pintelas P. Handling Imbalanced Datasets: A Review. *GESTS Int Transactions Comput Sci Eng* (2006) 30(1):25-36.
78. Chawla NV, Lazarevic A, Hall LO, Bowyer KW. SMOTEBoost: Improving Prediction of the Minority Class in Boosting. In: N Lavrač, D Gamberger, L Todorovski and H Blockeel, editors. *Knowledge Discovery in Databases: PKDD 2003*. Berlin, Heidelberg: Springer (2003). p. p.107–19. Lecture Notes in Computer Science.
79. Li GZ, Meng HH, Lu WC, Yang JY, Yang MQ. Asymmetric Bagging and Feature Selection for Activities Prediction of Drug Molecules. *BMC Bioinf* (2008) 9 Suppl 6:S7. doi: 10.1186/1471-2105-9-S6-S7
80. Tao D, Tang X, Li X, Wu X. Asymmetric Bagging and Random Subspace for Support Vector Machines-Based Relevance Feedback in Image Retrieval. *IEEE Trans Pattern Anal Mach Intell* (2006) 28(7):1088–99. doi: 10.1109/TPAMI.2006.134
81. Zhou ZH, Liu XY. Training Cost-Sensitive Neural Networks With Methods Addressing the Class Imbalance Problem. *IEEE Trans Knowledge Data Engineering* (2006) 18(1):63–77. doi: 10.1109/TKDE.2006.17
82. Hempstalk K, Frank E, Witten IH. One-Class Classification by Combining Density and Class Probability Estimation. In: W Daelemans, B Goethals and K Morik, editors. *Machine Learning and Knowledge Discovery in Databases*. Berlin, Heidelberg: Springer (2008). p. p.505–19. Lecture Notes in Computer Science.
83. Acs B, Rantalainen M, Hartman J. Artificial Intelligence as the Next Step Towards Precision Pathology. *J Intern Med* (2020) 288(1):62–81. doi: 10.1111/joim.13030
84. Yamamoto Y, Tsuzuki T, Akatsuka J, Ueki M, Morikawa H, Numata Y, et al. Automated Acquisition of Explainable Knowledge From Unannotated Histopathology Images. *Nat Commun* (2019) 10(1):5642. doi: 10.1038/s41467-019-13647-8
85. Praiss AM, Huang Y, St Clair CM, Tergas AI, Melamed A, Khoury-Collado F, et al. Using Machine Learning to Create Prognostic Systems for Endometrial Cancer. *Gynecol Oncol* (2020) 159(3):744–50. doi: 10.1016/j.ygyno.2020.09.047
86. Hodneland E, Dybvik JA, Wagner-Larsen KS, Šoltészová V, Munthe-Kaas AZ, Fasmer KE, et al. Automated Segmentation of Endometrial Cancer on MR Images Using Deep Learning. *Sci Rep* (2021) 11(1):179. doi: 10.1038/s41598-020-80068-9
87. Urushibara A, Saida T, Mori K, Ishiguro T, Inoue K, Masumoto T, et al. The Efficacy of Deep Learning Models in the Diagnosis of Endometrial Cancer

Using MRI: A Comparison With Radiologists. *BMC Med Imag* (2022) 22 (1):80. doi: 10.1186/s12880-022-00808-3
88. Pelikan HMP, Trum JW, Bakers FCH, Beets-Tan RGH, Smits LJM, Kruitwagen RFPM. Diagnostic Accuracy of Preoperative Tests for Lymph Node Status in Endometrial Cancer: A Systematic Review. *Cancer Imag* (2013) 13(3):314–22. doi: 10.1102/1470-7330.2013.0032
89. Çiçek Ö, Abdulkadir A, Lienkamp SS, Brox T, Ronneberger O. 3D U-Net: Learning Dense Volumetric Segmentation From Sparse Annotation. In: S Ourselin, L Joskowicz, MR Sabuncu, G Unal and W Wells, editors. *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2016*. Cham: Springer International Publishing (2016). p. 424–32. Lecture Notes in Computer Science.
90. Dong HC, Dong HK, Yu MH, Lin YH, Chang CC. Using Deep Learning With Convolutional Neural Network Approach to Identify the Invasion Depth of Endometrial Cancer in Myometrium Using MR Images: A Pilot Study. *Int J Environ Res Public Health* (2020) 17(16):E5993. doi: 10.3390/ijerph17165993
91. Chen X, Wang Y, Shen M, Yang B, Zhou Q, Yi Y, et al. Deep Learning for the Determination of Myometrial Invasion Depth and Automatic Lesion Identification in Endometrial Cancer MR Imaging: A Preliminary Study in a Single Institution. *Eur Radiol* (2020) 30(9):4985–94. doi: 10.1007/s00330-020-06870-1
92. Xu X, Li H, Wang S, Fang M, Zhong L, Fan W, et al. Multiplanar MRI-Based Predictive Model for Preoperative Assessment of Lymph Node Metastasis in Endometrial Cancer. *Front Oncol* (2019) 9:1007. doi: 10.3389/fonc.2019.01007
93. Norimatsu Y, Kouda H, Kobayashi TK, Shimizu K, Yanoh K, Tsukayama C, et al. Utility of Liquid-Based Cytology in Endometrial Pathology: Diagnosis of Endometrial Carcinoma. *Cytopathology* (2009) 20(6):395–402. doi: 10.1111/j.1365-2303.2008.00589.x
94. Wang Q, Wang Q, Zhao L, Han L, Sun C, Ma S, et al. Endometrial Cytology as a Method to Improve the Accuracy of Diagnosis of Endometrial Cancer: Case Report and Meta-Analysis. *Front Oncol* (2019) 9:256. doi: 10.3389/fonc.2019.00256
95. Makris GM, Pouliakis A, Siristatidis C, Margari N, Terzakis E, Koureas N, et al. Image Analysis and Multi-Layer Perceptron Artificial Neural Networks for the Discrimination Between Benign and Malignant Endometrial Lesions. *Diagn Cytopathol* (2017) 45(3):202–11. doi: 10.1002/dc.23649
96. Zhang Y, Wang Z, Zhang J, Wang C, Wang Y, Chen H, et al. Deep Learning Model for Classifying Endometrial Lesions. *J Transl Med* (2021) 19(1):10. doi: 10.1186/s12967-020-02660-x
97. Haraldsdottir S, Hampel H, Tomsic J, Frankel WL, Pearlman R, de la Chapelle A, et al. Colon and Endometrial Cancers With Mismatch Repair Deficiency can Arise From Somatic, Rather Than Germline, Mutations. *Gastroenterology* (2014) 147(6):1308–1316.e1. doi: 10.1053/j.gastro.2014.08.041
98. Ryan N a J, Glaire MA, Blake D, Cabrera-Dandy M, Evans DG, Crosbie EJ. The Proportion of Endometrial Cancers Associated With Lynch Syndrome: A Systematic Review of the Literature and Meta-Analysis. *Genet Med* (2019) 21(10):2167–80. doi: 10.1038/s41436-019-0536-8
99. Veeraraghavan H, Friedman CF, DeLair DF, Ninčević J, Himoto Y, Bruni SG, et al. Machine Learning-Based Prediction of Microsatellite Instability and High Tumor Mutation Burden From Contrast-Enhanced Computed Tomography in Endometrial Cancers. *Sci Rep* (2020) 10(1):17769. doi: 10.1038/s41598-020-72475-9
100. Qi R, Wu D, Sheng L, Henson D, Schwartz A, Xu E, et al. On an Ensemble Algorithm for Clustering Cancer Patient Data. *BMC Syst Biol* (2013) 7 Suppl 4:S9. doi: 10.1186/1752-0509-7-S4-S9
101. Chen P, Yang Y, Zhang Y, Jiang S, Li X, Wan J. Identification of Prognostic Immune-Related Genes in the Tumor Microenvironment of Endometrial Cancer. *Aging (Albany NY)* (2020) 12(4):3371–87. doi: 10.18632/aging.102817
102. Vincent KM, Findlay SD, Postovit LM. Assessing Breast Cancer Cell Lines as Tumour Models by Comparison of mRNA Expression Profiles. *Breast Cancer Res* (2015) 17:114. doi: 10.1186/s13058-015-0613-0
103. Jia D, Li S, Li D, Xue H, Yang D, Liu Y. Mining TCGA Database for Genes of Prognostic Value in Glioblastoma Microenvironment. *Aging (Albany NY)* (2018) 10(4):592–605. doi: 10.18632/aging.101415
104. Alonso MH, Aussó S, Lopez-Doriga A, Cordero D, Guinó E, Solé X, et al. Comprehensive Analysis of Copy Number Aberrations in Microsatellite Stable Colon Cancer in View of Stromal Component. *Br J Cancer* (2017) 117 (3):421–31. doi: 10.1038/bjc.2017.208
105. Yang S, Liu T, Nan H, Wang Y, Chen H, Zhang X, et al. Comprehensive Analysis of Prognostic Immune-Related Genes in the Tumor Microenvironment of Cutaneous Melanoma. *J Cell Physiol* (2020) 235 (2):1025–35. doi: 10.1002/jcp.29018
106. Bristow RE, Purinton SC, Santillan A, Diaz-Montes TP, Gardner GJ, Giuntoli RL. Cost-Effectiveness of Routine Vaginal Cytology for Endometrial Cancer Surveillance. *Gynecologic Oncol* (2006) 103(2):709–13. doi: 10.1016/j.ygyno.2006.05.013
107. Sarica A, Cerasa A, Quattrone A. Random Forest Algorithm for the Classification of Neuroimaging Data in Alzheimer's Disease: A Systematic Review. *Front Aging Neurosci* (2017) 9:329. doi: 10.3389/fnagi.2017.00329
108. Mount DW, Putnam CW, Centouri SM, Manziello AM, Pandey R, Garland LL, et al. Using Logistic Regression to Improve the Prognostic Value of Microarray Gene Expression Data Sets: Application to Early-Stage Squamous Cell Carcinoma of the Lung and Triple Negative Breast Carcinoma. *BMC Med Genomics* (2014) 7:33. doi: 10.1186/1755-8794-7-33
109. Kingsford C, Salzberg SL. What are Decision Trees? *Nat Biotechnol* (2008) 26 (9):1011–3. doi: 10.1038/nbt0908-1011
110. Huang S, Cai N, Pacheco PP, Narrandes S, Wang Y, Xu W. Applications of Support Vector Machine (SVM) Learning in Cancer Genomics. *Cancer Genomics Proteomics* (2018) 15(1):41–51. doi: 10.21873/cgp.20063
111. Zhang Z, Zhao Y, Canes A, Steinberg D, Lyashevska Owritten on behalf of AME Big-Data Clinical Trial Collaborative Group. Predictive Analytics With Gradient Boosting in Clinical Medicine. *Ann Transl Med* (2019) 7(7):152. doi: 10.21037/atm.2019.03.29
112. Akazawa M, Hashimoto K, Noda K, Yoshida K. The Application of Machine Learning for Predicting Recurrence in Patients With Early-Stage Endometrial Cancer: A Pilot Study. *Obstet Gynecol Sci* (2021) 64(3):266–73. doi: 10.5468/ogs.20248
113. Langarizadeh M, Moghbeli F. Applying Naive Bayesian Networks to Disease Prediction: A Systematic Review. *Acta Inform Med* (2016) 24(5):364–9. doi: 10.5455/aim.2016.24.364-369
114. Günakan E, Atan S, Haberal AN, Küçükyıldız İA, Gökçe E, Ayhan A. A Novel Prediction Method for Lymph Node Involvement in Endometrial Cancer: Machine Learning. *Int J Gynecol Cancer* (2019) 29(2):320–4. doi: 10.1136/ijgc-2018-000033
115. Reijnen C, Gogou E, Visser NCM, Engerud H, Ramjith J, van der Putten LJM, et al. Preoperative Risk Stratification in Endometrial Cancer (ENDORISK) by a Bayesian Network Model: A Development and Validation Study. *PloS Med* (2020) 17(5):e1003111. doi: 10.1371/journal.pmed.1003111
116. Arora P, Boyne D, Slater JJ, Gupta A, Brenner DR, Druzdzel MJ. Bayesian Networks for Risk Prediction Using Real-World Data: A Tool for Precision Medicine. *Value Health* (2019) 22(4):439–45. doi: 10.1016/j.jval.2019.01.006
117. Meyers L. A New View of Sex Education. *J Natl Med Assoc* (1988) 80(2):129.
118. Srikantia N BR, Rajeev AG, Kalyan SN. Endometrioid Endometrial Adenocarcinoma in a Premenopausal Woman With Multiple Organ Metastases. *Indian J Med Paediatr Oncol* (2009) 30(2):80–3. doi: 10.4103/0971-5851.60053
119. Yin F, Shao X, Zhao L, Li X, Zhou J, Cheng Y, et al. Predicting Prognosis of Endometrioid Endometrial Adenocarcinoma on the Basis of Gene Expression and Clinical Features Using Random Forest. *Oncol Lett* (2019) 18(2):1597–606. doi: 10.3892/ol.2019.10504
120. Bendifallah S, Ouldamer L, Lavoue V, Canlorbe G, Raimond E, Coutant C, et al. Patterns of Recurrence and Outcomes in Surgically Treated Women With Endometrial Cancer According to ESMO-ESGO-ESTRO Consensus Conference Risk Groups: Results From the FRANCOGYN Study Group. *Gynecol Oncol* (2017) 144(1):107–12. doi: 10.1016/j.ygyno.2016.10.025
121. Cancer Genome Atlas Research Network, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated Genomic Characterization of Endometrial Carcinoma. *Nature* (2013) 497(7447):67–73. doi: 10.1038/nature12325
122. Burki TK. Predicting Lung Cancer Prognosis Using Machine Learning. *Lancet Oncol* (2016) 17(10):e421. doi: 10.1016/S1470-2045(16)30436-3
123. Hart GR, Yan V, Huang GS, Liang Y, Nartowt BJ, Muhammad W, et al. Population-Based Screening for Endometrial Cancer: Human vs. Machine Intelligence. *Front Artif Intell* (2020) 3:539879. doi: 10.3389/frai.2020.539879

124. *Bishop: Pattern Recognition - Google Scholar* . Available at: https://scholar.google.com/scholar_lookup?title=Pattern+recognition+and+machine+learning&author=C.+M.+Bishop&publication_year=2006&.
125. Kitson SJ, Evans DG, Crosbie EJ. Identifying High-Risk Women for Endometrial Cancer Prevention Strategies: Proposal of an Endometrial Cancer Risk Prediction Model. *Cancer Prev Res (Phila)* (2017) 10(1):1–13. doi: 10.1158/1940-6207.CAPR-16-0224
126. Hutt S, Mihaies D, Karteris E, Michael A, Payne AM, Chatterjee J. Statistical Meta-Analysis of Risk Factors for Endometrial Cancer and Development of a Risk Prediction Model Using an Artificial Neural Network Algorithm. *Cancers (Basel)* (2021) 13(15):3689. doi: 10.3390/cancers13153689
127. Kelly CJ, Karthikesalingam A, Suleyman M, Corrado G, King D. Key Challenges for Delivering Clinical Impact With Artificial Intelligence. *BMC Med* (2019) 17(1):195. doi: 10.1186/s12916-019-1426-2
128. Collins GS, Moons KGM. Reporting of Artificial Intelligence Prediction Models. *Lancet* (2019) 393(10181):1577–9. doi: 10.1016/S0140-6736(19)30037-6
129. Saito T, Rehmsmeier M. The Precision-Recall Plot is More Informative Than the ROC Plot When Evaluating Binary Classifiers on Imbalanced Datasets. *PloS One* (2015) 10(3):e0118432. doi: 10.1371/journal.pone.0118432
130. Vickers AJ, Cronin AM, Elkin EB, Gonen M. Extensions to Decision Curve Analysis, a Novel Method for Evaluating Diagnostic Tests, Prediction Models and Molecular Markers. *BMC Med Inform Decis Mak* (2008) 8:53. doi: 10.1186/1472-6947-8-53
131. Shah NH, Milstein A, Bagley PhD SC. Making Machine Learning Models Clinically Useful. *JAMA* (2019) 322(14):1351–2. doi: 10.1001/jama.2019.10306
132. Dalton JE. Flexible Recalibration of Binary Clinical Prediction Models. *Stat Med* (2013) 32(2):282–9. doi: 10.1002/sim.5544
133. Debray TPA, Vergouwe Y, Koffijberg H, Nieboer D, Steyerberg EW, Moons KGM. A New Framework to Enhance the Interpretation of External Validation Studies of Clinical Prediction Models. *J Clin Epidemiol* (2015) 68(3):279–89. doi: 10.1016/j.jclinepi.2014.06.018
134. Kim DW, Jang HY, Kim KW, Shin Y, Park SH. Design Characteristics of Studies Reporting the Performance of Artificial Intelligence Algorithms for Diagnostic Analysis of Medical Images: Results From Recently Published Papers. *Korean J Radiol* (2019) 20(3):405–10. doi: 10.3348/kjr.2019.0025
135. Hersh WR, Weiner MG, Embi PJ, Logan JR, Payne PRO, Bernstam EV, et al. Caveats for the Use of Operational Electronic Health Record Data in Comparative Effectiveness Research. *Med Care* (2013) 51(8 Suppl 3):S30–37. doi: 10.1097/MLR.0b013e31829b1dbd
136. Mandel JC, Kreda DA, Mandl KD, Kohane IS, Ramoni RB. SMART on FHIR: A Standards-Based, Interoperable Apps Platform for Electronic Health Records. *J Am Med Inform Assoc* (2016) 23(5):899–908. doi: 10.1093/jamia/ocv189
137. Troyanskaya O, Trajanoski Z, Carpenter A, Thrun S, Razavian N, Oliver N. Artificial Intelligence and Cancer. *Nat Cancer* (2020) 1(2):149–52. doi: 10.1038/s43018-020-0034-6
138. Deasy JO, Stetson PD. A Platform for Continuous Learning in Oncology. *Nat Cancer* (2021) 2(7):675–6. doi: 10.1038/s43018-021-00239-z
139. Morin O, Vallières M, Braunstein S, Ginart JB, Upadhaya T, Woodruff HC, et al. An Artificial Intelligence Framework Integrating Longitudinal Electronic Health Records With Real-World Data Enables Continuous Pan-Cancer Prognostication. *Nat Cancer* (2021) 2(7):709–22. doi: 10.1038/s43018-021-00236-2
140. Coudray N, Tsirigos A. Deep Learning Links Histology, Molecular Signatures and Prognosis in Cancer. *Nat Cancer* (2020) 1(8):755–7. doi: 10.1038/s43018-020-0099-2
141. Kather JN, Heij LR, Grabsch HI, Loeffler C, Echle A, Muti HS, et al. Pan-Cancer Image-Based Detection of Clinically Actionable Genetic Alterations. *Nat Cancer* (2020) 1(8):789–99. doi: 10.1038/s43018-020-0087-6
142. Fu Y, Jung AW, Torne RV, Gonzalez S, Vöhringer H, Shmatko A, et al. Pan-Cancer Computational Histopathology Reveals Mutations, Tumor Composition and Prognosis. *Nat Cancer* (2020) 1(8):800–10. doi: 10.1038/s43018-020-0085-8
143. Hong R, Liu W, DeLair D, Razavian N, Fenyö D. Predicting Endometrial Cancer Subtypes and Molecular Features From Histopathology Images Using Multi-Resolution Deep Learning Models. *Cell Rep Med* (2021) 2(9):100400. doi: 10.1016/j.xcrm.2021.100400
144. Costas L, Frias-Gomez J, Guardiola M, Benavente Y, Pineda M, Pavón MÁ, et al. New Perspectives on Screening and Early Detection of Endometrial Cancer. *Int J Cancer* (2019) 145(12):3194–206. doi: 10.1002/ijc.32514
145. Wang Y, Li L, Douville C, Cohen JD, Yen TT, Kinde I, et al. Evaluation of Liquid from the Papanicolaou Test and Other Liquid Biopsies for the Detection of Endometrial and Ovarian Cancers. *Sci Transl Med* (2018) 10(433):eaap8793. doi: 10.1126/scitranslmed.aap8793
146. Backes FJ, Cohen D, Salani R, Cohn DE, O'Malley DM, Fanning E, et al. Prospective Clinical Trial of Robotic Sentinel Lymph Node Assessment With Isosulfane Blue (ISB) and Indocyanine Green (ICG) in Endometrial Cancer and the Impact of Ultrastaging (NCT01818739). *Gynecol Oncol* (2019) 153(3):496–9. doi: 10.1016/j.ygyno.2019.03.252
147. Abu-Rustum NR. Sentinel Lymph Node Mapping for Endometrial Cancer: A Modern Approach to Surgical Staging. *J Natl Compr Canc Netw* (2014) 12(2):288–97. doi: 10.6004/jnccn.2014.0026

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EDITED BY
Tea Lanisnik Rizner,
University of Ljubljana, Slovenia

REVIEWED BY
Komsun Suwannarurk,
Thammasat University, Thailand
Umberto Leone Roberti Maggiore,
Fondazione IRCCS Istituto Nazionale dei Tumori (IRCCS), Italy
Jure Knez,
Maribor University Medical Centre, Slovenia

*CORRESPONDENCE
Vít Weinberger
weinberger.vit@fnbrno.cz

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External validation study of endometrial cancer preoperative risk stratification model (ENDORISK)

Petra Vinklerová[1], Petra Ovesná[2], Jitka Hausnerová[3], Johanna M. A. Pijnenborg[4], Peter J. F. Lucas[5], Casper Reijnen[6], Stephanie Vrede[4] and Vít Weinberger[1]*

[1]Department of Gynecology and Obstetrics, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czechia, [2]Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czechia, [3]Department of Pathology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czechia, [4]Department of Obstetrics and Gynecology, Radboud University Medical Center, Nijmegen, Netherlands, [5]Department of Data Science, University of Twente, Enschede, Netherlands, [6]Department of Radiation Oncology, Radboud University Medical Center, Nijmegen, Netherlands

Introduction: Among industrialized countries, endometrial cancer is a common malignancy with generally an excellent outcome. To personalize medicine, we ideally compile as much information as possible concerning patient prognosis prior to effecting an appropriate treatment decision. Endometrial cancer preoperative risk stratification (ENDORISK) is a machine learning–based computational Bayesian networks model that predicts lymph node metastasis and 5-year disease-specific survival potential with percentual probability. Our objective included validating ENDORISK effectiveness in our patient cohort, assessing its application in the current use of sentinel node biopsy, and verifying its accuracy in advanced stages.

Methods: The ENDORISK model was evaluated with a retrospective cohort of 425 patients from the University Hospital Brno, Czech Republic. Two hundred ninety-nine patients were involved in our disease-specific survival analysis; 226 cases with known lymph node status were available for lymph node metastasis analysis. Patients were included undergoing either pelvic lymph node dissection (*N* = 84) or sentinel node biopsy (*N* =70) to explore the accuracy of both staging procedures.

Results: The area under the curve was 0.84 (95% confidence interval [CI], 0.77–0.9) for lymph node metastasis analysis and 0.86 (95% CI, 0.79–0.93) for 5-year disease-specific survival evaluation, indicating quite positive concordance between prediction and reality. Calibration plots to visualize results demonstrated an outstanding predictive value for low-risk cancers (grades 1–2), whereas outcomes were underestimated among high-risk patients (grade 3), especially in disease-specific survival. This phenomenon was even more obvious when patients were subclassified according to FIGO clinical stages.

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Conclusions: Our data confirmed ENDORISK model's laudable predictive ability, particularly among patients with a low risk of lymph node metastasis and expected favorable survival. For high-risk and/or advanced stages, the ENDORISK network needs to be additionally trained/improved.

KEYWORDS

Bayesian networks model, disease-specific survival, endometrial cancer, prognosis, risk stratification, sentinel node biopsy, lymph node metastasis

Introduction

In industrialized countries, endometrial cancer (EC) is a common malignancy with generally an excellent outcome and 5-year relative survival rate of 76% among European women (1). Despite its overall favorable prognosis, up to 15% of patients classified as low-risk will experience recurrence and may profit from adjuvant treatment (2). Conversely, a substantial number of patients classified as high-risk surprisingly evidence no disease recurrence many years after treatment. Respecting the current emphasis on personalized medicine, we ideally seek as much information as possible concerning a patient's prognosis prior to determine the most effective therapeutic approach, avoid overtreatment, and prevent treatment-related morbidity. Current European guidelines classify patients into five prognostic risk groups based on final tumor stage and histological characteristics (3). However, in the preoperative setting, risk stratification can be challenging owing to the lack of certain essential definitive histology information such as lymphovascular space invasion (LVSI) and myometrial invasion.

Lymph node (LN) involvement is an important issue that impacts treatment approach and is related to poor prognosis. Two large randomized trials (4, 5) renounced the curative significance of lymphadenectomy. Nowadays, pelvic and para-aortic lymphadenectomy (PLN and PALN) are mainly considered as staging tools with substantial morbidity (6). According to the recent European guidelines, sentinel node biopsy (SNB) is an alternative to full lymphadenectomy in low/intermediate-risk stage I/II EC and can also be considered in high-intermediate and high-risk stage I/II groups (3).

In order to identify preoperatively which patients are at risk for lymph node metastasis (LNM), the endometrial cancer preoperative risk stratification (ENDORISK) was constructed within the ENITEC network (European Network of Individual Treatment in Endometrial Cancer) (7). This is a machine learning–based computational Bayesian networks model, which predicts the probability of LNM and 5-year disease specific survival (DSS) in EC cases. This ENDORISK model has been validated forthwith using two multicentric cohorts: MoMaTEC (the Molecular Markers in Treatment in Endometrial Cancer) (8) and PIPENDO (the PIpelle Prospective ENDOmetrial carcinoma) study (9). The diagnostic accuracy was 0.82 and 0.84, respectively. Input data contains preoperative clinical and histological characteristics. Since the original model consisted of a notably heterogeneous patient group from many countries with possible treatment decision divergencies, we were questioning how this model would perform within our patient cohort with very well-structured and collected preoperative clinical/histological data, adjuvant treatment, and follow-up.

Our aim was to validate the ENDORISK model's accuracy and the applicability within the current SNB staging era. Since the model was constructed based on full lymphadenectomy, our further objective was to evaluate the model's potential accuracy bias by introducing the SNB method. Additionally, we wanted to verify the model's performance within advanced EC stages. Our study points out the weaknesses and strengths of the original ENDORISK model and proposes certain modifications in order to utilize the model within the actual and real clinical practice worldwide.

Methods

Patient cohort

We evaluated the ENDORISK model in our retrospectively collected study cohort including 425 patients treated at the University Hospital Brno, Czech Republic. Our cohort evolved from an EC database of 835 patients treated between January 2006 and May 2021. Cases that were incorporated in the original ENDORISK model (N = 150) and those without the minimally required data for using ENDORISK (N = 240) were excluded.

We assessed clinical and histological characteristics from the EC database and patients' medical records: age, BMI, follow-up length, preoperative tumor grade/histotype, estrogen receptor (ER), progesterone receptor (PR), L1 Cell Adhesion Molecule

(L1CAM), p53 expression, cancer antigen (Ca) 125 serum level, platelet count, preoperative cervical cytology result, lymphadenopathy according to imaging methods, myometrial/cervical invasion, LVSI, clinical/surgical staging, LN staging method, LNM, and adjuvant treatment.

All patients underwent preoperative biopsy *via* hysteroscopy or dilatation and curettage, imaging staging procedures with expert ultrasound and computed tomography (CT) scan to detect local or distant disease spread, and retroperitoneal lymphadenopathy. Patients were allocated to the clinical FIGO (International Federation of Gynecology and Obstetrics) (2009) stages. Subsequently, patients were classified into low- and high-risk groups. The low-risk group was defined as endometrioid/mucinous carcinoma, clinically FIGO stage 1A or 1B, grade 1; and endometrioid/mucinous cancer clinically FIGO 1A, grade 2, all without clinical or imaging evidence of lymphadenopathy or distant metastases. When the low-risk criteria were not met, patients were considered high risk.

Surgical treatment

Hysterectomy with bilateral salpingo-oophorectomy as basic surgical treatment was performed with an abdominal or laparoscopic approach. In addition, high-risk patients underwent systematic para-aortic/pelvic lymphadenectomy (historically pelvic lymphadenectomy only)—at least five LNs from each hemipelvis and 10 from the para-aortic region were removed. Since 2019, systematic lymphadenectomy has been replaced by SNB in all EC patients regardless of their preoperative risk group. Currently, lymphadenectomy is limited to patients experiencing bulky LNs on preoperative imaging or perioperative finding.

Sentinel node ultrastaging

Regarding sentinel node methodology, we used intracervical indocyanine green injections and searched for the nodes with an endoscopic fluorescence imaging camera (Novadaq Pinpoint).

All sentinel LNs were fixed in 10% buffered formalin, sliced at 2-mm lamellas, embedded in paraffin, and further examined by ultrastaging protocol. This protocol consists of two consecutive 4-µm thick sections obtained in regular 200-µm intervals, which are cut from each paraffin block. The first section was stained with hematoxylin and eosin, and the second section was examined with cytokeratins (AE1/3). We classified micrometastasis (0.2–2 mm) together with macrometastases (>2 mm) as LN positive, whereas isolated tumor cells (≤0.2 mm or single cells/clusters of cells ≤200 cells in a single LN cross-section) were considered LN negative.

Immunohistochemical analysis

The experienced gynecological histopathologist (J. H.) examined all hematoxylin and eosin-stained slides to confirm preoperative histological subtype and grade. Immunohistochemical staining was effected on formalin-fixed and paraffin-embedded tissue sections. L1CAM positivity was defined as distinct membrane staining in ≥10% of tumor cells. ER and PR were considered positive when there were ≥10% of tumor cells with nuclear staining. p53 was classified into wild type or mutant (strong diffuse overexpression in more than 90% of tumor cells or completely negative) phenotypes.

Statistical analysis

Following the original ENDORISK model validation, we used preoperative tumor grade, at least three IHC markers (ER, PR, p53, or L1CAM) and at least one of the clinical preoperative markers (CA 125 serum level, LN status according to imaging method, platelet count, or pap smear result) as the minimal input data. A five-year follow-up (in the 5-year DSS group) and LN staging procedure (in the LNM group) were available in all included cases.

Probabilities of LNM and 5-year DSS were calculated for each patient and compared with observed reality. (i) Discrimination testing was assessed using a receiver operating characteristic (ROC) curve generated by plotting sensitivity against 1-specificity. Discriminating performance was quantified based on the AUC (area under the curve). (ii) The model's overall performance was quantified by the Brier score, which is the mean squared difference between each predicted probability and the observed outcome; a lower Brier score indicates better accuracy of probabilistic predictions. (iii) Calibration was visualized using a calibration plot, in which the predicted outcome was plotted against the observed outcome. To quantify model calibration, the predicted number of events (i.e., sum of each predicted probability) was compared with the observed number. (iv) Concordance between the ENDORISK model, our data, and recent DSS prediction was undertaken by using U.K. Uterine cancer survival data for different FIGO stages (10). Sensitivity analysis was accomplished by omitting patients with only SNB. Analyses were achieved in R (4.1.1) with the bnlearn (4.7), pROC (1.18.0), DescTools (0.99.44), and caret (6.0–90) packages.

Ethics approval

Our study was approved by the University Hospital Brno Ethics Committee, Approval Number 06-151221/EK. All

patients signed informed consent for histology sample storage, scientific use, and publication purposes.

Results

Among the 835 patients in our EC database treated between January 2006 and May 2021, 299 patients were involved in our DSS analysis; 226 cases were available for LNM analysis (Figure 1). Table 1 summarizes clinical data, histological characteristics, and adjuvant treatment.

LNM analysis

A total of 226 patients were included in our LNM analysis: 84 (37%) PLN, 72 (32%) PLN+PALN, and 70 (31%) SNB. Forty-one patients had at least one LNM (18%): 24 (59%) in pelvic, three (7%) in para-aortic, and 14 (34%) in both localizations. A median of 27 and 22 LNs were removed during PLN and PALN, respectively.

The AUC (0.84) and Brier score (0.11) indicated good concordance between prediction and reality (Table 2, Figure 2). Predicted/observed ratio displayed non-significant underestimation (0.76; 95% CI 0.49–1.03). Results from sensitivity analysis, where cases with SNB were excluded, were comparable (Supplementary Material: Table 1, **Figure 1**), indicating that involvement in the main analysis did not alter the accuracy of ENDORISK.

Figure 3 shows LNM prediction and reality for the different clinical FIGO stages (Supplementary Figure 2 complements surgical stages).

DSS analysis

Only patients with at least 5 years of follow-up or who died from EC were included (N = 299). The AUC was 0.86 (95% CI,

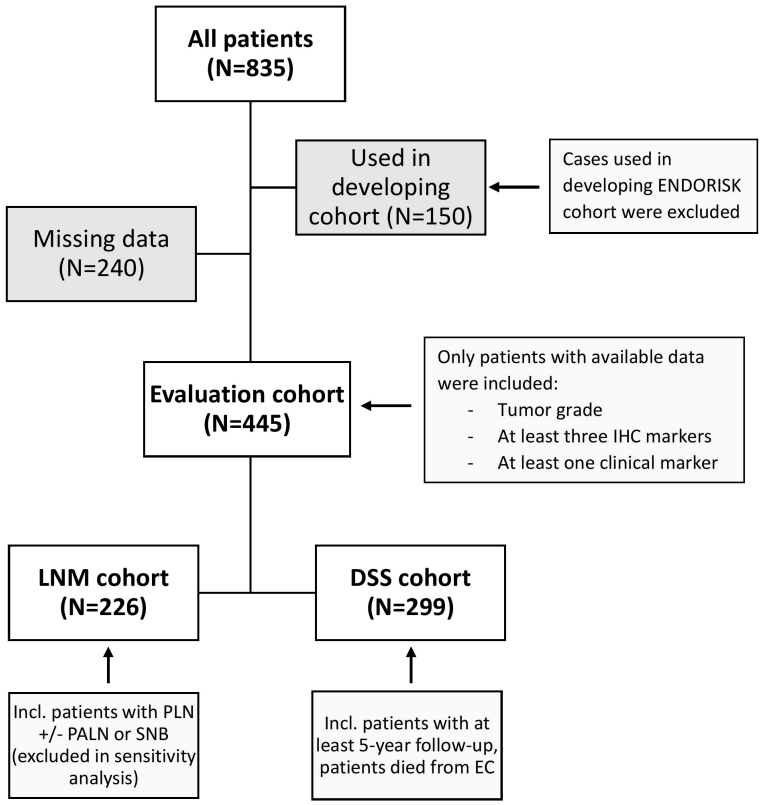

FIGURE 1
Cohort development. The evaluation cohort was developed using all patients from our clinical database treated between January 2006 and May 2021 with available data and lymph node staging (LNM cohort) and/or 5-year follow-up (DSS cohort). IHC, immunohistochemical; LNM, lymph node metastasis; DSS, disease specific survival; PLN, pelvic lymphadenectomy; PALN, para-aortal lymphadenectomy; SNB, sentinel node biopsy; EC, endometrial cancer.

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TABLE 1 Clinical and histological characteristics.

Variable		LNM cohort	5- year DSS cohort
Total N		N = 226*	N = 299*
Age (years)		64.5 (59.0 to 68.8)	65.0 (59.0 to 72.0)
BMI (kg/m^2)		30.0 (26.0 to 34.0)	32.0 (27.0 to 36.0)
Follow up length (month)		35.2 (13.0 to 90.6)	91.8 (64.5 to 122.2)
Preoperative tumor grade	1	38 (16.8)	62 (20.7)
	2	103 (45.6)	171 (57.2)
	3	85 (37.6)	66 (22.1)
ER expression	Negative	28 (12.4)	23 (7.7)
	Positive	198 (87.6)	276 (92.3)
PR expression	Negative	42 (18.6)	38 (12.7)
	Positive	184 (81.4)	261 (87.3)
L1CAM expression	Negative	175 (77.4)	258 (86.3)
	Positive	48 (21.2)	40 (13.4)
	Unknown	3 (1.3)	1 (0.3)
p53 expression	Wild type	185 (81.9)	244 (81.6)
	Muttated	37 (16.4)	37 (12.4)
	Missing	4 (1.8)	18 (6.0)
Ca-125	Negative (<35)	167 (73.9)	195 (65.2)
	Positive (35+)	47 (20.8)	63 (21.1)
	Unknown	12 (5.3)	41 (13.7)
Trombocytosis	No	215 (95.1)	279 (93.3)
	Yes	7 (3.1)	11 (3.7)
	Unknown	4 (1.8)	9 (3.0)
Imaging results	No lymphadenopathy	210 (92.9)	271 (90.6)
	Lymphadenopathy	11 (4.9)	10 (3.3)
	Unknown	5 (2.2)	18 (6.0)
Cervical cytology	Normal	143 (63.3)	200 (66.9)
	Abnormal	7 (3.1)	4 (1.3)
	Unknown	76 (33.6)	95 (31.8)
Histological subtype	Endometrioid	186 (82.3)	275 (92.0)
	Non-endometrioid	40 (17.7)	24 (8.0)
Myometrial invasion	less then 50%	129 (57.1)	200 (66.9)
	more then 50%	97 (42.9)	99 (33.1)
Cervical invasion	No	193 (85.4)	266 (89.0)
	Yes	33 (14.6)	33 (11.0)
FIGO stage (surgical)	IA	108 (47.8)	180 (60.2)
	IB	41 (18.1)	58 (19.4)
	II	29 (12.8)	30 (10.0)
	IIIA	5 (2.2)	9 (3.0)
	IIIB	1 (0.4)	1 (0.3)
	IIIC	38 (16.8)	16 (5.4)
	IV	4 (1.8)	5 (1.7)
LVSI	No	170 (75.2)	271 (90.6)
	Yes	53 (23.5)	24 (8.0)
	Unknown	3 (1.3)	4 (1.3)
Type of lymphadenectomy	PLN	84 (37.2)	68 (22.7)
	PLN+PALN	72 (31.9)	31 (10.4)
	SNB	70 (31.0)	1 (0.3)
	Unknown	0 (0.0)	199 (66.6)

(Continued)

TABLE 1 Continued

Variable		LNM cohort	5- year DSS cohort
Lymph nodes	Negative	185 (81.9)	87 (29.1)
	Positive	41 (18.1)	17 (5.7)
	Unknown	0 (0.0)	195 (65.2)
SNB	Negative	65 (28.8)	1 (0.3)
	Positive	5 (2.2)	0 (0.0)
	Unknown	156 (69.0)	298 (99.7)
Adjuvant treatment	None	84 (37.2)	163 (54.5)
	RT	94 (41.6)	106 (35.5)
	CHT	17 (7.5)	14 (4.7)
	CHRT	27 (11.9)	9 (3.0)
	Unknown	4 (1.8)	7 (2.3)

*n (%); Median (IQR).
DSS, disease-specific survival; LNM, lymph node metastasis; BMI, Body Mass Index; ER, Estrogen receptor; PR, Progesterone receptor; L1CAM, L1 cell adhesion molecule; LVSI, lymphovascular space invasion; PLN, pelvic lymphadenectomy; PALN, para-aortic lymphadenectomy; SNB, sentinel node biopsy; RT, radiotherapy; CHT, chemotherapy; CHRT, chemoradiotherapy.

0.79–0.93), Brier score 0.09. Five-year DSS prediction was well calibrated with a trend toward overestimating survival among the lower predicted survival rates (Figure 4 and Table 2).

Figure 5 displays the 5-year DSS prediction compared with reality and expected survival according to previously published probability (10) in different clinical FIGO stages (Supplementary Figure 3 expands on surgical stages).

Discussion

In the era of personalized medicine, we aim to have optimal information concerning a prognosis to facilitate adequate shared decision-making with the patient and define the most appropriate treatment decision. The ENDORISK model definitively contributes to the preoperative knowledge on risk of LNM and DSS.

Several EC predictive models have been published and focus on discriminating patients pre- and postoperatively into risk groups with predicting LNM or outcome. Previous models used traditional clinicopathological characteristics including LVSI, myometrial invasion, histotype, grade, age, and/or BMI (11, 12). So far, results were only moderate and, currently, additional immunohistochemical markers are already frequently used in the clinic: ER, PR, L1CAM, p53, Ki67 (13, 14). Some authors also included imaging information such as tumor diameter, myometrial/cervical invasion, or lymphadenopathy (15, 16).

The original multivariate analysis is based on a simple graphic calculating tool called nomogram. Jiang et al. published an LNM prediction model based on histological and IHC markers with a sensitivity of 82.8% and specificity of 82.7% (AUC 0.9) (14). However, this model cannot be applied when certain data are missing. Moreover, information on LVSI is required, which limits the use of the model in a preoperative setting. Similar results were presented with an effort to predict 3-year recurrence-free survival (sensitivity 76.5%, specificity 86.7%, AUC 0.82) with comparable limitations (including LVSI, all data required) (13).

With the development of computer technology, a Bayesian network has become more accessible, used for determining

TABLE 2 Model concordance statistics.

	LNM	5-year DSS
AUC (95% CI)	0.84 (0.77-0.9)	0.86 (0.79-0.93)
Brier score	0.11	0.09
Predicted no. of events	31.2	271.7
Observed no. of events	41	262
Predicted/observed ratio (95% CI)	0.76 (0.49-1.03)	1.04 (0.91-1.16)

Both AUC and Brier score substantiate very good concordance between prediction and reality in general across the dataset. Discriminative performance was quantified based on AUC (a higher AUC indicates better performance). Overall model performance was quantified by the Brier score (a lower Brier score characterizes better accuracy of the probabilistic predictions). The predicted/observed ratio <1 denotes a lower prediction than reality, whereas a ratio >1 signals overestimation compared with reality. If 95% CI includes value 1, the difference is non-significant.
AUC, area under the curve; CI, confidence interval; DSS, disease specific survival; LNM, lymph node metastasis.

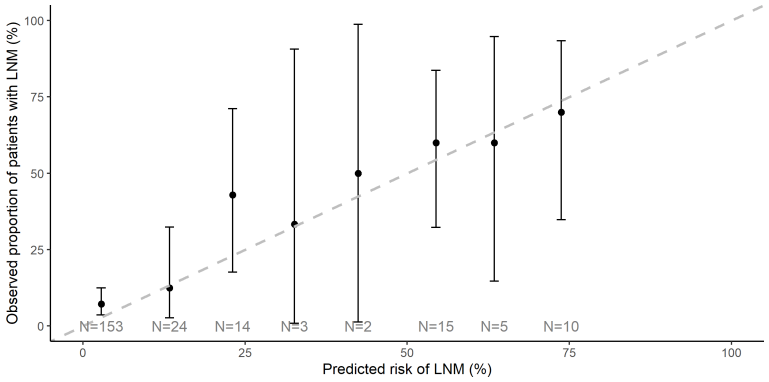

FIGURE 2
Lymph node metastasis calibration plot of observed versus predicted events. A dashed line displays the predicted value, and black dots represent the observed LNM. Ideally, all black marks are lying on the dashed line. LNM, lymph node metastasis.

probable relationships and causalities based on expert knowledge with machine learning. An enormous advantage is that it can be applied, even when some patient characteristics are absent, which often occurs in clinical practice. The ENDORISK model was established with a variety of pre- and postoperative information, yet it could be applied exclusively with preoperative data. Minimally required data to work properly include (1) preoperative tumor grade, (2) minimally three of four IHC markers (ER, PR, p53, or L1CAM), and (3) at least one clinical biomarker (CA 125 serum level, LN status according to imaging method, platelet count, or pap smear result) (7).

The original model was created cognizant of histologic results from pelvic and para-aortic LN staging. Nowadays, complete lymphadenectomy is not the standard practice with all patients, and less invasive SNB is recommended with a low/intermediate-risk disease (3). Certain authors prefer this method even in high-risk cases (17). Isolated para-aortic nodal metastasis (notwithstanding negative pelvic nodes) occurs in approximately 1% of surgically staged cases (18). Consequently, we decided to also include patients with only pelvic dissection or SNB, reflecting current diagnostic practice. Sensitivity analysis, excluding SNB cases, presented comparable results, supporting the results of the complete study cohort (Supplementary Figure 1).

Historically, knowing the potential preoperative risk of LNM guided whether or not para-aortic-pelvic lymphadenectomy was indicated. Currently, SNB is preferred not only in low- but also in high-risk EC and might reduce the benefit of preoperative risk stratification. Yet, based on the very low risk in EC patients without myometrial invasion, LN staging could be omitted in these cases (3). If patients with truly low risk of LNM (<5%)

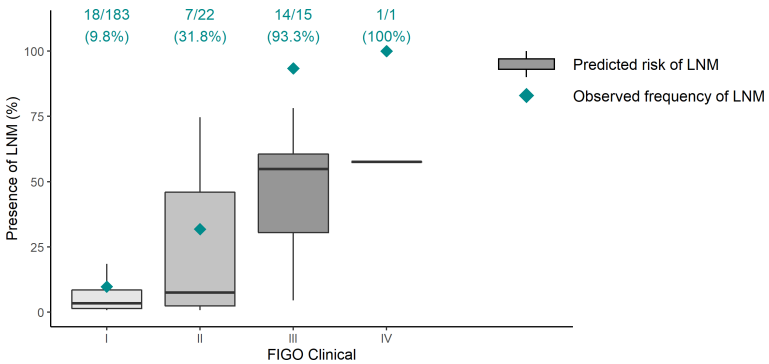

FIGURE 3
Lymph node metastasis prediction versus reality in different clinical FIGO stages. Gray boxes represent the model's prediction; green rhombuses indicate the real LNM frequency. Ideally, all green rhombuses lie in gray boxes. In clinical FIGO III–IV stages, ENDORISK predicts fewer cases of LNM than reality. LNM, lymph node metastasis; FIGO, International Federation of Gynecology and Obstetrics.

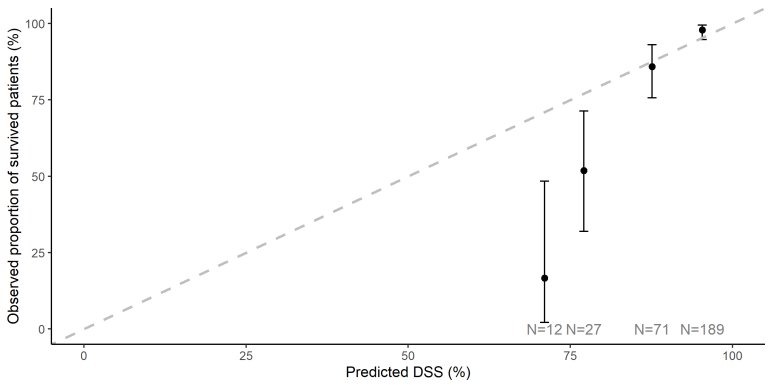

FIGURE 4
Five-year disease-specific survival calibration plot of observed versus predicted events. A dashed line displays the predicted value; black dots represent observed DSS. Ideally, all black marks are lying on the dashed line. There is a trend toward overestimating survival in the lower predicted survival rates. DSS, disease-specific survival.

could be properly identified preoperatively by using the ENDORISK model, SNB could be safely omitted in those hospitals where this technique is not available. An interesting question is whether it is necessary to provide LN staging in all EC types or if, according to other preoperative markers, we could abandon it. In an era of SNB staging practice, the ENDORISK model for LNM prediction could be used in hospitals, where this method is not available. Additionally, it could be supportive if SNB fails and side-specific lymphadenectomy is considered, especially in obese and fragile patients.

Our LNM prediction results were comparable with validation on MoMaTEC cohort: AUC 0.84 versus 0.82, Brier score 0.11 versus 0.09. The model very precisely predicts LNM in early stages, albeit underestimates clinically advanced carcinomas (Figure 3). For example, in patients with preoperative suspicion of LNM according to imaging methods, the ENDORISK model estimated an average probability of only 51% (25–78%). In fact, all were finally LNM positive. This might be explained by the low number of advanced cases; however, the model should be able to predict even worse stages.

ENDORISK model validation for 5-year DSS with our cohort displayed very similar results with previous cohorts MoMaTEC and PIPENDO, evaluated as well adjusted according to AUC (0.82, 0.84) and Brier score (0.12, 0.10) (7). Nevertheless, when using

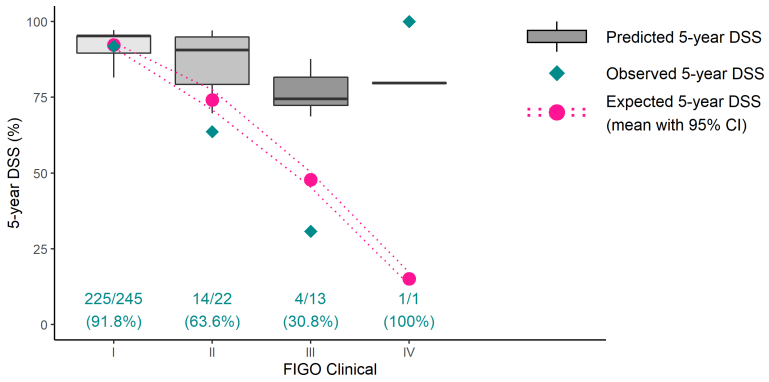

FIGURE 5
Five-year disease-specific survival prediction versus reality versus expectation in different clinical FIGO stages. Gray boxes represent the model's prediction, green rhombuses indicate the real 5-year DSS, and pink dots denote expected 5-year survival according to surgical FIGO stages (10). Ideally, all green rhombuses lie in gray boxes. In clinical FIGO II–III stages, ENDORISK predicts much better survival than reality. Only one patient was preoperatively categorized into FIGO IV stadium—the survival result implies her misclassification. DSS, disease-specific survival; FIGO, International Federation of Gynecology and Obstetrics.

calibration plots to visualize the results, predictive value was obviously outstanding only for low-risk patients and significantly overrated for high-risk patients. This phenomenon was even more evident when patients were classified according to clinical FIGO stages (Figure 5). Definitely, the most accurate results were achieved, when the final surgical stage was applied (Supplementary Figure 3); nevertheless, this information is unknown preoperatively.

The FIGO stage is an important independent factor affecting survival, even during molecular classification times. The average 5-year survival is declining from 92% in stage I, 74% within stage II, and 48% in stage III to only 15% in stage IV (10). The ENDORISK model, currently, does not include information about the clinical stage disease (except for "enlarged lymph nodes on imaging"), even though, there are other possibilities for attaining these data. An expert oncogynecologic ultrasound or magnetic resonance imaging (MRI) is suitable for myometrial and cervical invasion detection; a CT scan can identify distant metastasis (19). Although myometrial invasion <50% of >50% is incorporated in the ENDORISK network, it is currently based on final histology, yet might be a very valuable addition to the model when determined preoperatively by either ultrasound or MRI. In addition, ultrasound-measured tumor-free distance from the tumor to the uterine serosa is another promising marker for predicting deep myometrial invasion and poor prognosis (20), which might be incorporated in an updated version of the network.

Even when we situate the worst clinical and histological characteristics into the model, the lowest survival prediction was 66%. This seems not in line with the published survival data of only 48%/15% in stage III/IV (10). Nevertheless, the number of cases with advanced stage in our cohort was limited and, hence, validation in larger cohorts is needed.

ENDORISK is one of the most complex risk stratification models so far. The authors imperiously searched the literature for potential relevant risk factors and assigned them statistically significant prognostic values. Unlike other models, ENDORISK could be applied even with strictly preoperative and incomplete information. However, as we ascertained, there is a need for further improvement before introduction into clinical practice. Clinical FIGO stage extension would definitively increase the model's accuracy. Additionally, the incorporation of molecular classification would be highly relevant and is currently prepared in the ENDORISK 2.0.

Forthwith, we present the first unicentric ENDORISK model validation study, indicating a capacity for consistent treatment decisions and high-quality follow-up data. Innovatively, we have confirmed its application with SNB cases. Furthermore, we have suggested certain ancillary improvements to achieve better results among advanced cases that need to be considered when updating the ENDORISK network.

Conclusions

ENDORISK is one of the best and most complex preoperative risk stratification models promulgated at this point in time. Nevertheless, there is still a place for improvement, particularly with survival prediction. Including clinical FIGO staging would increase model accuracy in advanced disease cases. In this SNB era, preoperative LNM predictive importance is waning; however, since SNB is not yet standard procedure in all countries, ENDORISK could be a helpful factor in decision-making regarding lymphadenectomy. With molecular classification's inclusion into clinical practice, the ENDORISK model's authors should consider its incorporation as well.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Committee, University Hospital Brno, Brno, Czech Republic. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization, PV, PO, and VW; study design, PV, PO, VW, and JP; statistical analysis, PO and PL; pathological examination, JH; writing – original draft preparation, PV, VW, and PO; writing – review and editing, JP, JH, PL, SV, and CR; supervision, VW and JP. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.939226/full#supplementary-material

References

1. Sant M, Lopez MDC, Agresti R, Sánchez Pérez MJ, Holleczek B, Bielska-Lasota M, et al. Survival of women with cancers of breast and genital organs in Europe 1999–2007: Results of the EUROCARE-5 study. *Eur J Cancer*. (2015) 51 (15):2191–205. doi: 10.1016/j.ejca.2015.07.022

2. Salani R, Khanna N, Frimer M, Bristow RE, Chen LM. An update on post-treatment surveillance and diagnosis of recurrence in women with gynecologic malignancies: Society of gynecologic oncology (SGO) recommendations. *Gynecol Oncol* (2017) 146(1):3–10. doi: 10.1016/j.ygyno.2017.03.022

3. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer Off J Int Gynecol Cancer Soc* (2021) 31(1):12–39. doi: 10.1136/ijgc-2020-002230

4. ASTEC study group, Kitchener H, Swart AMC, Qian Q, Amos C, Parmar MKB. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. *Lancet Lond Engl* (2009) 373(9658):125–36. doi: 10.1016/S0140-6736(08)61766-3

5. Benedetti Panici P, Basile S, Maneschi F, Lissoni AA, Signorelli M, Scambia G, et al. Systematic pelvic lymphadenectomy vs. no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial. *J Natl Cancer Inst* (2008) 100(23):1707–16. doi: 10.1093/jnci/djn397

6. Frost JA, Webster KE, Bryant A, Morrison J. Lymphadenectomy for the management of endometrial cancer. *Cochrane Database Syst Rev* (2017) 10(10): CD007585. doi: 10.1002/14651858.CD007585.pub4

7. Reijnen C, Gogou E, Visser NCM, Engerud H, Ramijith J, van der Putten LJM, et al. Preoperative risk stratification in endometrial cancer (ENDORISK) by a Bayesian network model: A development and validation study. *PloS Med* (2020) 17 (5):e1003111. doi: 10.1371/journal.pmed.1003111

8. Trovik J, Wik E, Werner HMJ, Krakstad C, Helland H, Vandenput I, et al. Hormone receptor loss in endometrial carcinoma curettage predicts lymph node metastasis and poor outcome in prospective multicentre trial. *Eur J Cancer* (2013) 49(16):3431–41. doi: 10.1016/j.ejca.2013.06.016

9. Visser NCM, Bulten J, van der Wurff AAM, Boss EA, Bronkhorst CM, Feijen HWH, et al. PIpelle prospective ENDOmetrial carcinoma (PIPENDO) study, pre-operative recognition of high risk endometrial carcinoma: a multicentre prospective cohort study. *BMC Cancer* (2015) 15:487. doi: 10.1186/s12885-015-1487-3

10. Cancer survival in England - adults diagnosed - office for national statistics. Available at: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/datasets/cancersurvivalratescancersurvivalinenglandadultsdiagnosed (Accessed February 28, 2022).

11. Cong R, Kong F, Ma J, Li Q, Wu Q, Ma X. Combination of preoperative neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and monocyte-lymphocyte ratio: a superior prognostic factor of endometrial cancer. *BMC Cancer* (2020) 20 (1):464. doi: 10.1186/s12885-020-06953-8

12. Dong Y, Cheng Y, Tian W, Zhang H, Wang Z, Li X, et al. An externally validated nomogram for predicting lymph node metastasis of presumed stage I and II endometrial cancer. *Front Oncol* (2019) 9:1218. doi: 10.3389/fonc.2019.01218.

13. Jiang P, Jia M, Hu J, Huang Z, Deng Y, Hu Z. A nomogram model involving immunohistochemical markers for predicting the recurrence of stage i-ii endometrial cancer (2021) (Accessed April 9, 2022).

14. Jiang P, Huang Y, Tu Y, Li N, Kong W, Di F, et al. Combining clinicopathological parameters and molecular indicators to predict lymph node metastasis in endometrioid type endometrial adenocarcinoma. *Front Oncol* (2021) 11:682925. doi: 10.3389/fonc.2021.682925.

15. Zheng T, Yang L, Du J, Dong Y, Wu S, Shi Q, et al. Combination analysis of a radiomics-based predictive model with clinical indicators for the preoperative assessment of histological grade in endometrial carcinoma. *Front Oncol* (2021) 11:582495. doi: 10.3389/fonc.2021.582495.

16. Weinberger V, Bednarikova M, Hausnerova J, Ovesna P, Vinklerova P, Minar L, et al. A novel approach to preoperative risk stratification in endometrial cancer: the added value of immunohistochemical markers. *Front Oncol* (2019) 9:265. doi: 10.3389/fonc.2019.00265

17. Pineda VG, Zapardiel I, Gracia M, Siegrist J, Diestro MD, Alonso M, et al. Avoiding full lymphadenectomies in intermediate- and high-risk endometrial cancer by sentinel lymph node biopsy implementation. *Front Oncol* (2021) 11:654285. doi: 10.3389/fonc.2021.654285

18. Abu-Rustum NR, Gomez JD, Alektiar KM, Soslow RA, Hensley ML, Leitao MM Jr., et al. The incidence of isolated paraaortic nodal metastasis in surgically staged endometrial cancer patients with negative pelvic lymph nodes. *Gynecol Oncol* (2009) 115(2):236–8. doi: 10.1016/j.ygyno.2009.07.016

19. Faria SC, Devine CE, Rao B, Sagebiel T, Bhosale P. Imaging and staging of endometrial cancer. *Semin Ultrasound CT MR*. (2019) 40(4):287–94. doi: 10.1053/j.sult.2019.04.001

20. Liro M, Śniadecki M, Wycinka E, Wojtylak S, Brzeziński M, Stańczak A, et al. Ultrasound measurement of tumor-free distance from the serosal surface as the alternative to measuring the depth of myometrial invasion in predicting lymph node metastases in endometrial cancer. *Diagn Basel Switz* (2021) 11(8):1472. doi: 10.3390/diagnostics11081472

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EDITED BY
Andrzej Semczuk,
Medical University of Lublin, Poland

REVIEWED BY
Keith R. Laderoute,
Consultant, Redwood City, CA, United States
Pengming Sun,
Fujian Medical University, China

*CORRESPONDENCE
Feng Xie
fengxie10@fudan.edu.cn
Ming-Qing Li
mqli@fudan.edu.cn

†These authors share first authorship

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Glucose transporters: Important regulators of endometrial cancer therapy sensitivity

Xing Zhang[1,2†], Jia-Jing Lu[3†], Ayitila Abudukeyoumu[3], Ding-Yu Hou[2], Jing Dong[1], Jiang-Nan Wu[4], Li-Bing Liu[5], Ming-Qing Li[2,6,7*] and Feng Xie[1*]

[1]Medical Center of Diagnosis and Treatment for Cervical and Intrauterine Diseases, Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China, [2]Laboratory for Reproductive Immunology, Hospital of Obstetrics and Gynecology, Shanghai Medical School, Fudan University, Shanghai, China, [3]Department of Gynecology, Hospital of Obstetrics and Gynecology, Shanghai Medical School, Fudan University, Shanghai, China, [4]Clinical Epidemiology, Clinical Research Center, Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China, [5]Department of Gynecology, Changzhou No. 2 People's Hospital, affiliated with Nanjing Medical University, Changzhou, China, [6]National Health Commission (NHC) Key Lab of Reproduction Regulation, Shanghai Institute for Biomedical and Pharmaceutical Technologies, Fudan University, Shanghai, China, [7]Shanghai Key Laboratory of Female Reproductive Endocrine Related Diseases, Hospital of Obstetrics and Gynecology, Fudan University, Shanghai, China

Glucose is of great importance in cancer cellular metabolism. Working together with several glucose transporters (GLUTs), it provides enough energy for biological growth. The main glucose transporters in endometrial cancer (EC) are Class 1 (GLUTs 1–4) and Class 3 (GLUTs 6 and 8), and the overexpression of these GLUTs has been observed. Apart from providing abundant glucose uptake, these highly expressed GLUTs also participate in the activation of many crucial signaling pathways concerning the proliferation, angiogenesis, and metastasis of EC. In addition, overexpressed GLUTs may also cause endometrial cancer cells (ECCs) to be insensitive to hormone therapy or even resistant to radiotherapy and chemoradiotherapy. Therefore, GLUT inhibitors may hopefully become a sensitizer for EC precision-targeted therapies. This review aims to summarize the expression regulation, function, and therapy sensitivity of GLUTs in ECCs, aiming to provide a new clue for better diagnosis and treatment of EC.

KEYWORDS

glucose transporter, endometrial cancer, proliferation, angiogenesis, apoptosis, therapy sensitivity

Introduction

Endometrial cancer (EC) ranks as the sixth most common malignancy diagnosed among women. Most cases are diagnosed after menopause (1). However, morbidity has been increasing over the past years. Many risk factors are considered to be closely related to EC, such as obesity, estrogen exposure, insulin resistance, and age (2–4). Generally speaking, EC can be divided into two types. Type 1 refers to endometrioid carcinoma and accounts for 75%, which is believed to be closely associated with long-term estrogen stimulation, while type 2 tends to be high grade and with poor prognosis (5, 6). Moreover, approximately 5%–10% of endometrial carcinomas have a hereditary basis from hereditary non-polyposis colorectal cancer (7).

Glucose plays a deterministic role in cellular metabolism. Working together with several glucose transporters (GLUTs), it provides enough energy for biological growth through diffusion or secondary active transport. The major facilitator superfamily (MFS) of membrane transporters is encoded by *SLC2* genes, controlling the transmembrane movement of various substrates. As vital facilitative sugar transporters, these GLUTs can be categorized into three classes in the light of respective sequence similarity and substrate specificity: Class 1 (GLUTs 1–4 and 14), Class 2 (GLUTs 5, 7, 9, and 11), and Class 3 (GLUTs 6, 8, 10, 12, and 13/HMIT). Their physiological substrate is generally a hexose, but their substrates can be urate (8), dehydro-ascorbate (9), polyols (10), myo-inositol (11), and trehalose (12). Among this SLC2 family of 14 members, GLUT2/4/12 mainly functions as insulin-dependent transporters, GLUT5/7/11 chiefly refers to fructose transport (13–17), and GLUT6 is a lysosomal transporter (18). All in all, GLUTs 1–4 play a predominant role in maintaining cellular glucose uptake and functional metabolic homeostasis.

Various physiological functions of the proteins mainly depend on factors including the difference of principal substrates and the cell type distribution, and the relevance between the proteins and subcellular compartments. Some GLUT proteins can translocate between subcellular compartments, and this effectively promotes their control of long- and short-time scales in regulating the supply of glucose to tissues (19). The occurrence of their dysfunction means a number of pathological disorders, such as GLUT1 deficiency syndrome and the Fanconi–Bickel syndrome, type 2 diabetes mellitus, and cancers.

It is widely acknowledged that various malignant tumors have an activated glucose metabolic rate under some adverse circumstances, such as hypoxia, inflammation, and malnutrition (20, 21). However, a special pattern called the Warburg effect shows that even under an oxygen-rich environment, the glucose metabolism of tumor cells still remains quite active (22). Generally, a high expression of GLUTs accelerates glucose metabolism, which is indispensable for endometrial proliferation and differentiation (23). This review aims to summarize the expression regulation, function, and therapy sensitivity of GLUTs in EC.

Glucose transporters in endometrial cancer

It has been reported that the main GLUTs in EC are mainly Class 1 (GLUTs 1–4) and Class 3 (GLUTs 6 and 8), and their basic functions have been mentioned above. Next, we will try to elaborate on the expression of these GLUTs in endometrial cancer (Table 1).

Class 1 (GLUTs 1–4): GLUT1 protein mainly localizes in the luminal epithelium, glandular epithelium, endometrium stroma, and endothelial cells (24–29). Of note, either in healthy endometrium or EC tissues, the relative expression of GLUT1 is greater than that of any other GLUTs, suggesting that it is the most important transporter in endometrial tissues. In addition, studies have confirmed that the expression of GLUT1 is related to tumor differentiation. Compared with well-differentiated tumors, the expression of GLUT1 is significantly elevated in poorly differentiated tumors, which may be of great significance for predicting prognosis and survival estimates of EC (30). Previous reports have indicated that GLUT2 has a low expression in EC and is not controlled by a hormone, but it is

TABLE 1 Expression of glucose transporters in endometrial cancer.

Subtypes	Highly expressed localization			The relationship with grade/prognosis	
	Tissues	Stromal cells	Epithelial cell	Grade	Prognosis
GLUT1	+	+	+	+	Not mentioned
GLUT2	+	+	+	Not mentioned	Not mentioned
GLUT3	+	+	+	+	–
GLUT4	+	+	+	+	Not mentioned
GLUT6	+	+	+	+	+
GLUT8	+	+	+	+	+

+, expression or positive relationship; –, no relationship.

not observed in normal endometrium (31, 32). The mRNA level of GLUT3 seems to be much lower than that of GLUT1 in EC. Compared with the expression of estrogen/progesterone receptor (ER/PR)-negative EC, that of GLUT3 in ER/PR-positive EC is much higher (23). However, the relationship between the expression of GLUT3 and prognosis in EC has not been clarified. A report has shown that GLUT4 is barely present in healthy endometrium; nevertheless, it is upregulated in EC and might have a similar level of expression to GLUT3 (24, 33).

Class 3 (GLUTs 6 and 8): Moststudies show that the level of GLUT6 is quite low in normal endometrial epithelial and stromal cells, while it is upregulated in early-stage EC cells. Furthermore, the mutations and amplifications of GLUT6 are observed more frequently in EC than in any other malignancies. However, a study carried out by Byrne et al. showed that GLUT6 (instead of GLUT1) is the most significantly elevated GLUT in the malignant endometrium and is especially highly expressed in cancerous glandular epithelial cells, which are closest to blood vessels in the surrounding stroma. This finding indicates that GLUT6 is pivotal for the occurrence of EC and that it may have some other unknown functions that remain to be discovered (34). GLUT8 is predominantly localized in the endoplasmic reticulum and has a moderate expression level, which can translocate to the cell surface under insulin stimulation, assisting in the indispensable glucose consumption for glycosylation of protein. GLUT8 is highly expressed in EC. Similar to GLUT1, the expression of GLUT8 is also related to tumor differentiation, and a higher level is observed in poorly differentiated tumors. Of note, its expression reached a peak in endometrial serous carcinoma (35, 36). Interestingly, whether in mammary epithelial cells or 3T3-L1 adipocyte cells, the expression of GLUT8 seems to be reduced by hypoxia but is not affected by the small interfering RNA (siRNA) knockdown of hypoxia-inducible factor-1α (HIF-1α), indicating that hypoxia may not play a predominant role in regulating GLUT8 expression, which totally differs from other hypoxia-dependent increased GLUTs (GLUT1/3) (24, 37). However, whether its expression level in hypoxia-related endometrial cancer cells (ECCs) is similar to the level of these cells is unclear until now.

Upstream regulators of glucose transporters

Estrogen or progesterone

Most studies support that estrogen can significantly increase the expression level of GLUT1, far more than the effect on other GLUTs (Table 2) (32). Differing from traditional two nuclear ERs that function as ligand-activated transcription factors, G-protein-coupled ER 1 (GPER), formerly known as GPR30, has an increased expression in the intracellular location of various cancer cells (e.g., breast, ovaries, and ECCs) and becomes involved in transcriptional activities, such as the production of cyclic adenosine monophosphate (cAMP), phosphatidylinositol 3-kinase (PI3K)/serine-threonine kinase (AKT), and AMP-activated protein kinase (MAPK) pathways, which indirectly strengthen the combination between ERs and other transcriptional factors (38). High levels of GPER expression are in consistence with poor survival (39). Here these estrogen-induced GLUTs may be achieved by activating independent transcription GPER or its downstream factor 6-phosphofructo-

TABLE 2 Upstream regulators of GLUTs in endometrial cancer.

Upstream regulators		GLUT1	GLUT3	GLUT4	GLUT6	Regulatory mechanisms
Hormones	Estrogen	+		+		ER
	Progesterone	+				PGRMC1, IR
High insulin		+		+		IRAP, IGF1R, PI3K/AKT pathway
High glucose		+	+	+		ER, AMPK/mTOR/S6 pathway
Hypoxia		+	+			HIF-1α, ATP, HtrA3
Cytokines	IL-3/IL-7	+				PI3K/AKT/mTOR pathway
	TNF-α				+	NF-κB, RELA
	VEGF	+				/
Enzymes	ABHD5	+				AKT pathway
	ALDH	+				/
Natural compounds	Flavonoids	–				/
	Vitamin C	–				HIF-1α

+, positive regulation; –, negative regulation.
HIF-1α, hypoxia-inducible factor-1α; VEGF, vascular endothelial growth factor; IL-3/IL-7, interleukin 3/7; ABHD5, abhydrolase domain containing 5; ALDH, high-level aldehyde dehydrogenase; Vitamin C, ascorbic acid; ER, estrogen receptor; PGRMC1, the progesterone receptor membrane component 1; IR, insulin receptor; IRAP, insulin-regulated aminopeptidase; IGF1R, insulin-like growth factor 1 receptor; PI3K, phosphatidylinositol 3-kinase; AKT, the serine-threonine kinase; AMPK, adenosine 5′-monophosphate (AMP)-activated protein kinase; mTOR, mammalian target of rapamycin; ATP, adenosine triphosphate; HtrA3, high-temperature requirement A3; NF-κB, nuclear factor kappa-B.

2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) levels in human healthy endothelial cells (40, 41). Progesterone also has a role in promoting the expression of GLUT1, while this effect is not as strong as that of estrogen. However, the combination treatment of estrogen and progesterone eliminates the two effects; namely, the combination therapy of the two hormones reduces the expression of GLUTs. Upregulated GLUT4 is predominantly associated with estrogen combined with its receptor, which poses an important role in the epithelial–mesenchymal transition (EMT) process of EC by stimulating vascular endothelial growth factor (VEGF) secretion (42). The expression of GLUT4 can also be elevated *via* estrogen-induced ESR1 regulation, which may be through SRC (proto-oncogene tyrosine-protein kinases)-mediated phosphorylation of ESR1 in normal muscle and adipose cells (43). In type 2 diabetes, this may induce GLUT4 expression and plasma membrane GLUT4 translocation in adipocytes (44). Another study shows that estrogen indirectly activates primary gene transcription-specificity protein 1, which directly functions as the GLUT4 gene promoter, increasing the expression of GLUT4 in type 2 diabetes (45). However, recent studies show that both estrogen and progesterone have little influence on the activity of the low-affinity transporter GLUT2/3/6.

As is commonly known, long-time estrogen exposure is closely associated with hyperplastic proliferation of the endometrial glands; however, the effect of progesterone is quite the opposite. There is multiple evidence elucidating that progesterone plays an antagonistic role in inhibiting cell growth, invasiveness, and differentiation in type 1 EC (46). The progesterone receptor membrane component 1 (PGRMC1) is the first identified progesterone-binding membrane protein and has a high expression in various cancer cells. PGRMC1 can stimulate the expression of insulin receptors (IRs) in the plasma membrane and increase the level of GLUT1 and GLUT4 in the plasma membrane (47). However, whether the upregulation of GLUT1/4 is induced by IR remains uncertain.

High insulin and high glucose

Insulin can increase the expression, transport, and oxidation of GLUT1. In pancreatic cancer cells, insulin can stimulate DNA synthesis by activating PI3K and phosphatidylinositol kinase (PIPK) in a concentration- and time-dependent manner (48). Previous studies have suggested that insulin can activate the PI3K/AKT pathway in muscle and adipose cells, which shows a beneficial effect on GLUT4 vesicle trafficking to the cell membrane (49). Furthermore, placental leucine aminopeptidase (P-LAP) is a cell surface aminopeptidase and a synonym for oxytocinase, referred to as insulin-regulated membrane aminopeptidase (IRAP). Hyperglycemia or hyperinsulinemia can be a signal to facilitate GLUT4 expression and PI3K/AKT pathway, which can be mediated by P-LAP/IRAP pathway (50, 51). A study has shown that insulin-like growth factor 1 receptor (IGF-1R) can inhibit the translocation of GLUT8 to the cytoplasm, achieving redistribution of cell survival in the murine blastocyst. Nevertheless, how insulin regulates GLUT8 expression in EC has not been reported so far (52).

High glucose directly activates the expression of GLUT1 and GLUT3 *via* the modulation of AMPK/mammalian target of rapamycin (mTOR)/S6 signaling in ECCs (53). Another study found that high glucose can upregulate the level of ER-mediated GLUT4 and facilitate the expression of VEGF/VEGFR, which in turn increases the viability and invasion of ECCs (42).

Hypoxia

Hypoxia increases the expression of GLUT1 and GLUT3 in endometrial stromal cells (54, 55). It seems that hypoxia can increase intracellular adenosine triphosphate (ATP), which sequentially triggers GLUT1 translocation to the plasma membrane *via* ATP-sensitive potassium channels (KATP channels) (56). Some studies have confirmed that glucose consumption increases significantly under hypoxic conditions, and the key regulator HIF-1α seems to play an important role in the process. In both type 1 and type 2 EC, HIF-1α is widely expressed in epithelial and stromal components; however, its role may vary. By activating its downstream genes such as GLUT1, VEGF, and epidermal growth factor (EGF), HIF-1α accelerates the occurrence and development of EC (57, 58). Nevertheless, it has been reported that HIF-1α activity can be constitutively induced by oxygen-insensitive pathways in addition to its induction by hypoxia/anoxia, such as ubiquitination, acetylation, sumoylation, hydroxylation, and phosphorylation. These pathways may make a joint effort to promote HIF-1α activity and expression, which further activate GLUT1-induced glucose uptake and crucial genes including VEGF and matrix metalloproteinases (MMPs). This mechanism has been reported in various cancers except in EC (59, 60). Increased SHARP1 is a physiological transcription factor to decrease the levels of HIF-1α, VEGF, and so forth, functioning as a protective molecule to repress the development of EC (61). This indicates that it may indirectly inhibit the expression the GLUT1 *via* in a HIF-1α-dependent manner. High-temperature requirement A3 (HtrA3) is a member of the ATP-independent serine protease family. Many studies have demonstrated that the expression of HtrA3 downregulates in some cancers, indicating that it may act as a pro-apoptotic protein in carcinogenesis (62). Hypoxia can further reduce the expression of HtrA3, promoting the development of EC (63). It has been reported that in vulvar squamous cell carcinoma, the expression of GLUT1 may be dependent on neither hypoxia nor aerobic glycolysis. However, this mode seems to be crucial for protecting DNA's integrity from oxygen radical damage as well as promoting the regeneration of membranes (64).

Various cytokines

Cytokines play an important role in synthesizing GLUTs. Furthermore, previous evidence supports that all the known risk factors for EC are directly or indirectly involved in inflammation pathways, such as estrogen, obesity-related factors, and diabetes mellitus (65). Tumor necrosis factor-α (TNF-α), one of the most powerful cytokines, may activate the downstream mediator-RELA *via* the nuclear factor kappa-B (NF-κB) signaling pathway, increasing the expression of GLUT6, in addition to enhancing local estrogen synthesis, insulin resistance, and the like (66). There is evidence that TNF-α may act alone or together with GLUT6, promoting the occurrence and progression of obesity-related EC (67). A high expression of TNF-α seems to play a part in some advanced and worse overall survival EC. Interleukin-3 (IL-3) activates the PI3K/AKT/mTOR pathway, promoting the activity, recycling, and internalization of GLUT1 in lymphoid/myeloid hematopoietic precursor cells (68). Interleukin-7 (IL-7) can upregulate reactive oxygen species, which rely on PI3K/AKT/mTOR pathway, upregulating GLUT1 expression in EC (69). In one previous EPIC cohort study, Dossus et al. concluded that IL-6 was associated with an increased risk of EC in obese women in addition to TNF-α (70). Trabert et al. confirmed the positive correlation between these cytokines (e.g., adipokines, inflammatory cytokines, and angiogenic factors) and obese women with EC in a nested case–control study (71). Further, with the VEGF-A becoming the most important component of the VEGF family, both VEGF-A binding to VEGFR1/2 and GLUT1 can be activated by HIF-1α under hypoxic conditions, accelerating the occurrence and development of EC (60, 72). Similarly, there exists a broad consensus that VEGF-A stimulated by estrogen also has a positive correlation with GLUT4 in promoting the angiogenesis process of EC. Sahoo et al. investigated visceral adipocytes that could induce VEGF activation in the angiogenesis of EC (73).

Other regulators

Abhydrolase domain containing 5 (ABHD 5) acts as a carcinogenic component and is overexpressed in EC, which significantly increases the expression of GLUT1 and glycolysis enzymes *via* the AKT pathway, which is believed to be closely related to cell proliferation, invasion, and EMT in EC (74). High-level aldehyde dehydrogenase (ALDH) expression is significantly correlated with increased expression of GLUT1, and the glycolytic pathways' activation plays a crucial role in the prognostic evaluation of EC (75). As natural compounds with antiproliferative activities, flavonoids can regulate the expression of GLUT1 and glucose uptake, which may be of help in controlling the growth of prostate cancer cells (76). Ascorbic acid (also named vitamin C) can downregulate the activity of many GLUTs, including GLUT1/3/4, which may be due to its repressive effect on HIF-1α under normoxic or hypoxic conditions. This mechanism has been delineated in many tumors, such as pancreatic cancer, liver cancer, and ovarian cancer (77).

The functions of glucose transporters in endometrial cancer

Cell proliferation and apoptosis

Increased GLUT1 is always associated with cell proliferation and tumorigenesis in various tumors *via* glucose supply (78–80). Additionally, GLUT1 was regarded as a downstream target of miR-150-5p, which can protect cells by inhibiting GLUT1 expression (81). Another report indicates that overexpression of GLUT1 is regulated by lncRNA-plasmacytoma variant translocation 1 (PVT1) through the PVT1/miR−150−5p/GLUT1 signaling axis to promote cell proliferation and invasion and inhibit apoptosis in oral squamous cell carcinoma (82). It has been reported that the expression of GLUT1 is positively related to that of CASC9 (a long non-coding RNA). In laryngeal carcinoma cells, through activating PI3K/AKT/mTOR and EGFR signal pathways, CASC9 facilitates cell proliferation and inhibits cell apoptosis (83), while the level of GLUT1 is also regarded as an independent prognostic predictor.

Previous studies show that ECCs rely on complicated macromolecule synthesis to promote cell proliferation. Through stimulating different GLUT (GLUT1/3/6) production, the inactivation of proto-oncogenes phosphatase and tensin homolog (PTEN) and mutation of oncogenes (e.g., BRAF and KRAS) occur in the endometrium (Figure 1), activating the PI3K/AKT pathway (84, 85). GLUT1-related glucose uptake is tightly associated with ATM (an insulin-responsive protein kinase). As a crucial regulator of tumorigenesis, ATM facilitates the production of ATP and the activation signaling of AKT, promoting cell proliferation and inhibiting apoptosis in aggressive breast and prostate cancer cells (86).

Another study has also elucidated that GLUT3 expression can be upregulated by the Hippo-Yes-associated protein (YAP) at a transcriptional level. AMPK directly phosphorylates YAP at S61 and inhibits YAP transcriptional activity, maintaining glucose homeostasis in HEK293T and HeLa cells (87). In liver cancer, therapy with the sodium-glucose transporter 2 (SGLT2) inhibitor induces AMPK/mTOR-mediated cell cycle arrest, antiproliferation, and apoptosis, which may be regarded as a novel way of treatment. Nevertheless, the expression of SGLY2 has not been identified in EC (88). Estrogen is greatly associated with the expression of GLUT1/4. It is reported that estrogen has positive effects on excessive cellular proliferation and tumor differentiation *via* the dysregulation of Wnt signaling-related molecules including secreted frizzled-related protein 1 (sFRP1) and sFRP4 and the upregulation of the IGF pathway (32, 89).

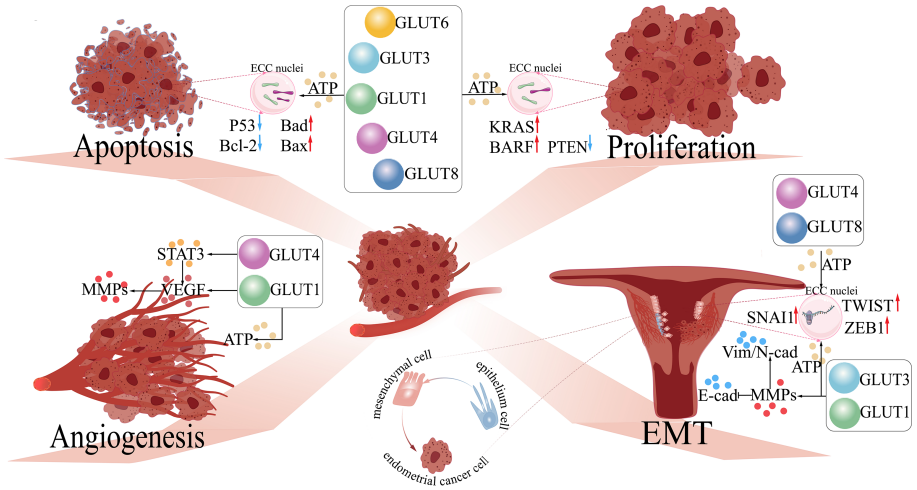

FIGURE 1
The functions of GLUTs in endometrial cancer. 1) Proliferation: GLUTs upregulate the expression of oncogenes (e.g., KRAS and BARF) and inhibit anti-oncogenes (e.g., PTEN) in endometrial cancer cells (ECCs) by providing abundant ATP for cellular metabolism. 2) Apoptosis: GLUTs downregulate pro-apoptosis genes (e.g., P53 and Bcl-2) and upregulate anti-apoptosis genes (e.g., Bad and Bax) in ECCs. 3) Angiogenesis: GLUT1 regulates VEGF and its downstream molecule (MMPs), further accelerating angiogenesis in ECCs by activating STAT3. 4) EMT: GLUT1/3 regulates the expression of EMT-related proteins (Vim, N-cad, and E-cad) by upregulating the levels of MMPs in ECCs, facilitating the development of EMT in ECCs; GLUT4/8 activates EMT-related transcription factors (TWIST, SNAI1, ZEB1) of ECCs. GLUTs, glucose transporters; STAT3, signal transducer and activator of transcription 3; VEGF, vascular and epidermal growth factor; MMPs, matrix metalloproteinases; EMT, epithelial–mesenchymal transition; Bcl-2, B-cell lymphoma-2; Bad, Bcl-2 agonist of cell death; Bax, BCL2-associated X; KRAS, Kirsten rat sarcoma; BARF: *Bam*HI A right frame 1; PTEN, phosphatase and tensin homolog; TWIST, time without significant symptoms of toxicity protein; SNAI, Snail-1 protein. .

Furthermore, studies show that GLUT4 is significantly correlated with IRAP, which induces the activation of the PI3K/AKT pathway (4, 51, 90). Increasing evidence supports that activated GLUT1/4 expression and translocation from the cytoplasm to the membrane are positively induced by AKT, which may facilitate cell proliferation and lead to drug resistance in EC treatment (91). GLUT6 plays the most important role in glucose transport and glycolytic–lipogenic metabolism, providing glucose for ECCs (34, 92, 93). GLUT8 plays a key role in glucose supply by supporting serine/glycine biosynthesis of KRAS/the Kelch-like ECH-associated protein 1 double mutants in non-small cell lung cancer, while it has also been found to be importantly upregulated in EC (94).

Epithelial–mesenchymal transition

Studies have confirmed that EMT is closely related to the occurrence, progression, metastasis, and even treatment resistance in EC. An early report indicated that estrogen-induced GLUT1/4 plays a crucial role in the malignant transformation of benign epithelial EC (95). Further, the expression of ER-related GLUT4 can be upregulated by high glucose, which in turn activates the transcription of many EMT-related molecules in EC (42). By providing abundant glucose, they meet the glucose metabolism demand of these tumor cells, which are far from stromal blood vessels (86). Estrogen activates the binding of ERα to the expression of ubiquitin-conjugating enzyme E2C promoter region and negatively modulates the expression of p53 (96). In addition, this combination can also improve the level of miR-200c, which inhibits the expression of PTEN and PTENP1, leading to the activation of the PI3K/AKT pathway (97). In a later study, the activation of the PI3K/AKT/mTOR pathway and the inhibition of the level of E-cad under the estrogen stimuli are both involved in the EMT process of EC (98). These mechanisms facilitate cell migration, invasion, and EMT-related vimentin in EC. Collectively, GLUTs may play a pivotal role in the process of EMT in an estrogen-dependent manner in EC.

There is increasing evidence that hypoxia is one of the microenvironmental factors that directly promote the EMT process and GLUT production in multiple cancers (99, 100). The expressions of glycolysis-related GLUT1/3 and EMT-related proteins (Vim, N-cad except for E-cad) are both increased under hypoxia conditions (101–103). A study concerning laryngeal carcinoma showed that by regulating the activity of MMPs, hypoxia-induced GLUT1/3 may induce EMT and promote cell invasion and metastasis (104). In addition, some studies showed that hypoxia-induced GLUT1 expression not only is closely correlated with tumor proliferation and angiogenesis but also

has a strong positive correlation with Ki-67 expression (EMT-related) in epithelial ovarian carcinoma and diffuse large B-cell lymphoma (105, 106). Although such a mechanism has not been found in EC, we speculate that hypoxia-induced GLUTs may also participate in the regulation of EMT-related factors (such as MMP, SNAI1, TWIST, and ZEB1).

High glucose and estrogen can upregulate GLUT4, facilitating the expression of VEGF/VEGFR and the progression of EMT, which finally improves the viability and invasion of ECCs (42). As mentioned, the overexpression of GLUT8 is related to the differentiation, proliferation, and invasion of EC. Evidence suggests that the abnormal transposition of GLUT8 is significantly associated with the malignant transformation of ECCs. There are three possible mechanisms that might explain this phenomenon: intracellular phosphorylation events similar to those of GLUT4, mutation of the di-leucine motifs, and the inhibition of IGF-1R by antisense oligonucleotides (35).

Angiogenesis

Angiogenesis acts as a critical part of tumor growth and invasion, providing a new colony for tumor immune escape (72). Factors participating in angiogenesis include fibroblast growth factor, VEGF, platelet-derived growth factor, and EGF. Among them, VEGF plays the most important role (107). As mentioned before, in EC, the expression of GLUT1 is related to tumor differentiation; the expression of GLUT1 is significantly elevated in poorly differentiated types compared with well-differentiated types. In several EC-related clinical studies, tumor aggressiveness has a positive correlation expression of GLUT1 in patients with early EC; nevertheless, the aggression-related molecules of GLUT1 have not been identified (108). Further studies confirm that the expression of GLUT1 is positively correlated with VEGF and its downstream component MMPs, which is induced by HIF-1α (60).

A large amount of evidence shows a tight relationship between estrogen and angiogenesis and that estrogen can activate the PI3K/AKT signaling pathway in a HIF-1α-dependent manner, sequentially stimulating VEGF and GLUT1/4 expression levels (109, 110). As mentioned above, we speculate that SHARP1(a basic helix-loop-helix transcription repressor) may indirectly regulate GLUT1 overexpression and VEGF levels *via* a HIF-1α-dependent manner, which is negatively associated with hypoxia-related angiogenesis in EC (61). In addition, GLUT4 induced by high glucose or estrogen may also play a role in promoting cell proliferation and endothelial cell migration. Through activating the signal transducer and activator of the transcription 3 (STAT3) signaling pathway, GLUT4 upregulates the expression of VEGF (42, 111, 112). We speculate that GLUT1/4 may promote the invasion ability and angiogenesis in EC by influencing VEGF and its downstream gene, although the exact mechanism remains unknown (113–115).

As mentioned before, progesterone can increase the expression of GLUT1. Studies show that through activating the GLUT1-related PI3K/AKT pathway, progesterone can also downregulate the expression of progesterone receptor B (PRB), promoting the proliferation and angiogenesis of ECCs (116).

Glucose transporters in endometrial cancer therapies

Glucose transporter-related radiotherapy sensitivity

Radiation therapy is one of the important complementary treatments for EC. In recent years, research on how to enhance the sensitivity of radiation therapy in EC has received more and more attention. Autophagy is of great importance in the formation of radiotherapy sensitivity in solid tumors, and studies have verified that the PI3K/AKT/mTOR signaling pathway may negatively regulate intracellular autophagy (117, 118). GLUT1 siRNA is the targeted inhibitor of GLUT1-mediated glucose uptake, which can increase radiosensitivity through activating autophagy in a PI3K/AKT pathway-dependent manner and reducing DNA repair capability (Figure 2) (119, 120). In addition, it can also interfere with the active glucose metabolism to a large extent. This mechanism has been certified in laryngeal carcinoma and prostate cancer (121, 122).

The overexpression of GLUT1 is closely related to the radiation therapy resistance in EC. It has been reported that in rat glioma tumor cells, oleanolic acid (OA) shows a radio-sensitizing effect by decreasing the expression of many significant factors, including GLUT1 and its upstream molecule HIF-1α, Ki-67, and P53 (123).

A large amount of data indicate that the anti-radiosensitivity in EC refers to PI3K/PTEN/AKT/mTOR signaling pathway, MAPK signaling pathway, and NF-kB signaling pathway; each of them is directly or indirectly involved in tumor radio-resistance and GLUT1-induced malignant processes that include proliferation, angiogenesis, and EMT in EC (124, 125). It is clear that either upstream elements' inhibitors of these significant pathways or inhibitors of GLUT1 itself should play a therapeutic role in the radio-resistance of EC. Among them, a prominently activated PI3K/AKT/mTOR pathway could increase the expression and translocation to the plasma membrane of GLUT1/3, and numerous preclinical setup and clinical trials have been launched with some of their inhibitors approved to be used in trials (126). For example, using sunitinib (one AKT inhibitor) as a neoadjuvant treatment could promote autophagy along with radiosensitivity to recalcitrant EC, which only provides a novel point for clinical implementation of sunitinib (127).

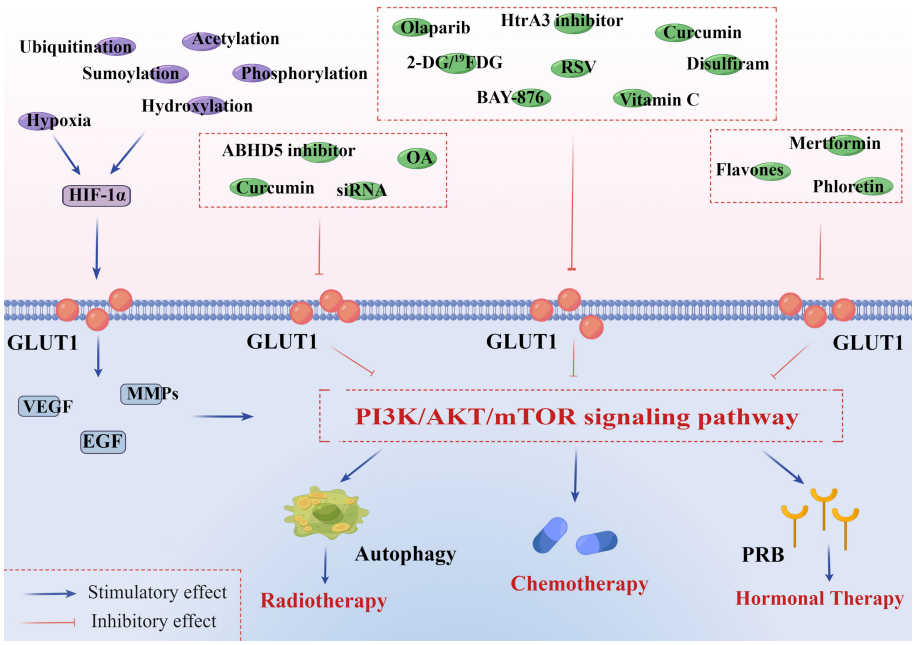

FIGURE 2
PI3K/AKT/mTOR signaling pathway is associated with GLUT1 overexpression and endometrial cancer therapies. HIF-1α is implicated in both GLUT1 expression and aberrant PI3K/AKT/mTOR signaling pathway in tumor microenvironments. In addition to hypoxia, HIF-1α can be constitutively induced by oxygen-insensitive pathways, such as ubiquitination, acetylation, sumoylation, hydroxylation, and phosphorylation. These pathways jointly promote the expression of HIF-1α and further activate the downstream genes of GLUT1, including VEGF, EGF, and MMPs. 1) Radiotherapy: curcumin inhibits a site overlapping the cytochalasin B of GLUT1 and metabolism-related enzymes. OA decreases the expression of many significant factors, including GLUT1, HIF-1α, Ki67, and P53. GLUT1-siRNA interferes with the targeted gene, inhibiting the synthesis of GLUT1. ABHD 5 plays an oncogenic role in the development of EC, and its knockdown can notably suppress ECC proliferation and invasion *in vivo*. All of them can inhibit the PI3K/AKT/mTOR pathway and activate autophagy of endometrial cancer cells (ECCs), increasing the sensitivity to radiotherapy. 2) Chemotherapy: olaparib inhibits the activity of GLUT1 in plasma in a concentration-dependent manner. BAY-876 can suppress cell viability and decrease stemness oncogene (Nanog and c-Myc) expression of ECCs. RSV inhibits GLUT1-induced glycolysis in a PI3K/AKT/mTOR-dependent manner, enhancing the anti-endometrial cancer (anti-EC) effects of cisplatin and doxorubicin. Curcumin inhibits a site overlapping the cytochalasin B of GLUT1 and metabolism-related enzymes. Vitamin C inhibits the expression of HIF-1α and GLUT1 in ECCs. 2-DG/^{19}FDG and disulfiram show notably antiproliferative and anti-angiogenesis effects by downregulating the level of GLUT1. Targeting HtrA3 can enhance the cytotoxic effect of chemotherapy *via* the X-linked inhibitor of apoptosis protein cleavage. 3) Hormonal therapy: GLUT1 participates in regulating PR of ECCs in a PI3K/AKT/mTOR pathway-dependent manner. Flavones, phloretin, and metformin can greatly increase the sensitivity of hormonal therapy in EC by strengthening PR transcriptional activity. This figure was drawn by Figdraw (www.figdraw.com).
GLUT1, glucose transporter 1; HIF-1α, hypoxia-inducible factor-1α; VEGF, vascular and epidermal growth factor; EGF, epidermal growth factor; MMPs, matrix metalloproteinases; OA, oleanolic acid; siRNA, small interfering RNA; ABHD 5, abhydrolase domain containing 5; RSV, resveratrol; DG, deoxyglucose; PR, progesterone receptor.

In the tumor microenvironment, hypoxia, high glucose and insulin, and various cytokines and all their inhibitors could play different roles in the inhibition of the expression of GLUTs and the final development of EC. Both hypoxia and its inducible factor HIF-1α known as an aggressive biomarker are positively correlated with the expression of GLUT1 and VEGF, which makes their inhibitors a great potential treatment in the prevention of angiogenesis, EMT process, and increased radiosensitivity of EC (128). As a HIF-1α inhibitor, curcumin (diferuloylmethane) has been assumed to decrease glucose uptake in many cancers, such as lung, cervical, prostate, and breast cancers. It might function as a novel anticancer drug to assist chemoradiotherapy by inhibiting a site overlapping the cytochalasin B of GLUT1 and metabolism-related enzymes (129–131). In some studies concerning cervical cancer, the theory that curcumin enhances radiosensitivity by inhibiting GLUT1-induced PI3K/AKT/mTOR pathway or MAPK/mTOR/ULK1 pathway and subsequently activating autophagy has also been confirmed (121, 125, 132). Therefore, we speculate that there may be a similar mechanism in EC. Several studies also indicate that P-LAP siRNA (an inhibitor of hyperinsulinemia) may be a potential agent of molecular-targeted therapy for EC *via* the downregulation of GLUT4 expression and the prevention of tumor cells' proliferation and angiogenesis (133). As mentioned before, ABHD5 may play an oncogenic role in GLUT1 expression and the EMT process of EC *via* the AKT pathway, and its

knockdown notably suppresses tumor cell proliferation and invasion *in vivo*. This illustrates that it may act as a potential therapeutic target in radio-resistance of EC (74).

Of course, large amounts of cytokines also play a unique role in EC. Among them, VEGF as an upstream regulator in PI3K/AKT/mTOR signaling pathway illustrates that either its inhibitors or mTOR inhibitors could be a valid treatment of angiogenesis and EMT of EC and the level of GLUT1 (73). Likewise, in the epithelial cells, TNF-α also efficiently binds to its receptor to activate the NF-κB transcription factor and subsequently regulates the expression of genes and Snail-like proteins, which control E-cadherin transcription in tumor invasion (134). TNF-α inhibitors (e.g., adalimumab) are known as effective agents in both suppressing the level of GLUT6 and restraining the proliferation, angiogenesis, and EMT process in EC (66, 135). Therefore, it is possible that not only GLUT1 inhibitors but also blocking crucial pathways or upstream regulative factors (e.g., hypoxia, hyperinsulinemia, and cytokines) could promote apoptosis procedure and radiosensitivity in EC.

Glucose transporter-related chemotherapy sensitivity

The resistance of tumor cells to drugs is a major obstacle in cancer chemotherapy. Thus, GLUTs as a novel therapeutic target might be of great importance in the chemotherapy of EC (136). At present, there are four inhibitor-bound hGLUTs, hGLUT 1–4, and each of them can provide a significant inhibitory effect on glucose uptake and cancer cell proliferation (137). BAY-876, as a specific inhibitor of GLUT1, can suppress ALDH-dependent glycolytic activation, cell viability, and stemness marker (e.g., Nanog and c-Myc) expression in ALDH-high ECCs. It can strongly suppress the proliferation of endometrial cancer stem cells (CSCs) when used in combination with paclitaxel (75). However, despite some current progress in the treatment of hepatocellular carcinoma, administration or intravenous infusion of BAY-876 can cause the drug to be distributed systemically, which greatly interferes with the physical uptake of glucose in the body in addition to the negligible dose distribution at the lesion site of cancer cells (138, 139).

As mentioned before, ALDH also plays an important role in the maintenance of CSCs and chemoresistance through upregulating GLUT1-induced glycolysis. Of note, the pan-ALDH-specific inhibitor disulfiram (DSF) can improve the paclitaxel-resistance effect in EC by suppressing GLUT1 and crucial pathways in several processes (e.g., proliferation, EMT, and angiogenesis) (75). Although it has not been confirmed in EC treatment, this combination therapy has already been applied in many preclinical trials for the treatment of several other cancers (e.g., non-small cell lung cancer, glioblastoma, and breast cancer) (140, 141). Pharmacodynamics reveals that DSF has a risk of reversible neurological toxicities, which readily occur after treatment with 1,000 mg per day (142).

Olaparib is an inhibitor of poly(ADP-ribose) polymerase (PARP)-1/2/3 (143–145). It can inhibit the activity of GLUT1 in plasma in a concentration-dependent manner and reduce the expression of cyclin D1 *via* a PARP-1 level-dependent manner. In several EGFR inhibitor-resistant cancers, such as glioblastoma and lung cancer, olaparib reduces lactate production and glucose uptake in a pyruvate kinase 2 (PKM2)-dependent manner (146). The most common adverse effects are nausea, fatigue, anemia, and vomiting (147).

Substantial work has constantly sought to target glucose metabolism. Among them, directly downregulating glucose levels through a special compound known as 2-deoxyglucose (2-DG) or inhibiting lactate production and excretion could be more prominent than others. 2-DG has been used for antiproliferation in numerous preclinical studies and partial clinical testing. The example here is that 2-DG shows notable antiproliferative effects and increased sensitization of resistant cells on oral cancer by downregulating the level of PARP, LDHA, and GLUT1 when it is used in a combination therapy with paclitaxel and erlotinib (148). Nevertheless, how to manage serious hypoglycemia symptoms caused by its higher dose along with an insufficient therapeutic response to its lower dose limits its clinical efficacy (149). Thus, ^{19}FDG, as an alternative to 2-DG, shows a better ability to inhibit GLUT-dependent glycolysis, prevent cell viability and proliferation, and induce apoptosis *in vitro* under normoxic and hypoxic conditions. Niccoli et al. confirmed it in HeLa cells *via* by combining ^{19}FDG with doxorubicin and comparing its efficacy with that of 2-DG and doxorubicin (150). Excessive lactate production could promote angiogenesis and tumor vascularization through the induction of HIF-1α-stimulated VEGF increase, and dysregulated pH is also involved in chemotherapeutic drug resistance (e.g., vinblastine, doxorubicin, and paclitaxel) (151). The cardiac Na^+/H^+ exchanger (NHE1) is a membrane glycoprotein for multiple housekeeping tasks relying on cell function, including regulation of intracellular pH, Na^+ concentration, and cell volume. Therefore, clinical NHE1 inhibitors and much more selective inhibitors (e.g., KR-33028 and cariporide) might be taken into consideration to attain increased chemotherapeutic effectiveness in EC; this has been assessed in a triple-negative breast cancer model (152). It is proven to be well tolerated in people with cardiovascular disease. However, some side effects are inevitable, mainly related to drug accumulation and cerebrovascular complications (153).

Resveratrol (RSV) has no effect on GLUT1 mRNA and protein expressions but disturbs intracellular GLUT1 trafficking to the plasma membrane by suppressing AKT/mTOR activation, which ultimately impairs glucose uptake

and induces apoptosis in ovarian cancer cells (154). In EC, RSV can enhance the antitumor effects of cisplatin and doxorubicin in a time-dependent manner (155–157). It may regulate the expression of EGF/VEGF and angiogenesis to promote chemosensitivity in an estrogen-dependent or estrogen-independent manner (158, 159). This inhibitor has been controversial because its biologically effective concentration is hard to confirm. Moreover, it acts as a natural reservoir for body antioxidants and is accompanied by many toxic effects, such as high dosage-associated hormetic effects, systemic inhibition of P450 cytochromes, and attenuation of the activities of drugs (160).

Curcumin analog (EF24) could exert antiproliferative and anti-angiogenic effects on three ovarian cancer cells (SKOV-3, A2780, and OVCAR-3) *in vivo via* the downregulation of GLUT1-related glucose glycolysis, lactate production, and its upstream molecule HIF-1α (161). Anti-GLUT1 antibody or curcumin combined with doxorubicin could significantly enhance the ability in killing colorectal adenocarcinoma cells (162). This result leads us to speculate that both curcumin and anti-GLUT1 antibody may also have the effect of sensitizing chemotherapeutic drugs of EC (121, 132). It is reported that curcumin can cause diarrhea, and other toxic and adverse effects have not been confirmed. However, in the long-term rat trials, adverse effects are noticeable, such as incidence of ulcers, chronic inflammation, and hyperplasia of the cecum as well as carcinogenesis (163).

Ritonavir displays inhibitory effects on GLUT4 expression and induces the apoptosis of multiple myeloma (MM) cells by reducing myeloid cell leukemia-1 expression. It is regarded as a sensitizer in the therapy of MM, which can make tumor cells more sensitive to drugs such as doxorubicin, dexamethasone, and melphalan (164, 165). Patients who are treated with ritonavir at a dose higher than 7.9 ml/L may be at a higher risk of experiencing neurological or gastrointestinal side effects. Although ritonavir's sensitizing effect in the chemotherapy of EC has not been reported so far, related studies have already been carried out in many clinical trials for the treatment of cancers, such as multiple myeloma, prostate cancer, and breast cancer (166, 167).

Vitamin C plays an important role in VEGF-related angiogenesis and anti-chemoresistance in many cancers by inhibiting the expression of HIF-1α and GLUT1/3/4 (77, 168). For example, through inhibiting extracellular signal-regulated kinase 1/2 and PKM2 phosphorylation, the combination of vitamin C and cetuximab can significantly downregulate the expression of GLUT1 in KRAS colon cancer (169). Therefore, we infer that perhaps vitamin C is also valid in enhancing the sensitivity of chemotherapy in EC. Targeting HtrA3 might be a potential therapeutic measure to reverse the negative effects induced by hypoxia and enhance the cytotoxic of conventional chemotherapy *via* the X-linked inhibitor of apoptosis protein (XIAP) cleavage in EC (170). However, a more detailed understanding of the molecular mechanisms and cellular targets in clinical treatment agents is needed.

Hormonal therapy sensitivity

Progestogen is the most commonly used drug in the conservative treatment of early EC (171, 172). However, the response rate to progestin therapy varies from person to person. As mentioned before, GLUT1 expression is positively correlated with the activation of PI3K/AKT pathways. Recent evidence has implicated that PI3K/AKT pathway increases the drug resistance of progestin in EC by weakening PRB transcriptional activity. GLUT1 may become involved in regulating PR in the PI3K/AKT pathway-dependent manner; therefore, GLUT1 inhibitors may be an effective therapeutic strategy for increasing hormone-insensitivity therapies in EC (116, 173). Metformin has been a well-tolerated biguanide drug to treat type 2 diabetes mellitus for decades. In the context of hyperinsulinemia easily accompanying EC patients, some studies have demonstrated that metformin could suppress the proliferation of ECCs by changing GLUT1-related glucose metabolism and inhibiting the PI3K/AKT/mTOR signaling pathway (174). Moreover, metformin could facilitate the expression of PR, which greatly promotes the sensitivity of medroxyprogesterone acetate (MPA)-induced apoptosis progestin in resistant ECCs (175). However, direct data concerning metformin plus progestin producing a better therapeutic effect than progestin alone have not been found. Its side effects include diarrhea, dyspepsia, and flatulence.

In addition, it has been reported that some flavonoids, such as flavones and phloretin, show a well-established inhibition of GLUT1 *via* against ERs (176, 177). This suggests that GLUT1 inhibitors seem to be more effective in ER-positive EC.

Glucose transporter-related clinical trials

Until now, most advances in the GLUT inhibitors are in the early preclinical stage, while a few are in the clinical trial stages of many cancers except EC (148). Several indirect data still exist; yet in a phase I trial of glioblastoma, 2-DG performed notable effectiveness in asymptomatic QTc prolongation and restriction of dose escalation (178). In addition, 2-DG combined with radiation therapy shows improvement including better tolerance of 2-DG toxicity and lower incidences of late radiation effects in glioblastoma patients (179). There are as few as 20 ongoing clinical trials on curcumin combination therapy, with two being related to EC. Both are in phase II trials: one was completed in 2016, which showed an increased therapeutic effect on standard treatment through disturbing tumor-induced inflammation. However,

the other trial concerning pembrolizumab, radiation, and immune modulation is still ongoing (180). Furthermore, in obese women with early EC, a phase II non-controlled trial has reported that metformin combined with MPA could lead to a better complete response (CR) rate and a recurrence rate than MPA alone (181). Another randomized controlled clinical trial also confirms that the addition of metformin is associated with a higher early CR compared with megestrol acetate (MA) alone (182). In a phase III CONFIRM clinical trial, the VEGFR inhibitor PTK787/ZK 222584 (vatalanib) has been confirmed to have greatly increased benefit as compared to original agents in metastatic colorectal cancer patients (183).

If combined with other radio- or chemo-therapeutic agents and hormone therapy, these inhibitors could become an excellent helper to enhance therapeutic sensitivity and reduce toxicity and dosage. In fact, there are many studies focusing on the combination of GLUT inhibitors with various glycolytic inhibitors (e.g., hexokinase 2 (HK2) inhibitors, PKM2 activators, and lactate dehydrogenase (LDH) inhibitors). Their joint functions to confront glycolytic and mitochondrial metabolism also make promising effectiveness in the treatment of active proliferative cancer. Thus, preclinical and clinical trials are needed for GLUT-related inhibitors and GLUT inhibitors to be used for EC patients.

Perspectives

The main GLUTs in EC are Class 1 (GLUTs 1–4) and Class 3 (GLUTs 6, 8), and the overexpression of these GLUTs has been observed. As mentioned above, such abnormal overexpression of GLUTs may be related to the regulation of estrogen or progesterone, insulin and high glucose, microenvironment (such as hypoxia and cytokines), and so on. On the one hand, these overexpressed GLUTs provide abundant glucose uptake for various metabolic pathways; on the other hand, they also participate in the activation of many crucial signaling pathways and the regulation of key genes concerning proliferation and apoptosis, EMT, and angiogenesis in EC. In addition, overexpressed GLUTs may also cause ECCs to be insensitive to hormone therapy or even resistant to chemoradiotherapy, which has become a huge challenge in the treatment of EC in recent years. Nevertheless, from what has been discussed in this review, we can conclude that with more and more attention to the regulation of various GLUTs and GLUT-related inhibitors in EC, patients are bound to receive more effective treatment strategies and better outcomes. For future EC therapies, there is a consensus to monitor GLUT expression in tumors that are being treated with several appropriate therapies (e.g., hormonal, chemotherapy, and radiotherapy), to ascertain how their expression levels and activity change under these treatments.

However, there are still many problems that remain to be solved. For example, the specific mechanism of GLUTs in the regulation of endometrium, especially the heterogeneity of GLUTs in EC, has not been clarified yet. Breakthroughs in these fields will promote the development of personalized and precise treatment of EC. In addition, the regulation of GLUTs on the immune microenvironment in EC also deserves to be further studied. The expression characteristics and metabolic regulation mechanisms of GLUTs (e.g., GLUT1, GLUT4, GLUT6, and GLUT8) in the EC microenvironment are likely to be a hotspot, which will provide a basis for the realization of immunometabolism typing of EC. Last but not least, how GLUT inhibitors can reach maximum utilization in EC precision-targeted therapies also remains to be explored. In particular, how to achieve an efficient synergistic effect of GLUT inhibitors and hormone therapy may be a focus of future research.

Author contributions

XZ and JJ-L drafted and revised the manuscript. AA, DY-H, JD, JN-W, and LB-L edited the manuscript. FX and MQ-L conceived and designed the review and edited the manuscript. All the authors were involved in writing the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2018) 68(6):394–424. doi: 10.3322/caac.21492

2. Lortet-Tieulent J, Ferlay J, Bray F, Jemal A. International patterns and trends in endometrial cancer incidence, 1978-2013. *J Natl Cancer Inst* (2018) 110(4):354–61. doi: 10.1093/jnci/djx214

3. Raglan O, Kalliala I, Markozannes G, Cividini S, Gunter MJ, Nautiyal J, et al. Risk factors for endometrial cancer: An umbrella review of the literature. *Int J Cancer* (2019) 145(7):1719–30. doi: 10.1002/ijc.31961

4. Onstad MA, Schmandt RE, Lu KH. Addressing the role of obesity in endometrial cancer risk, prevention, and treatment. *J Clin Oncol* (2016) 34 (35):4225–30. doi: 10.1200/jco.2016.69.4638

5. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the women's health initiative randomized controlled trial. *JAMA* (2002) 288(3):321–33. doi: 10.1001/jama.288.3.321

6. Xu Y, Burmeister C, Hanna RK, Munkarah A, Elshaikh MA. Predictors of survival after recurrence in women with early-stage endometrial carcinoma. *Int J Gynecol Cancer* (2016) 26(6):1137–42. doi: 10.1097/igc.0000000000000733

7. Clarke MA, Long BJ, Del Mar Morillo A, Arbyn M, Bakkum-Gamez JN, Wentzensen N. Association of endometrial cancer risk with postmenopausal bleeding in women: A systematic review and meta-analysis. *JAMA Intern Med* (2018) 178(9):1210–22. doi: 10.1001/jamainternmed.2018.2820

8. Ruiz A, Gautschi I, Schild L, Bonny O. Human mutations in Slc2a9 (Glut9) affect transport capacity for urate. *Front Physiol* (2018) 9:476. doi: 10.3389/fphys.2018.00476

9. Wang L, Pavlou S, Du X, Bhuckory M, Xu H, Chen M. Glucose transporter 1 critically controls microglial activation through facilitating glycolysis. *Mol Neurodegener* (2019) 14(1):2. doi: 10.1186/s13024-019-0305-9

10. Jiang CL, Jen WP, Tsao CY, Chang LC, Chen CH, Lee YC. Glucose transporter 10 modulates adipogenesis *Via* an ascorbic acid-mediated pathway to protect mice against diet-induced metabolic dysregulation. *PLoS Genet* (2020) 16 (5):e1008823. doi: 10.1371/journal.pgen.1008823

11. Uldry M, Ibberson M, Horisberger JD, Chatton JY, Riederer BM, Thorens B. Identification of a mammalian h(+)-Myo-Inositol symporter expressed predominantly in the brain. *EMBO J* (2001) 20(16):4467–77. doi: 10.1093/emboj/20.16.4467

12. Narita H, Tanji K, Miki Y, Mori F, Wakabayashi K. Trehalose intake and exercise upregulate a glucose transporter, Glut8, in the brain. *Biochem Biophys Res Commun* (2019) 514(3):672–77. doi: 10.1016/j.bbrc.2019.05.017

13. Setiawan R, Priyadarshana C, Tajima A, Travis AJ, Asano A. Localisation and function of glucose transporter Glut1 in chicken (Gallus gallus domesticus) spermatozoa: Relationship between atp production pathways and flagellar motility. *Reprod Fertil Dev* (2020) 32(7):697–705. doi: 10.1071/RD19240

14. Rogers RC, Burke SJ, Collier JJ, Ritter S, Hermann GE. Evidence that hindbrain astrocytes in the rat detect low glucose with a glucose transporter 2-phospholipase c-calcium release mechanism. *Am J Physiol Regul Integr Comp Physiol* (2020) 318(1):38–48. doi: 10.1152/ajpregu.00133.2019

15. Amir Shaghaghi M, Zhouyao H, Tu H, El-Gabalawy H, Crow GH, Levine M, et al. The Slc2a14 gene, encoding the novel Glucose/Dehydroascorbate transporter Glut14, is associated with inflammatory bowel disease. *Am J Clin Nutr* (2017) 106 (6):1508–13. doi: 10.3945/ajcn.116.147603

16. Matsuo S, Hiasa M, Omote H. Functional characterization and tissue localization of the facilitative glucose transporter Glut12. *J Biochem* (2020) 168 (6):611–20. doi: 10.1093/jb/mvaa090

17. Merigo F, Brandolese A, Facchin S, Missaggia S, Bernardi P, Boschi F, et al. Glucose transporter expression in the human colon. *World J Gastroenterol* (2018) 24(7):775–93. doi: 10.3748/wjg.v24.i7.775

18. Maedera S, Mizuno T, Ishiguro H, Ito T, Soga T, Kusuhara H. Glut6 is a lysosomal transporter that is regulated by inflammatory stimuli and modulates glycolysis in macrophages. *FEBS Lett* (2019) 593(2):195–208. doi: 10.1002/1873-3468.13298

19. Holman GD. Structure, function and regulation of mammalian glucose transporters of the Slc2 family. *Pflugers Arch* (2020) 472(9):1155–75. doi: 10.1007/s00424-020-02411-3

20. Jing X, Yang F, Shao C, Wei K, Xie M, Shen H, et al. Role of hypoxia in cancer therapy by regulating the tumor microenvironment. *Mol Cancer* (2019) 18 (1):157. doi: 10.1186/s12943-019-1089-9

21. Baczewska M, Bojczuk K, Kołakowski A, Dobroch J, Guzik P, Knapp P. Obesity and energy substrate transporters in ovarian cancer-review. *Molecules* (2021) 26(6):1659. doi: 10.3390/molecules26061659

22. Liberti MV, Locasale JW. The warburg effect: How does it benefit cancer cells? *Trends Biochem Sci* (2016) 41(3):211–18. doi: 10.1016/j.tibs.2015.12.001

23. Krzeslak A, Wojcik-Krowiranda K, Forma E, Jozwiak P, Romanowicz H, Bienkiewicz A, et al. Expression of Glut1 and Glut3 glucose transporters in endometrial and breast cancers. *Pathol Oncol Res* (2012) 18(3):721–8. doi: 10.1007/s12253-012-9500-5

24. Frolova AI, Moley KH. Glucose transporters in the uterus: An analysis of tissue distribution and proposed physiological roles. *Reproduction* (2011) 142 (2):211–20. doi: 10.1530/rep-11-0114

25. Kim ST, Moley KH. Regulation of facilitative glucose transporters and Akt/Mapk/Prkaa signaling *Via* estradiol and progesterone in the mouse uterine epithelium. *Biol Reprod* (2009) 81(1):188–98. doi: 10.1095/biolreprod.108.072629

26. Frolova A, Flessner L, Chi M, Kim ST, Foyouzi-Yousefi N, Moley KH. Facilitative glucose transporter type 1 is differentially regulated by progesterone and estrogen in murine and human endometrial stromal cells. *Endocrinology* (2009) 150(3):1512–20. doi: 10.1210/en.2008-1081

27. Yamaguchi M, Sakata M, Ogura K, Miyake A. Gestational changes of glucose transporter gene expression in the mouse placenta and decidua. *J Endocrinol Invest* (1996) 19(8):567–9. doi: 10.1007/bf03349018

28. von Wolff M, Ursel S, Hahn U, Steldinger R, Strowitzki T. Glucose transporter proteins (Glut) in human endometrium: Expression, regulation, and function throughout the menstrual cycle and in early pregnancy. *J Clin Endocrinol Metab* (2003) 88(8):3885–92. doi: 10.1210/jc.2002-021890

29. Korgun ET, Demir R, Hammer A, Dohr G, Desoye G, Skofitsch G, et al. Glucose transporter expression in rat embryo and uterus during decidualization, implantation, and early postimplantation. *Biol Reprod* (2001) 65(5):1364–70. doi: 10.1095/biolreprod65.5.1364

30. Anagnostou E, Miliaras D, Meditskou S, Grimbizis G. Immunohistochemical investigation of metabolic markers fatty acid synthase (Fasn) and glucose transporter 1 (Glut1) in normal endometrium, endometrial hyperplasia, and endometrial malignancy. *Hippokratia* (2017) 21(4):169–74.

31. Frolova AI, Moley KH. Quantitative analysis of glucose transporter mrnas in endometrial stromal cells reveals critical role of Glut1 in uterine receptivity. *Endocrinology* (2011) 152(5):2123–8. doi: 10.1210/en.2010-1266

32. Medina RA, Meneses AM, Vera JC, Guzman C, Nualart F, Rodriguez F, et al. Differential regulation of glucose transporter expression by estrogen and progesterone in ishikawa endometrial cancer cells. *J Endocrinol* (2004) 182 (3):467–78. doi: 10.1677/joe.0.1820467

33. Knapp P, Chabowski A, Harasiuk D, Górski J. Reversed glucose and fatty acids transporter expression in human endometrial cancer. *Horm Metab Res* (2012) 44(6):436–41. doi: 10.1055/s-0031-1301301

34. Byrne FL, Poon IK, Modesitt SC, Tomsig JL, Chow JD, Healy ME, et al. Metabolic vulnerabilities in endometrial cancer. *Cancer Res* (2014) 74(20):5832–45. doi: 10.1158/0008-5472.Can-14-0254

35. Goldman NA, Katz EB, Glenn AS, Weldon RH, Jones JG, Lynch U, et al. Glut1 and Glut8 in endometrium and endometrial adenocarcinoma. *Mod Pathol* (2006) 19(11):1429–36. doi: 10.1038/modpathol.3800656

36. Doege H, Schürmann A, Bahrenberg G, Brauers A, Joost HG. Glut8, a novel member of the sugar transport facilitator family with glucose transport activity. *J Biol Chem* (2000) 275(21):16275–80. doi: 10.1074/jbc.275.21.16275

37. Shao Y, Wellman TL, Lounsbury KM, Zhao FQ. Differential regulation of Glut1 and Glut8 expression by hypoxia in mammary epithelial cells. *Am J Physiol Regul Integr Comp Physiol* (2014) 307(3):237–47. doi: 10.1152/ajpregu.00093.2014

38. Prossnitz ER, Barton M. The G-Protein-Coupled estrogen receptor gper in health and disease. *Nat Rev Endocrinol* (2011) 7(12):715–26. doi: 10.1038/nrendo.2011.122

39. Smith HO, Leslie KK, Singh M, Qualls CR, Revankar CM, Joste NE, et al. Gpr30: A novel indicator of poor survival for endometrial carcinoma. *Am J Obstet Gynecol* (2007) 196(4):386. doi: 10.1016/j.ajog.2007.01.004

40. Dama A, Baggio C, Boscaro C, Albiero M, Cignarella A. Estrogen receptor functions and pathways at the vascular immune interface. *Int J Mol Sci* (2021) 22 (8):4254. doi: 10.3390/ijms22084254

41. Boscaro C, Carotti M, Albiero M, Trenti A, Fadini GP, Trevisi L, et al. Non-genomic mechanisms in the estrogen regulation of glycolytic protein levels in endothelial cells. *FASEB J* (2020) 34(9):12768–84. doi: 10.1096/fj.202001130R

42. Gu CJ, Xie F, Zhang B, Yang HL, Cheng J, He YY, et al. High glucose promotes epithelial-mesenchymal transition of uterus endometrial cancer cells by

increasing Er/Glut4-mediated vegf secretion. *Cell Physiol Biochem* (2018) 50 (2):706–20. doi: 10.1159/000494237

43. Gregorio KCR, Laurindo CP, Machado UF. Estrogen and glycemic homeostasis: The fundamental role of nuclear estrogen receptors Esr1/Esr2 in glucose transporter Glut4 regulation. *Cells* (2021) 10(1):99. doi: 10.3390/cells10010099

44. Campello RS, Fátima LA, Barreto-Andrade JN, Lucas TF, Mori RC, Porto CS, et al. Estradiol-induced regulation of Glut4 in 3t3-L1 cells: Involvement of Esr1 and akt activation. *J Mol Endocrinol* (2017) 59(3):257–68. doi: 10.1530/jme-17-0041

45. Barreto-Andrade JN, de Fátima LA, Campello RS, Guedes JAC, de Freitas HS, Machado M. Estrogen receptor 1 (Esr1) enhances Slc2a4/Glut4 expression by a Sp1 cooperative mechanism. *Int J Med Sci* (2018) 15(12):1320–28. doi: 10.7150/ijms.26774

46. Kim JJ, Kurita T, Bulun SE. Progesterone action in endometrial cancer, endometriosis, uterine fibroids, and breast cancer. *Endocr Rev* (2013) 34(1):130–62. doi: 10.1210/er.2012-1043

47. Hampton KK, Anderson K, Frazier H, Thibault O, Craven RJ. Insulin receptor plasma membrane levels increased by the progesterone receptor membrane component 1. *Mol Pharmacol* (2018) 94(1):665–73. doi: 10.1124/mol.117.110510

48. Ding XZ, Fehsenfeld DM, Murphy LO, Permert J, Adrian TE. Physiological concentrations of insulin augment pancreatic cancer cell proliferation and glucose utilization by activating map kinase, Pi3 kinase and enhancing glut-1 expression. *Pancreas* (2000) 21(3):310–20. doi: 10.1097/00006676-200010000-00014

49. Welsh GI, Hers I, Berwick DC, Dell G, Wherlock M, Birkin R, et al. Role of protein kinase b in insulin-regulated glucose uptake. *Biochem Soc Trans* (2005) 33 (Pt 2):346–49. doi: 10.1042/bst0330346

50. Shibata K, Kajiyama H, Ino K, Nawa A, Nomura S, Mizutani S, et al. P-Lap/Irap-Induced cell proliferation and glucose uptake in endometrial carcinoma cells *Via* insulin receptor signaling. *BMC Cancer* (2007) 7:15. doi: 10.1186/1471-2407-7-15

51. Shibata K, Kikkawa F, Kondo C, Mizokami Y, Kajiyama H, Ino K, et al. Placental leucine aminopeptidase (P-lap) expression is associated with chemosensitivity in human endometrial carcinoma. *Gynecol Oncol* (2004) 95 (2):307–13. doi: 10.1016/j.ygyno.2004.07.054

52. Pinto AB, Carayannopoulos MO, Hoehn A, Dowd L, Moley KH. Glucose transporter 8 expression and translocation are critical for murine blastocyst survival. *Biol Reprod* (2002) 66(6):1729–33. doi: 10.1095/biolreprod66.6.1729

53. Han J, Zhang L, Guo H, Wysham WZ, Roque DR, Willson AK, et al. Glucose promotes cell proliferation, glucose uptake and invasion in endometrial cancer cells *Via* Ampk/Mtor/S6 and mapk signaling. *Gynecol Oncol* (2015) 138 (3):668–75. doi: 10.1016/j.ygyno.2015.06.036

54. Al-Sharaky DR, Abdou AG, Wahed MM, Kassem HA. Hif-1alpha and glut-1 expression in atypical endometrial hyperplasia, type I and ii endometrial carcinoma: A potential role in pathogenesis. *J Clin Diagn Res* (2016) 10(5): EC20–27. doi: 10.7860/JCDR/2016/19576.7805

55. Airley RE, Mobasheri A. Hypoxic regulation of glucose transport, anaerobic metabolism and angiogenesis in cancer: Novel pathways and targets for anticancer therapeutics. *Chemotherapy* (2007) 53(4):233–56. doi: 10.1159/000104457

56. Mamun AA, Hayashi H, Yamamura A, Nayeem MJ, Sato M. Hypoxia induces the translocation of glucose transporter 1 to the plasma membrane in vascular endothelial cells. *J Physiol Sci* (2020) 70(1):44. doi: 10.1186/s12576-020-00773-y

57. Horree N, van Diest PJ, van der Groep P, Sie-Go DM, Heintz AP. Hypoxia and angiogenesis in endometrioid endometrial carcinogenesis. *Cell Oncol* (2007) 29 (3):219–27. doi: 10.1155/2007/434731

58. Ozbudak IH, Karaveli S, Simsek T, Erdogan G, Pestereli E. Neoangiogenesis and expression of hypoxia-inducible factor 1alpha, vascular endothelial growth factor, and glucose transporter-1 in endometrioid type endometrium adenocarcinomas. *Gynecol Oncol* (2008) 108(3):603–08. doi: 10.1016/j.ygyno.2007.11.028

59. Singh D, Arora R, Kaur P, Singh B, Mannan R, Arora S. Overexpression of hypoxia-inducible factor and metabolic pathways: Possible targets of cancer. *Cell Biosci* (2017) 7:62. doi: 10.1186/s13578-017-0190-2

60. Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: Causes, consequences, challenges and opportunities. *Cell Mol Life Sci* (2020) 77(9):1745–70. doi: 10.1007/s00018-019-03351-7

61. Liao Y, Lu W, Che Q, Yang T, Qiu H, Zhang H, et al. Sharp1 suppresses angiogenesis of endometrial cancer by decreasing hypoxia-inducible factor-1α level. *PLoS One* (2014) 9(6):e99907. doi: 10.1371/journal.pone.0099907

62. Singh H, Li Y, Fuller PJ, Harrison C, Rao J, Stephens AN, et al. Htra3 is downregulated in cancer cell lines and significantly reduced in primary serous and granulosa cell ovarian tumors. *J Cancer* (2013) 4(2):152–64. doi: 10.7150/jca.5702

63. Lv Q, Yang B, Ning C, Xie B, Nie G, Chen X, et al. Hypoxia is involved in the reduction of Htra3 in patients with endometrial hyperplasia and cancer. *Biochem Biophys Res Commun* (2018) 503(4):2918–23. doi: 10.1016/j.bbrc.2018.08.070

64. Mayer A, Schmidt M, Seeger A, Serras AF, Vaupel P, Schmidberger H. Glut-1 expression is largely unrelated to both hypoxia and the warburg phenotype in squamous cell carcinomas of the vulva. *BMC Cancer* (2014) 14:760. doi: 10.1186/1471-2407-14-760

65. Modugno F, Ness RB, Chen C, Weiss NS. Inflammation and endometrial cancer: A hypothesis. *Cancer Epidemiol Biomarkers Prev* (2005) 14(12):2840–7. doi: 10.1158/1055-9965.Epi-05-0493

66. Dossus L, Becker S, Rinaldi S, Lukanova A, Tjønneland A , Olsen A, et al. Tumor necrosis factor (Tnf)-A, soluble tnf receptors and endometrial cancer risk: The epic study. *Int J Cancer* (2011) 129(8):2032–7. doi: 10.1002/ijc.25840

67. Caruana BT, Byrne FL. The nf-Kb signalling pathway regulates Glut6 expression in endometrial cancer. *Cell Signal* (2020) 73:109688. doi: 10.1016/j.cellsig.2020.109688

68. Wieman HL, Wofford JA, Rathmell JC. Cytokine stimulation promotes glucose uptake *Via* phosphatidylinositol-3 Kinase/Akt regulation of Glut1 activity and trafficking. *Mol Biol Cell* (2007) 18(4):1437–46. doi: 10.1091/mbc.e06-07-0593

69. Wahl H, Daudi S, Kshirsagar M, Griffith K, Tan L, Rhode J, et al. Expression of metabolically targeted biomarkers in endometrial carcinoma. *Gynecol Oncol* (2010) 116(1):21–7. doi: 10.1016/j.ygyno.2009.10.040

70. Dossus L, Lukanova A, Rinaldi S, Allen N, Cust AE, Becker S, et al. Hormonal, metabolic, and inflammatory profiles and endometrial cancer risk within the epic cohort–a factor analysis. *Am J Epidemiol* (2013) 177(8):787–99. doi: 10.1093/aje/kws309

71. Trabert B, Eldridge RC, Pfeiffer RM, Shiels MS, Kemp TJ, Guillemette C, et al. Prediagnostic circulating inflammation markers and endometrial cancer risk in the prostate, lung, colorectal and ovarian cancer (Plco) screening trial. *Int J Cancer* (2017) 140(3):600–10. doi: 10.1002/ijc.30478

72. Olsen JJ, Pohl S, Deshmukh A, Visweswaran M, Ward NC, Arfuso F, et al. The role of wnt signalling in angiogenesis. *Clin Biochem Rev* (2017) 38(3):131–42.

73. Sahoo SS, Lombard JM, Ius Y, O'Sullivan R, Wood LG, Nahar P, et al. Adipose-derived vegf-mtor signaling promotes endometrial hyperplasia and cancer: Implications for obese women. *Mol Cancer Res* (2018) 16(2):309–21. doi: 10.1158/1541-7786.Mcr-17-0466

74. Zhou Q, Wang F, Zhou K, Huang K, Zhu Q, Luo X, et al. Oncogenic role of Abhd5 in endometrial cancer. *Cancer Manag Res* (2019) 11:2139–50. doi: 10.2147/CMAR.S188648

75. Mori Y, Yamawaki K, Ishiguro T, Yoshihara K, Ueda H, Sato A, et al. Aldh-dependent glycolytic activation mediates stemness and paclitaxel resistance in patient-derived spheroid models of uterine endometrial cancer. *Stem Cell Rep* (2019) 13(4):730–46. doi: 10.1016/j.stemcr.2019.08.015

76. Gonzalez-Menendez P, Hevia D, Rodriguez-Garcia A, Mayo JC, Sainz RM. Regulation of glut transporters by flavonoids in androgen-sensitive and -insensitive prostate cancer cells. *Endocrinology* (2014) 155(9):3238–50. doi: 10.1210/en.2014-1260

77. Blaszczak W, Barczak W, Masternak J, Kopczyński P, Zhitkovich A, Rubiś B. Vitamin c as a modulator of the response to cancer therapy. *Molecules* (2019) 24 (3):453. doi: 10.3390/molecules24030453

78. Lee SY, Park JY. Glut1 variants for predicting prognosis after surgery in non-small cell lung cancer. *Ann Surg Oncol* (2018) 25(Suppl 3):948–49. doi: 10.1245/s10434-018-6847-1

79. Xiao H, Wang J, Yan W, Cui Y, Chen Z, Gao X, et al. Glut1 regulates cell glycolysis and proliferation in prostate cancer. *Prostate* (2018) 78(2):86–94. doi: 10.1002/pros.23448

80. Chen J, Cao L, Li Z, Li Y. Sirt1 promotes Glut1 expression and bladder cancer progression *Via* regulation of glucose uptake. *Hum Cell* (2019) 32(2):193–201. doi: 10.1007/s13577-019-00237-5

81. Yuan G, Zhao Y, Wu D, Gao C. Mir-150 up-regulates Glut1 and increases glycolysis in osteosarcoma cells. *Asian Pac J Cancer Prev* (2017) 18(4):1127–31. doi: 10.22034/apjcp.2017.18.4.1127

82. Li X, Ren H. Long noncoding rna Pvt1 promotes tumor cell proliferation, invasion, migration and inhibits apoptosis in oral squamous cell carcinoma by regulating Mir−150−5p/Glut−1. *Oncol Rep* (2020) 44(4):1524–38. doi: 10.3892/or.2020.7706

83. Xi J, Wang Y, Liu H. Glut-1 participates in the promotion of lncrna Casc9 in proliferation and metastasis of laryngeal carcinoma cells. *Gene* (2020) 726:144194. doi: 10.1016/j.gene.2019.144194

84. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, et al. Integrated genomic characterization of endometrial carcinoma. *Nature* (2013) 497 (7447):67–73. doi: 10.1038/nature12113

85. Urick ME, Bell DW. Clinical actionability of molecular targets in endometrial cancer. *Nat Rev Cancer* (2019) 19(9):510–21. doi: 10.1038/s41568-019-0177-x

86. Harris BRE, Zhang Y, Tao J, Shen R, Zhao X, Cleary MP, et al. Atm inhibitor Ku-55933 induces apoptosis and inhibits motility by blocking Glut1-mediated glucose uptake in aggressive cancer cells with sustained activation of akt. *FASEB J* (2021) 35(4):e21264. doi: 10.1096/fj.202001415RR

87. Wang W, Xiao ZD, Li X, Aziz KE, Gan B, Johnson RL, et al. Ampk modulates hippo pathway activity to regulate energy homeostasis. *Nat Cell Biol* (2015) 17(4):490–99. doi: 10.1038/ncb3113

88. Zhou J, Zhu J, Yu SJ, Ma HL, Chen J, Ding XF, et al. Sodium-glucose Co-Transporter-2 (Sglt-2) inhibition reduces glucose uptake to induce breast cancer cell growth arrest through Ampk/Mtor pathway. *BioMed Pharmacother* (2020) 132:110821. doi: 10.1016/j.biopha.2020.110821

89. Westin SN, Broaddus RR, Deng L, McCampbell A, Lu KH, Lacour RA, et al. Molecular clustering of endometrial carcinoma based on estrogen-induced gene expression. *Cancer Biol Ther* (2009) 8(22):2126–35. doi: 10.4161/cbt.8.22.9740

90. Folsom AR, Anderson KE, Sweeney C, Jacobs DRJr. Diabetes as a risk factor for death following endometrial cancer. *Gynecol Oncol* (2004) 94(3):740–45. doi: 10.1016/j.ygyno.2004.06.027

91. Wofford JA, Wieman HL, Jacobs SR, Zhao Y, Rathmell JC. Il-7 promotes Glut1 trafficking and glucose uptake *Via* Stat5-mediated activation of akt to support T-cell survival. *Blood* (2008) 111(4):2101–11. doi: 10.1182/blood-2007-06-096297

92. Dang CV, Kim JW. Convergence of cancer metabolism and immunity: An overview. *Biomol Ther (Seoul)* (2018) 26(1):4–9. doi: 10.4062/biomolther.2017.194

93. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. *Cell Metab* (2016) 23(1):27–47. doi: 10.1016/j.cmet.2015.12.006

94. McMillan EA, Ryu MJ, Diep CH, Mendiratta S, Clemenceau JR, Vaden RM, et al. Chemistry-first approach for nomination of personalized treatment in lung cancer. *Cell* (2018) 173(4):864–78.e29. doi: 10.1016/j.cell.2018.03.028

95. Ashton-Sager A, Paulino AF, Afify AM. Glut-1 is preferentially expressed in atypical endometrial hyperplasia and endometrial adenocarcinoma. *Appl Immunohistochem Mol Morphol* (2006) 14(2):187–92. doi: 10.1097/01.pai.0000162003.43334.c7

96. Liu Y, Zhao R, Chi S, Zhang W, Xiao C, Zhou X, et al. Ube2c is upregulated by estrogen and promotes epithelial-mesenchymal transition *Via* P53 in endometrial cancer. *Mol Cancer Res* (2020) 18(2):204–15. doi: 10.1158/1541-7786.Mcr-19-0561

97. Chen R, Zhang M, Liu W, Chen H, Cai T, Xiong H, et al. Estrogen affects the negative feedback loop of Ptenp1-Mir200c to inhibit pten expression in the development of endometrioid endometrial carcinoma. *Cell Death Dis* (2018) 10 (1):4. doi: 10.1038/s41419-018-1207-4

98. Chiu HC, Li CJ, Yiang GT, Tsai AP, Wu MY. Epithelial to mesenchymal transition and cell biology of molecular regulation in endometrial carcinogenesis. *J Clin Med* (2019) 8(4):439. doi: 10.3390/jcm8040439

99. Jung HY, Fattet L, Yang J. Molecular pathways: Linking tumor microenvironment to epithelial-mesenchymal transition in metastasis. *Clin Cancer Res* (2015) 21(5):962–68. doi: 10.1158/1078-0432.Ccr-13-3173

100. Schito L, Rey S. Hypoxic pathobiology of breast cancer metastasis. *Biochim Biophys Acta Rev Cancer* (2017) 1868(1):239–45. doi: 10.1016/j.bbcan.2017.05.004

101. Marín-Hernández Á, Gallardo-Pérez JC, Hernández-Reséndiz I, Del Mazo-Monsalvo I, Robledo-Cadena DX, Moreno-Sánchez R, et al. Hypoglycemia enhances epithelial-mesenchymal transition and invasiveness, and restrains the warburg phenotype, in hypoxic hela cell cultures and microspheroids. *J Cell Physiol* (2017) 232(6):1346–59. doi: 10.1002/jcp.25617

102. Zuo J, Wen J, Lei M, Wen M, Li S, Lv X, et al. Hypoxia promotes the invasion and metastasis of laryngeal cancer cells *Via* emt. *Med Oncol* (2016) 33 (2):15. doi: 10.1007/s12032-015-0716-6

103. Lee M, Jeon JY, Neugent ML, Kim JW, Yun M. 18f-fluorodeoxyglucose uptake on positron emission Tomography/Computed tomography is associated with metastasis and epithelial-mesenchymal transition in hepatocellular carcinoma. *Clin Exp Metastasis* (2017) 34(3-4):251–60. doi: 10.1007/s10585-017-9847-9

104. Starska K, Forma E, Jóźwiak P, Bryś M, Lewy-Trenda I, Brzezińska-Błaszczyk E, et al. Gene and protein expression of glucose transporter 1 and glucose transporter 3 in human laryngeal cancer-the relationship with regulatory hypoxia-inducible factor-1α expression, tumor invasiveness, and patient prognosis. *Tumour Biol* (2015) 36(4):2309–21. doi: 10.1007/s13277-014-2838-4

105. Semaan A, Munkarah AR, Arabi H, Bandyopadhyay S, Seward S, Kumar S, et al. Expression of glut-1 in epithelial ovarian carcinoma: Correlation with tumor cell proliferation, angiogenesis, survival and ability to predict optimal cytoreduction. *Gynecol Oncol* (2011) 121(1):181–86. doi: 10.1016/j.ygyno.2010.11.019

106. Xu Y, Zhou X, Zhang S, Nanding A, Xuan Q. Expression and prognostic value of glucose transporter 3 in diffuse Large b cell lymphoma. *Onco Targets Ther* (2022) 15:181–91. doi: 10.2147/ott.S338826

107. Chung AS, Ferrara N. Developmental and pathological angiogenesis. *Annu Rev Cell Dev Biol* (2011) 27:563–84. doi: 10.1146/annurev-cellbio-092910-154002

108. Lee DW, Chong GO, Lee YH, Hong DG, Cho YL, Jeong SY, et al. Role of suvmax and glut-1 expression in determining tumor aggressiveness in patients with clinical stage I endometrioid endometrial cancer. *Int J Gynecol Cancer* (2015) 25 (5):843–49. doi: 10.1097/IGC.0000000000000301

109. Kazi AA, Koos RD. Estrogen-induced activation of hypoxia-inducible factor-1alpha, vascular endothelial growth factor expression, and edema in the uterus are mediated by the phosphatidylinositol 3-Kinase/Akt pathway. *Endocrinology* (2007) 148(5):2363–74. doi: 10.1210/en.2006-1394

110. Berger AA, Dao F, Levine DA. Angiogenesis in endometrial carcinoma: Therapies and biomarkers, current options, and future perspectives. *Gynecol Oncol* (2021) 160(3):844–50. doi: 10.1016/j.ygyno.2020.12.016

111. Cao D, Zhou H, Zhao J, Jin L, Yu W, Yan H, et al. Pgc-1α integrates glucose metabolism and angiogenesis in multiple myeloma cells by regulating vegf and glut-4. *Oncol Rep* (2014) 31(3):1205–10. doi: 10.3892/or.2014.2974

112. Lee H, Lee HJ, Bae IJ, Kim JJ, Kim SH. Inhibition of Stat3/Vegf/Cdk2 axis signaling is critically involved in the antiangiogenic and apoptotic effects of arsenic herbal mixture pros in non-small lung cancer cells. *Oncotarget* (2017) 8 (60):101771–83. doi: 10.18632/oncotarget.21973

113. Salvesen HB, Iversen OE, Akslen LA. Prognostic significance of angiogenesis and ki-67, P53, and P21 expression: A population-based endometrial carcinoma study. *J Clin Oncol* (1999) 17(5):1382–90. doi: 10.1200/jco.1999.17.5.1382

114. Mahecha AM, Wang H. The influence of vascular endothelial growth factor-a and matrix metalloproteinase-2 and -9 in angiogenesis, metastasis, and prognosis of endometrial cancer. *Onco Targets Ther* (2017) 10:4617–24. doi: 10.2147/ott.S132558

115. Molina F, Del Moral ML, Peinado M, Rus A. Angiogenesis is vegf-independent in the aged striatum of Male rats exposed to acute hypoxia. *Biogerontology* (2017) 18(5):759–68. doi: 10.1007/s10522-017-9709-5

116. Lee II, Maniar K, Lydon JP, Kim JJ. Akt regulates progesterone receptor b-dependent transcription and angiogenesis in endometrial cancer cells. *Oncogene* (2016) 35(39):5191–201. doi: 10.1038/onc.2016.56

117. Classen F, Kranz P, Riffkin H, Pompsch M, Wolf A, Göpelt K, et al. Autophagy induced by ionizing radiation promotes cell death over survival in human colorectal cancer cells. *Exp Cell Res* (2019) 374(1):29–37. doi: 10.1016/j.yexcr.2018.11.004

118. Taylor MA, Das BC, Ray SK. Targeting autophagy for combating chemoresistance and radioresistance in glioblastoma. *Apoptosis* (2018) 23(11-12):563–75. doi: 10.1007/s10495-018-1480-9

119. Lin W, Yin CY, Yu Q, Zhou SH, Chai L, Fan J, et al. Expression of glucose transporter-1, hypoxia inducible factor-1α and beclin-1 in head and neck cancer and their implication. *Int J Clin Exp Pathol* (2018) 11(7):3708–17.

120. Chang L, Graham PH, Hao J, Ni J, Bucci J, Cozzi PJ, et al. Pi3k/Akt/Mtor pathway inhibitors enhance radiosensitivity in radioresistant prostate cancer cells through inducing apoptosis, reducing autophagy, suppressing nhej and hr repair pathways. *Cell Death Dis* (2014) 5(10):e1437. doi: 10.1038/cddis.2014.415

121. Dai LB, Yu Q, Zhou SH, Bao YY, Zhong JT, Shen LF, et al. Effect of combination of curcumin and glut-1 as-odn on radiosensitivity of laryngeal carcinoma through regulating autophagy. *Head Neck* (2020) 42(9):2287–97. doi: 10.1002/hed.26180

122. Zhong JT, Yu Q, Zhou SH, Yu E, Bao YY, Lu ZJ, et al. Glut-1 sirna enhances radiosensitization of laryngeal cancer stem cells *Via* enhanced DNA damage, cell cycle redistribution, and promotion of apoptosis in vitro and in vivo. *Onco Targets Ther* (2019) 12:9129–42. doi: 10.2147/ott.S221423

123. Wang H, Zhang Y, Yu W, Xue Y, Xiao L, Xu H. Hypoxia imaging and biological evaluation of the radiosensitizing effect of oleanolic acid. *BioMed Res Int* (2018) 2018:2694679. doi: 10.1155/2018/2694679

124. Sorolla MA, Parisi E, Sorolla A. Determinants of sensitivity to radiotherapy in endometrial cancer. *Cancers (Basel)* (2020) 12(7):1906. doi: 10.3390/cancers12071906

125. Dai LB, Zhong JT, Shen LF, Zhou SH, Lu ZJ, Bao YY, et al. Radiosensitizing effects of curcumin alone or combined with Glut1 sirna on laryngeal carcinoma cells through ampk pathway-induced autophagy. *J Cell Mol Med* (2021). doi: 10.1111/jcmm.16450

126. Alzahrani AS. Pi3k/Akt/Mtor inhibitors in cancer: At the bench and bedside. *Semin Cancer Biol* (2019) 59:125–32. doi: 10.1016/j.semcancer.2019.07.009

127. Wang E, Sorolla A. Sensitizing endometrial cancer to ionizing radiation by multi-tyrosine kinase inhibition. *J Gynecol Oncol* (2020) 31(3):e29. doi: 10.3802/jgo.2020.31.e29

128. Ye Y, Hu Q, Chen H, Liang K, Yuan Y, Xiang Y, et al. Characterization of hypoxia-associated molecular features to aid hypoxia-targeted therapy. *Nat Metab* (2019) 1(4):431–44. doi: 10.1038/s42255-019-0045-8

129. Gunnink LK, Alabi OD, Kuiper BD, Gunnink SM, Schuiteman SJ, Strohbehn LE, et al. Curcumin directly inhibits the transport activity of Glut1. *Biochimie* (2016) 125:179–85. doi: 10.1016/j.biochi.2016.03.014

130. Perrone D, Ardito F, Giannatempo G, Dioguardi M, Troiano G, Lo Russo L, et al. Biological and therapeutic activities, and anticancer properties of curcumin. *Exp Ther Med* (2015) 10(5):1615–23. doi: 10.3892/etm.2015.2749

131. Feng T, Wei Y, Lee RJ, Zhao L. Liposomal curcumin and its application in cancer. *Int J Nanomedicine* (2017) 12:6027–44. doi: 10.2147/ijn.S132434

132. Assad DX, Borges GA, Avelino SR, Guerra ENS. Additive cytotoxic effects of radiation and mtor inhibitors in a cervical cancer cell line. *Pathol Res Pract* (2018) 214(2):259–62. doi: 10.1016/j.prp.2017.10.019

133. Kondo C, Shibata K, Terauchi M, Kajiyama H, Ino K, Nomura S, et al. A novel role for placental leucine aminopeptidase (P-lap) as a determinant of chemoresistance in endometrial carcinoma cells. *Int J Cancer* (2006) 118 (6):1390–4. doi: 10.1002/ijc.21509

134. Bigatto V, De Bacco F, Casanova E, Reato G, Lanzetti L , Isella C, et al. Tnf-A promotes invasive growth through the met signaling pathway. *Mol Oncol* (2015) 9(2):377–88. doi: 10.1016/j.molonc.2014.09.002

135. Borghi C, Indraccolo U, Scutiero G, Iannone P, Martinello R, Greco P, et al. Biomolecular basis related to inflammation in the pathogenesis of endometrial cancer. *Eur Rev Med Pharmacol Sci* (2018) 22(19):6294–99. doi: 10.26355/eurrev_201810_16038

136. Shi Y, Liu S, Ahmad S, Gao Q. Targeting key transporters in tumor glycolysis as a novel anticancer strategy. *Curr Top Med Chem* (2018) 18(6):454–66. doi: 10.2174/1568026618666180523105234

137. Kapoor K, Finer-Moore JS, Pedersen BP, Caboni L, Waight A, Hillig RC, et al. Mechanism of inhibition of human glucose transporter Glut1 is conserved between cytochalasin b and phenylalanine amides. *Proc Natl Acad Sci U.S.A.* (2016) 113(17):4711–16. doi: 10.1073/pnas.1603735113

138. Wang J, Liu R, Zhao Y, Ma Z, Sang Z, Wen Z, et al. Novel microcrystal formulations of sorafenib facilitate a long-acting antitumor effect and relieve treatment side effects as observed with fundus microcirculation imaging. *Front Oncol* (2021) 11:743055. doi: 10.3389/fonc.2021.743055

139. Yang H, Zhang MZ, Sun HW, Chai YT, Li X, Jiang Q, et al. A novel microcrystalline bay-876 formulation achieves long-acting antitumor activity against aerobic glycolysis and proliferation of hepatocellular carcinoma. *Front Oncol* (2021) 11:783194. doi: 10.3389/fonc.2021.783194

140. Wu L, Meng F, Dong L, Block CJ, Mitchell AV, Wu J, et al. Disulfiram and Bkm120 in combination with chemotherapy impede tumor progression and delay tumor recurrence in tumor initiating cell-rich tnbc. *Sci Rep* (2019) 9(1):236. doi: 10.1038/s41598-018-35619-6

141. Januchowski R, Wojtowicz K, Sterzyńska K, Sosińska P, Andrzejewska M, Zawierucha P, et al. Inhibition of Aldh1a1 activity decreases expression of drug transporters and reduces chemotherapy resistance in ovarian cancer cell lines. *Int J Biochem Cell Biol* (2016) 78:248–59. doi: 10.1016/j.biocel.2016.07.017

142. Lu C, Li X, Ren Y, Zhang X. Disulfiram: A novel repurposed drug for cancer therapy. *Cancer Chemother Pharmacol* (2021) 87(2):159–72. doi: 10.1007/s00280-020-04216-8

143. Romero I, Rubio MJ, Medina M, Matias-Guiu X, Santacana M, Schoenenberger JA, et al. An olaparib window-of-Opportunity trial in patients with early-stage endometrial carcinoma: Polen study. *Gynecol Oncol* (2020) 159 (3):721–31. doi: 10.1016/j.ygyno.2020.09.013

144. Zhong Q, Hu Z, Li Q, Yi T, Li J, Yang H. Cyclin D1 silencing impairs DNA double strand break repair, sensitizes Brca1 wildtype ovarian cancer cells to olaparib. *Gynecol Oncol* (2019) 152(1):157–65. doi: 10.1016/j.ygyno.2018.10.027

145. Bundred N, Gardovskis J, Jaskiewicz J, Eglitis J, Paramonov V, McCormack P, et al. Evaluation of the pharmacodynamics and pharmacokinetics of the parp inhibitor olaparib: A phase I multicentre trial in patients scheduled for elective breast cancer surgery. *Invest New Drugs* (2013) 31(4):949–58. doi: 10.1007/s10637-012-9922-7

146. Li N, Feng L, Liu H, Wang J, Kasembeli M, Tran MK, et al. Parp inhibition suppresses growth of egfr-mutant cancers by targeting nuclear Pkm2. *Cell Rep* (2016) 15(4):843–56. doi: 10.1016/j.celrep.2016.03.070

147. Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, et al. Adjuvant olaparib for patients with Brca1- or Brca2-mutated breast cancer. *N Engl J Med* (2021) 384(25):2394–405. doi: 10.1056/NEJMoa2105215

148. Tilekar K, Upadhyay N, Iancu CV, Pokrovsky V, Choe JY, Ramaa CS. Power of two: Combination of therapeutic approaches involving glucose transporter (Glut) inhibitors to combat cancer. *Biochim Biophys Acta Rev Cancer* (2020) 1874(2):188457. doi: 10.1016/j.bbcan.2020.188457

149. Luengo A, Gui DY, Vander Heiden MG. Targeting metabolism for cancer therapy. *Cell Chem Biol* (2017) 24(9):1161–80. doi: 10.1016/j.chembiol.2017.08.028

150. Niccoli S, Boreham DR, Phenix CP, Lees SJ. Non-radioactive 2-Deoxy-2-Fluoro-D-Glucose inhibits glucose uptake in xenograft tumours and sensitizes hela cells to doxorubicin in vitro. *PLoS One* (2017) 12(11):e0187584. doi: 10.1371/journal.pone.0187584

151. Asgharzadeh MR, Barar J, Pourseif MM, Eskandani M, Jafari Niya M, Mashayekhi MR, et al. Molecular machineries of ph dysregulation in tumor microenvironment: Potential targets for cancer therapy. *Bioimpacts* (2017) 7 (2):115–33. doi: 10.15171/bi.2017.15

152. Amith SR, Wilkinson JM, Fliegel L. Kr-33028, a potent inhibitor of the Na (+)/H(+) exchanger Nhe1, suppresses metastatic potential of triple-negative breast cancer cells. *Biochem Pharmacol* (2016) 118:31–9. doi: 10.1016/j.bcp.2016.08.010

153. Harguindey S, Arranz JL, Polo Orozco JD, Rauch C, Fais S, Cardone RA, et al. Cariporide and other new and powerful Nhe1 inhibitors as potentially selective anticancer drugs–an integral Molecular/Biochemical/Metabolic/Clinical approach after one hundred years of cancer research. *J Transl Med* (2013) 11:282. doi: 10.1186/1479-5876-11-282

154. Gwak H, Haegeman G, Tsang BK, Song YS. Cancer-specific interruption of glucose metabolism by resveratrol is mediated through inhibition of Akt/Glut1 axis in ovarian cancer cells. *Mol Carcinog* (2015) 54(12):1529–40. doi: 10.1002/mc.22227

155. León D, Uribe E, Zambrano A, Salas M. Implications of resveratrol on glucose uptake and metabolism. *Molecules* (2017) 22(3):398. doi: 10.3390/molecules22030398

156. Skrobuk P, von Kraemer S, Semenova MM, Zitting A, Koistinen HA. Acute exposure to resveratrol inhibits ampk activity in human skeletal muscle cells. *Diabetologia* (2012) 55(11):3051–60. doi: 10.1007/s00125-012-2691-1

157. Bahrami A, Ayen E, Razi M, Behfar M. Effects of atorvastatin and resveratrol against the experimental endometriosis; evidence for glucose and monocarboxylate transporters, neoangiogenesis. *Life Sci* (2021) 272:119230. doi: 10.1016/j.lfs.2021.119230

158. Rauf A, Imran M, Butt MS, Nadeem M, Peters DG, Mubarak MS. Resveratrol as an anti-cancer agent: A review. *Crit Rev Food Sci Nutr* (2018) 58 (9):1428–47. doi: 10.1080/10408398.2016.1263597

159. Rezk YA, Balulad SS, Keller RS, Bennett JA. Use of resveratrol to improve the effectiveness of cisplatin and doxorubicin: Study in human gynecologic cancer cell lines and in rodent heart. *Am J Obstet Gynecol* (2006) 194(5):e23–6. doi: 10.1016/j.ajog.2005.11.030

160. Shaito A, Posadino AM, Younes N, Hasan H, Halabi S, Alhababi D, et al. Potential adverse effects of resveratrol: A literature review. *Int J Mol Sci* (2020) 21 (6):2084. doi: 10.3390/ijms21062084

161. Granchi C, Fortunato S, Minutolo F. Anticancer agents interacting with membrane glucose transporters. *Medchemcomm* (2016) 7(9):1716–29. doi: 10.1039/c6md00287k

162. Rastogi S, Banerjee S, Chellappan S, Simon GR. Glut-1 antibodies induce growth arrest and apoptosis in human cancer cell lines. *Cancer Lett* (2007) 257 (2):244–51. doi: 10.1016/j.canlet.2007.07.021

163. Wan Mohd Tajuddin WNB, Lajis NH, Abas F, Othman I, Naidu R. Mechanistic understanding of curcumin's therapeutic effects in lung cancer. *Nutrients* (2019) 11(12):2989. doi: 10.3390/nu11122989

164. McBrayer SK, Cheng JC, Singhal S, Krett NL, Rosen ST, Shanmugam M. Multiple myeloma exhibits novel dependence on Glut4, Glut8, and Glut11: Implications for glucose transporter-directed therapy. *Blood* (2012) 119 (20):4686–97. doi: 10.1182/blood-2011-09-377846

165. Wei C, Bajpai R, Sharma H, Heitmeier M, Jain AD, Matulis SM, et al. Development of Glut4-selective antagonists for multiple myeloma therapy. *Eur J Med Chem* (2017) 139:573–86. doi: 10.1016/j.ejmech.2017.08.029

166. Vermunt MAC, van der Heijden LT, Hendrikx J, Schinkel AH, de Weger VA, van der Putten E, et al. Pharmacokinetics of docetaxel and ritonavir after oral administration of Modradoc006/R in patients with prostate cancer versus patients with other advanced solid tumours. *Cancer Chemother Pharmacol* (2021) 87 (6):855–69. doi: 10.1007/s00280-021-04259-5

167. Takahashi S, Karayama M, Takahashi M, Watanabe J, Minami H, Yamamoto N, et al. Pharmacokinetics, safety, and efficacy of trastuzumab deruxtecan with concomitant ritonavir or itraconazole in patients with Her2-expressing advanced solid tumors. *Clin Cancer Res* (2021) 27(21):5771–80. doi: 10.1158/1078-0432.Ccr-21-1560

168. Böttger F, Vallés-Martí A, Cahn L, Jimenez CR. High-dose intravenous vitamin c, a promising multi-targeting agent in the treatment of cancer. *J Exp Clin Cancer Res* (2021) 40(1):343. doi: 10.1186/s13046-021-02134-y

169. Aguilera O, Muñoz-Sagastibelza M, Torrejón B, Borrero-Palacios A, Del Puerto-Nevado L, Martínez-Useros J, et al. Vitamin c uncouples the warburg metabolic switch in kras mutant colon cancer. *Oncotarget* (2016) 7(30):47954–65. doi: 10.18632/oncotarget.10087

170. Wu L, Li X, Li Z, Cheng Y, Wu F, Lv C, et al. Htra serine proteases in cancers: A target of interest for cancer therapy. *BioMed Pharmacother* (2021) 139:111603. doi: 10.1016/j.biopha.2021.111603

171. Edey KA, Rundle S, Hickey M. Hormone replacement therapy for women previously treated for endometrial cancer. *Cochrane Database Syst Rev* (2018) 5(5): Cd008830. doi: 10.1002/14651858.CD008830.pub3

172. Garzon S, Uccella S, Zorzato PC, Bosco M, Franchi MP, Student V, et al. Fertility-sparing management for endometrial cancer: Review of the literature. *Minerva Med* (2021) 112(1):55–69. doi: 10.23736/s0026-4806.20.07072-x

173. Gu C, Zhang Z, Yu Y, Liu Y, Zhao F, Yin L, et al. Inhibiting the Pi3k/Akt pathway reversed progestin resistance in endometrial cancer. *Cancer Sci* (2011) 102 (3):557–64. doi: 10.1111/j.1349-7006.2010.01829.x

174. Lee TY, Martinez-Outschoorn UE, Schilder RJ, Kim CH, Richard SD, Rosenblum NG, et al. Metformin as a therapeutic target in endometrial cancers. *Front Oncol* (2018) 8:341. doi: 10.3389/fonc.2018.00341

175. Meireles CG, Pereira SA, Valadares LP, Rêgo DF, Simeoni LA, Guerra ENS, et al. Effects of metformin on endometrial cancer: Systematic review and meta-analysis. *Gynecol Oncol* (2017) 147(1):167–80. doi: 10.1016/j.ygyno.2017.07.120

176. Hillerns PI, Zu Y, Fu YJ, Wink M. Binding of phytoestrogens to rat uterine estrogen receptors and human sex hormone-binding globulins. *Z Naturforsch C J Biosci* (2005) 60(7-8):649–56. doi: 10.1515/znc-2005-7-823

177. Granchi C, Tuccinardi T, Minutolo F. Design, synthesis, and evaluation of glut inhibitors. *Methods Mol Biol* (2018) 1713:93–108. doi: 10.1007/978-1-4939-7507-5_8

178. Dwarakanath BS, Singh D, Banerji AK, Sarin R, Venkataramana NK, Jalali R, et al. Clinical studies for improving radiotherapy with 2-Deoxy-D-Glucose: Present status and future prospects. *J Cancer Res Ther* (2009) 5 Suppl 1:S21–6. doi: 10.4103/0973-1482.55136

179. Singh D, Banerji AK, Dwarakanath BS, Tripathi RP, Gupta JP, Mathew TL, et al. Optimizing cancer radiotherapy with 2-Deoxy-D-Glucose dose escalation studies in patients with glioblastoma multiforme. *Strahlenther Onkol* (2005) 181 (8):507–14. doi: 10.1007/s00066-005-1320-z

180. Batra H, Pawar S, Bahl D. Curcumin in combination with anti-cancer drugs: A nanomedicine review. *Pharmacol Res* (2019) 139:91–105. doi: 10.1016/j.phrs.2018.11.005

181. Mitsuhashi A, Sato Y, Kiyokawa T, Koshizaka M, Hanaoka H, Shozu M. Phase ii study of medroxyprogesterone acetate plus metformin as a fertility-sparing treatment for atypical endometrial hyperplasia and endometrial cancer. *Ann Oncol* (2016) 27(2):262–6. doi: 10.1093/annonc/mdv539

182. Yang BY, Gulinazi Y, Du Y, Ning CC, Cheng YL, Shan WW, et al. Metformin plus megestrol acetate compared with megestrol acetate alone as fertility-sparing treatment in patients with atypical endometrial hyperplasia and well-differentiated endometrial cancer: A randomised controlled trial. *Bjog* (2020) 127(7):848–57. doi: 10.1111/1471-0528.16108

183. Wilson PM, Yang D, Azuma M, Shi MM, Danenberg KD, Lebwohl D, et al. Intratumoral expression profiling of genes involved in angiogenesis in colorectal cancer patients treated with chemotherapy plus the vegfr inhibitor Ptk787/Zk 222584 (Vatalanib). *Pharmacogenomics J* (2013) 13(5):410–6. doi: 10.1038/tpj.2012.23

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EDITED BY
Tea Lanisnik Rizner,
University of Ljubljana, Slovenia

REVIEWED BY
Matthew Schlumbrecht,
University of Miami Health System, United States
Rita Trozzi,
Agostino Gemelli University Polyclinic (IRCCS), Italy
Elena Teodorico,
Agostino Gemelli University Polyclinic (IRCCS), Italy

*CORRESPONDENCE
Tjalling Bosse
t.bosse@lumc.nl

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The evolving role of morphology in endometrial cancer diagnostics: From histopathology and molecular testing towards integrative data analysis by deep learning

Sarah Fremond[1], Viktor Hendrik Koelzer[2], Nanda Horeweg[3] and Tjalling Bosse[1]*

[1]Department of Pathology, Leiden University Medical Center (LUMC), Leiden, Netherlands, [2]Department of Pathology and Molecular Pathology, University Hospital and University of Zürich, Zürich, Switzerland, [3]Department of Radiotherapy, Leiden University Medical Center, Leiden, Netherlands

Endometrial cancer (EC) diagnostics is evolving into a system in which molecular aspects are increasingly important. The traditional histological subtype-driven classification has shifted to a molecular-based classification that stratifies EC into *DNA polymerase epsilon* mutated (*POLE*mut), mismatch repair deficient (MMRd), and p53 abnormal (p53abn), and the remaining EC as no specific molecular profile (NSMP). The molecular EC classification has been implemented in the World Health Organization 2020 classification and the 2021 European treatment guidelines, as it serves as a better basis for patient management. As a result, the integration of the molecular class with histopathological variables has become a critical focus of recent EC research. Pathologists have observed and described several morphological characteristics in association with specific genomic alterations, but these appear insufficient to accurately classify patients according to molecular subgroups. This requires pathologists to rely on molecular ancillary tests in routine workup. In this new era, it has become increasingly challenging to assign clinically relevant weights to histological and molecular features on an individual patient basis. Deep learning (DL) technology opens new options for the integrative analysis of multi-modal image and molecular datasets with clinical outcomes. Proof-of-concept studies in other cancers showed promising accuracy in predicting molecular alterations from H&E-stained tumor slide images. This suggests that some morphological characteristics that are associated with molecular alterations could be identified in EC, too, expanding the current understanding of the molecular-driven EC classification. Here in this review, we report the morphological characteristics of the molecular EC classification currently identified in the literature. Given the new challenges in EC diagnostics, this review discusses, therefore, the potential supportive role that DL could have, by providing an outlook on all

relevant studies using DL on histopathology images in various cancer types with a focus on EC. Finally, we touch upon how DL might shape the management of future EC patients.

KEYWORDS

endometrial carcinoma, tumour morphology, computer vision, deep learning, molecular classification, phenotype, whole slide image, histopathology image

Introduction

The incorporation of the molecular endometrial cancer (EC) classification in the fifth edition of the World Health Organization (WHO) classification of female genital tumors and the 2021 European treatment guidelines (1, 2) has marked a new era in EC diagnostics. This moved the field farther away from the classic dualistic model proposed by Bockman in 1983 (3), who divided endometrial carcinomas into type I and type II cancers. Type I EC is endometrioid and estrogen driven and can be graded using the International Federation of Gynaecology and Obstetrics (FIGO) grading system (4). Type II EC includes a variety of non-endometrioid histological subtypes, such as uterine serous carcinoma, clear cell carcinoma, mixed carcinomas, un-/dedifferentiated carcinomas, and uterine carcinosarcomas. The new molecular EC classification that is now recommended by the WHO (1, 2) completely changes the diagnostic perspective by placing histological subtype secondary to molecular class. It utilizes surrogate markers paralleling the four molecular classes originally described by The Cancer Genome Atlas (TCGA) (5). First, targeted sequencing (Sanger or panel next-generation sequencing, NGS) of exons 9–14 of *DNA polymerase epsilon* (*POLE*) can identify *POLE*-mutated (*POLE*mut) EC. Second, mismatch repair-deficient (MMRd) EC is defined by loss of expression of one of the mismatch repair proteins (MLH1, PMS2, MSH6, and MSH2) by immunohistochemistry (IHC). Third, p53 IHC is performed to identify EC with abnormal p53 expression patterns (p53abn) (6, 7). Finally, EC without a pathogenic *POLE* variant, with retained MMR protein expression, and wild-type p53 IHC, is classified as "no specific molecular profile" (NSMP) EC. Studies on EC with more than one molecular alteration, commonly referred to as "multiple-classifiers," have served to identify the order by which these tests should be performed (8). It has resulted in the EC diagnostic algorithm endorsed by the WHO 2020 classification that first assesses *POLE* status regardless of other markers, then MMR, and finally p53 (9) (Figure 1).

The molecular classification resolves one of the main issues of the histology-driven EC classification, which is the high level of interobserver variability (10). Particularly high-grade and non-endometrioid histological subtypes are only moderately reproducible (11), which provides a poor basis for patient management (12). The recent paradigm shift in EC diagnostics follows preceding developments in surgical pathology, in which a series of technological breakthroughs such as immunohistochemistry and molecular testing have continually improved diagnostic accuracy (13).

The molecular EC classification has also shown a prognostic value across cohorts of different risk populations and is predictive of response to treatment, specifically in p53abn EC, which has a poor outcome and may benefit from addition of adjuvant chemotherapy (14), and in *POLE*mut EC, which has an excellent outcome regardless of adjuvant treatment, whereas MMRd and NSMP EC have intermediate prognoses (5, 14–20). This has been the rationale for adapting the clinical risk stratification system of EC patients (21) wherein it is encouraged to apply the molecular classification in the management of EC, especially high-risk EC (1, 2); ongoing and new trials such as PORTEC-4a (22) and RAINBO (23) exploit the molecular classification as a basis for targeted adjuvant therapy (Figure 1) (24). Consequently, the gynecological oncology community has started to utilize the molecular classification; however, the current risk stratification system does not clearly indicate which of the histological or molecular variables are most clinically relevant, or leverage the combination of both.

New technological breakthroughs in pathology are now driving progress in cancer diagnostics. Since the emergence of convolutional neural networks in 2012 (25), deep learning (DL) models have continuously demonstrated their high accuracy for the classification of medical (26) and non-medical image datasets (27). This was followed by the start of a digital revolution in pathology, wherein state-of-the-art DL models from the computer vision community were used on digital histopathology slides. Hematoxylin and eosin (H&E) staining procedure is the most common in cancer diagnostics, and large, well-characterized retrospective cohorts and clinical trial sets are available, enabling the collection of large-scale histopathology imaging datasets to train state-of-the-art DL models. A number of proof-of-concept papers showed the potential of DL models to aid the diagnosis and molecular classification of cancers (28–55) or predicting patient prognosis (56–60), by recognizing

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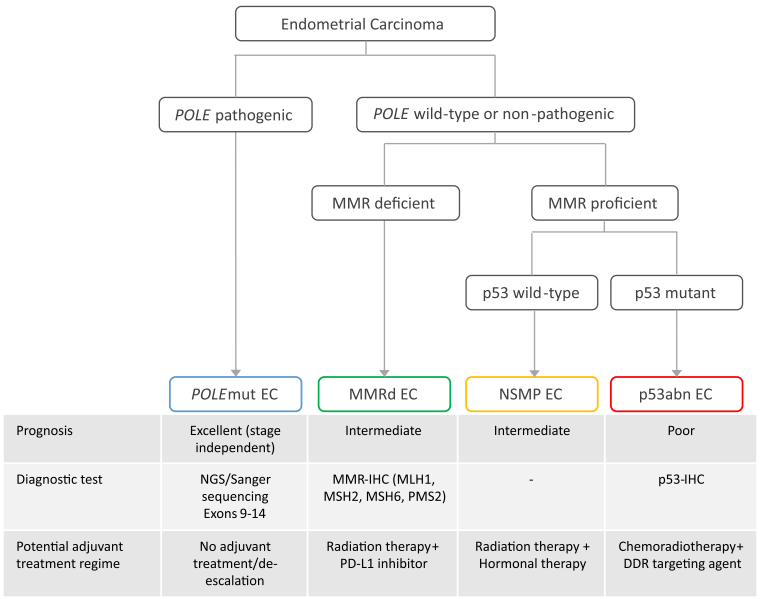

	POLEmut EC	MMRd EC	NSMP EC	p53abn EC
Prognosis	Excellent (stage independent)	Intermediate	Intermediate	Poor
Diagnostic test	NGS/Sanger sequencing Exons 9-14	MMR-IHC (MLH1, MSH2, MSH6, PMS2)	-	p53-IHC
Potential adjuvant treatment regime	No adjuvant treatment/de-escalation	Radiation therapy+ PD-L1 inhibitor	Radiation therapy + Hormonal therapy	Chemoradiotherapy+ DDR targeting agent

FIGURE 1
The diagnostic algorithm of the molecular classification of endometrial cancer, associated prognosis, diagnostic test, and potential adjuvant treatment regime. EC, endometrial cancer; NGS, panel next-generation sequencing; *POLE*mut, polymerase epsilon mutated; MMRd, mismatch repair deficient; NSMP, no specific molecular profile; p53abn, p53 abnormal; DDR, DNA damage response; PD-L1, programmed death ligand.

phenotypes on H&E-stained tumor slide images. Image-based DL models have been frequently trained onto colorectal cancer (29–37) and breast cancer (35, 38–44). However, in EC, only two studies (53, 54) so far have been published using DL for predicting one to various EC molecular alterations or gene mutations from publicly available datasets. They have obtained promising performance; however, the size of the dataset and application to a few non-endometrioid histological EC subtypes limit the generalizability of the findings. Furthermore, in these studies, DL models have not been trained to predict the newly established four-class molecular classification in EC diagnostics. Opportunities for future image-based DL models to impact EC diagnostics and thus guide clinical management decisions include the following: improve EC diagnostic classification by serving as a pre-screening tool to prioritize molecular testing, refine EC risk stratification by identifying morphological features with prognostic relevance, and predict patient outcome or even response to treatment.

In the light of the redefinition of EC on the basis of molecular features, we here provide a concise summary of the histopathological features associated with the four molecular classes (Table 1). These morpho-molecular correlates may serve to explore the feasibility of histology-directed molecular testing, particularly in low-income countries, and deepen our understanding of the underlying biological processes. We also present possible avenues by which image-based DL may be able to support these objectives, by discussing the landmark studies that have used DL onto histopathology slide images in EC and other cancers, which illustrates how these novel DL applications may impact the field of EC diagnostics in the future (Figure 2).

Morpho-molecular correlates of the current EC classification

POLE-mutated endometrial cancers

Pathogenic mutations in the exonuclease domain of *DNA polymerase epsilon* (*POLE*) in EC, *POLE*mut EC, were first described by Church et al. (61) and quickly thereafter by the TCGA (5). Mutations in *POLE* result in a defective proofreading domain during DNA leading-strand replication, yielding a very high mutation burden and increased neoantigen load. In these original publications, only a limited number of non-endometrioid EC were tested, and these did not carry *POLE* mutations. This led to the assumption that *POLE* mutations could only occur in endometrioid-type EC. However, subsequent larger studies challenged this idea by showing that *POLE* mutations can be identified in non-endometrioid carcinomas, including uterine carcinosarcomas, serous carcinomas, clear cell carcinomas, and un-/dedifferentiated carcinomas, albeit in low frequencies (14, 62–65). A search for a *POLE*mut EC-specific phenotypic trait resulted in the identification of specific morphological features (Figure 3): first, approximately two-thirds of *POLE*mut EC show at least 50% solid growth (also referred to as FIGO grade 3) (66, 67),

TABLE 1 Summary of the histopathological and immunohistochemical features correlated with the molecular endometrial cancer (EC) classification, dividing EC into *POLE*-mutated (*POLE*mut) EC, mismatch repair deficient (MMRd) EC, p53 abnormal (p53abn) EC, and non-specific molecular profile (NSMP) EC.

	*POLE*mut EC	MMRd EC	p53abn EC	NSMP EC
Prototypical histopathological features				
Glands with smooth luminal borders	++	+	–	+++
Glands with hobnailing (ragged luminal surface)	–	+	+++	–
Solid growth (at least 50%)	+++	++	+	+
Squamous differentiation (including morulae)	+	+	–	+++
Nuclear atypia	++	+	+++	+
Tumor giant cells	+++	–	+	–
Peri-tumoral and intra-epithelial infiltrate of lymphocytes	+++	++	–	–
Tertiary lymphoid structures (TLS)	+++	++	+	+
Microcystic elongated and fragmented (MELF)	–	+	–	++
Lymphovascular space invasion (LVSI)	+	++	+	+
Immunohistochemical features				
Abnormal MMR staining	+	+++	–	–
Abnormal p53 staining	+	+	+++	–

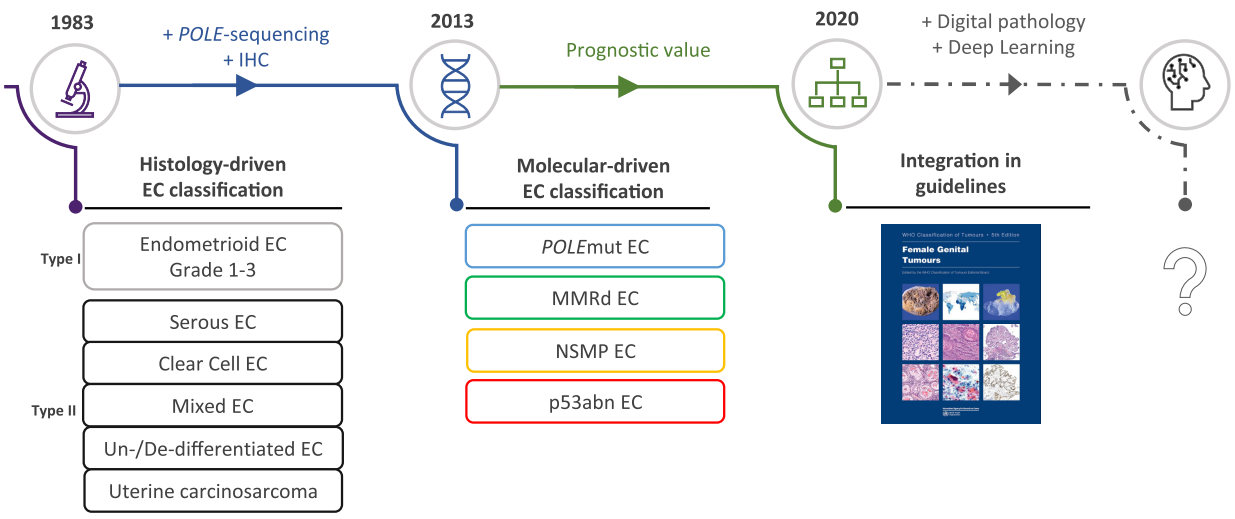

FIGURE 2
The evolving role of morphology in endometrial cancer diagnostics. EC, endometrial cancer; *POLE*mut, polymerase epsilon mutated; MMRd, mismatch repair deficient; NSMP, no specific molecular profile; p53abn, p53 abnormal.

and the glandular component, if present, usually consists of glands with smooth luminal borders without hobnailing (66); second, hyperchromatic (multi-nucleated) tumor giant cells scattered throughout the solid sheets of tumor cells have been described as a recurring feature (66, 68); third, a dense peri-tumoral and intra-epithelial infiltrate of lymphocytes is frequently noted, likely the phenotypic response of its high mutational load (66, 67, 69–71); finally, a more recent addition to these features is the presence of (often numerous) tertiary lymphoid structures (TLS) within the myometrial wall of *POLE*mut EC (72–74).

To date, there is no single immunohistochemical stain that can serve to identify *POLE*mut EC, and only few studies have studied specific markers (75). Among the IHC stains commonly used in diagnostics, abnormal staining for MMR proteins is present in about 20% of *POLE*mut EC (72). Mutant-type abnormal p53 staining can be identified in 12%–30% of *POLE*mut EC (5, 7, 8), but no specific morphological substrate has been detected in this subset of cases (8). Among the *POLE*mut EC with secondary p53 abnormality, subclonal/regional mutant-type overexpression of p53 is a relatively

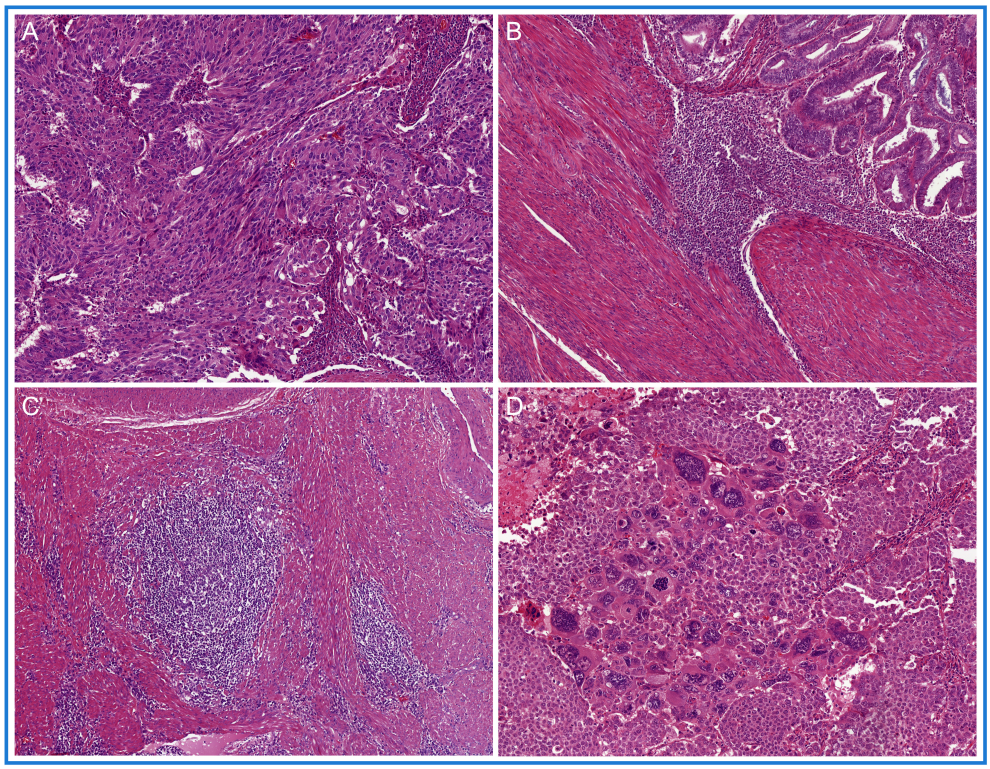

FIGURE 3
A selection of prototypical morphological features found in *POLE*-mutated endometrial cancer (*POLE*mut EC): **(A)** at least 50% solid growth; **(B)** hyperchromatic tumor giant cells; **(C)** a dense peri-tumoral and intra-epithelial infiltrate of lymphocytes; and **(D)** tertiary lymphoid structures (TLS).

common finding (7). Sequencing of the exonuclease domain of *POLE* thus remains required to accurately identify *POLE*mut EC, since morphology and adjunct studies alone are insufficient.

Mismatch repair-deficient endometrial cancers

Damage in the DNA mismatch repair (MMR) pathway leaves unrepaired post-DNA replication errors. Thus, the phenotype of MMR-deficient EC (MMRd EC) much like *POLE*mut EC is most likely shaped by the biological consequences of a high mutational load, leading to a similar morphological representation (Figure 4). In fact, several studies have described the abundance of stromal and intra-epithelial lymphocytes and, more recently, the presence of TLS in MMRd EC (69, 70, 72, 73, 76), yet in a somewhat lower abundance than in *POLE*mut EC (69, 72). Furthermore, endometrioid-type EC is the dominant histological subtype of MMRd EC (5, 15, 17–19, 77, 78). Endometrioid EC with solid growth (FIGO grade 3) is relatively more frequent in MMRd EC than in NSMP EC but less frequent than in *POLE*mut EC (5, 14, 15, 18). However, a variety of other non-endometrioid histological subtypes have also been reported within the MMRd EC subclass. For example, one recent study identified MMR deficiency in uterine carcinosarcomas and interestingly noted that their epithelial components often had an endometrioid morphology (79). A small proportion of MMRd EC has also been observed with a serous or clear cell morphology (14, 18). This serous-like phenotype was found not to be associated with acquired *TP53* mutations in these MMRd tumors (8). Instead, there is some indication that MMRd EC with serous-like morphology is more often seen in MMRd EC with underlying germline mutations (Lynch syndrome associated); however this, observation needs to be validated (62, 80, 81). Interestingly, about two-thirds of the un-/dedifferentiated EC have been shown to be MMR deficient (82). Finally, for yet unknown reasons, multiple reports described an association between the presence of lymphovascular space invasion (LVSI) and MMR deficiency in EC (15, 77). Hence, morphology alone is insufficient to accurately detect and classify MMRd EC.

After excluding pathogenic *POLE* mutations, immunohistochemical staining of the four MMR proteins is therefore used to identify MMRd EC. Approximately one quarter of all EC show loss of expression of one of the MMR proteins. The most common combination (about 70%) in MMRd EC is loss of MLH1 and PMS2 expression, which is

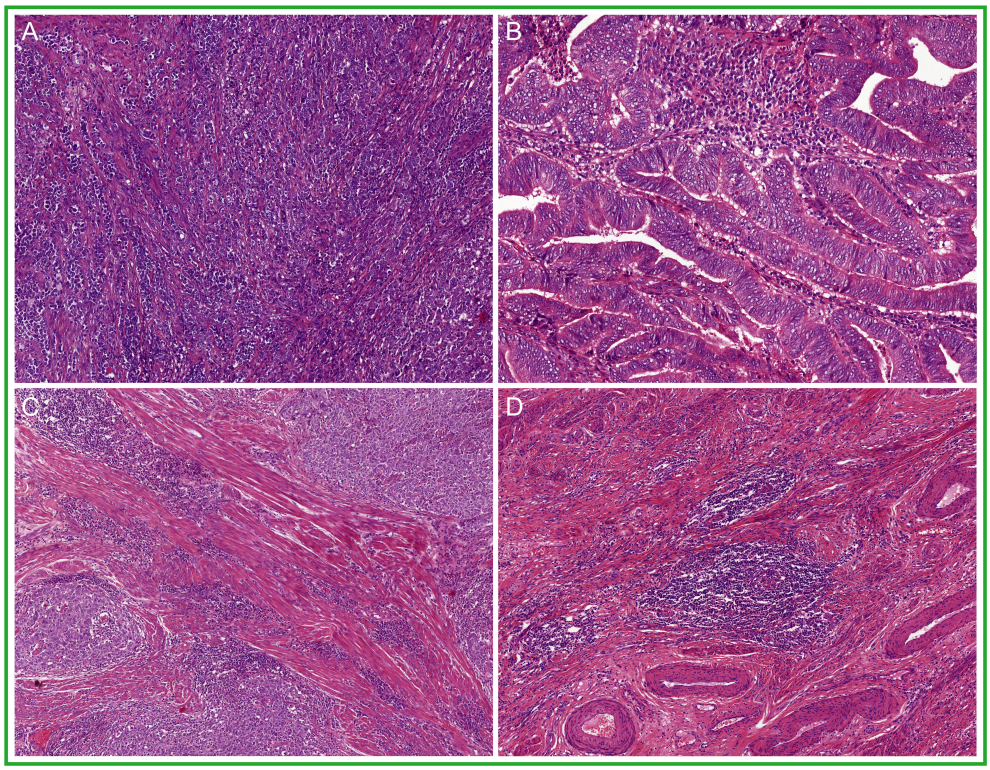

FIGURE 4
A selection of prototypical morphological features found in mismatch repair deficient endometrial cancer (MMRd EC): **(A)** solid growth; **(B)** glandular architecture; **(C)** a dense to moderate peri-tumoral and intra-epithelial infiltrate of lymphocytes; and **(D)** tertiary lymphoid structures (TLS).

usually caused by promotor hypermethylation of the *MLH1* gene. The remaining cases show loss of protein expression in other combinations, namely, single loss of MSH6, single loss of PMS2, or MSH2/MSH6 loss, of which about 10% is Lynch syndrome associated (80). p53 abnormal staining can be seen in 10% of MMRd EC (72), of which approximatively three quarters show p53 subclonal mutant-type overexpression (7).

p53 abnormal endometrial cancers

The prototypical p53 abnormal endometrial cancers (p53abn EC) has a classic serous histology with a (micro-) papillary or (pseudo-) glandular architecture. The papillae or glands are lined by a single layer of tumor cells with strong cytonuclear atypia resulting in a ragged luminal surface (Figure 5). Furthermore, a brisk mitotic activity is consistently found (14, 18, 78, 83–85). The p53abn EC molecular subgroup, however, has a broader histological spectrum, as it also includes uterine carcinosarcomas (78), clear cell carcinomas (14, 18), and FIGO grade 3 endometrioid carcinomas (5, 14, 18, 84, 85). Intriguingly, some studies described that p53abn EC can also present with low-grade endometrioid morphology (15, 70, 84). Whether this observation is true, or that these cases rather represent misclassified pseudo-glandular serous endometrial carcinomas, remains to be determined. The low abundance of tumor-infiltrating lymphocytes and lack of TLS are other histological features that differentiate p53abn EC from MMRd EC and *POLE*mut EC (69, 70, 72).

p53abn ECs, per definition, are MMR proficient and *POLE* wild type and displays one of the well-described mutant-like immunohistochemical p53 staining patterns (9). This includes abnormal p53 nuclear overexpression in 65%, abnormal null-mutant pattern in 13%, or cytoplasmic p53 overexpression (6, 7). In addition, strong and diffuse positive membranous Her2Neu staining (3+), found in 20%–25% of p53abn EC, may be p53abn subclass specific (7, 86).

No specific molecular profile endometrial cancers

The group of EC that does not carry a pathogenic *POLE* mutation is MMR proficient and shows wild-type expression of p53 is currently referred to as “no specific molecular profile” (NSMP) EC. The majority shows a predominant glandular

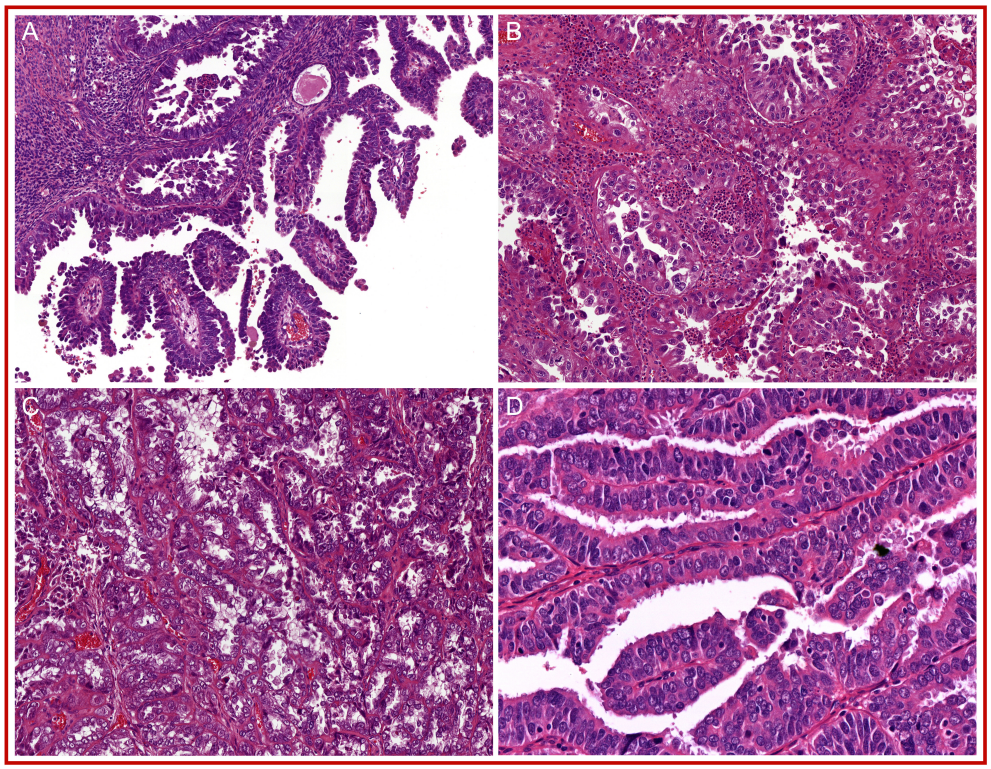

FIGURE 5
A selection of prototypical morphological features found in p53 abnormal endometrial cancer (p53abn EC): **(A)** (micro-)papillary glandular architecture; **(B)** glands with ragged luminal surface; **(C)** brisk mitotic activity; and **(D)** strong nuclear atypia.

proliferation in which the glands have smooth luminal borders and the nuclei have mild to moderate atypia (FIGO grades 1 and 2) (5, 15, 17, 18, 78). A subset of these low-grade NSMP EC present (morular) squamous differentiation to a varying degree, a distinct morphological feature that has been linked to underlying *CTNNB1* mutations (87–90). In addition to these prototypical features (Figure 6), approximately 20% of the low-grade NSMP EC present with a specific type of invasion, referred to as "microcystic elongated and fragmented" (MELF) type of invasion (91–93), which is rarely seen outside NSMP EC. A lower abundance of TILS and TLS in the NSMP EC group than in MMRd EC and *POLE*mut EC has also been reported (69, 70, 72). Finally, non-endometrioid or high-grade NSMP ECs are uncommon but have been described (14, 17–19, 78).

Emerging data suggest that NSMP EC may be further stratified into two groups with a distinct prognosis based on hormone receptor expression status (94). Although the majority of NSMP EC shows high expression of estrogen receptors (ER alpha) and progesterone receptors (PR A/B), a notable subset of approximately 10% of the NSMP EC show complete lack of ER and PR expression. Interestingly, this subgroup is enriched with non-endometrioid morphology, particularly clear cell morphology. It is also conceivable that the recently described mesonephric-like endometrial carcinomas and the gastric-type endometrial carcinomas fall in this group of hormone-receptor-negative NSMP EC (78).

The current role of morphology within the molecular EC classification

All these outlined human-identified morpho-molecular correlates are presently insufficient to accurately predict molecular class on H&E features only, and no exclusive phenotypic trait has been identified for any of the molecular classes. The observed histopathological heterogeneity within defined molecular classes clearly challenges the role of morphology in the context of the molecular EC classification. Morphological information may still refine prognosis within a specific molecular context such as histological subtype and grade in NSMP EC (95) or dense immune infiltrate or presence of TLS in MMRd EC (69, 70). Yet, at the same time, some morphological features may arguably no longer carry prognostic value in some

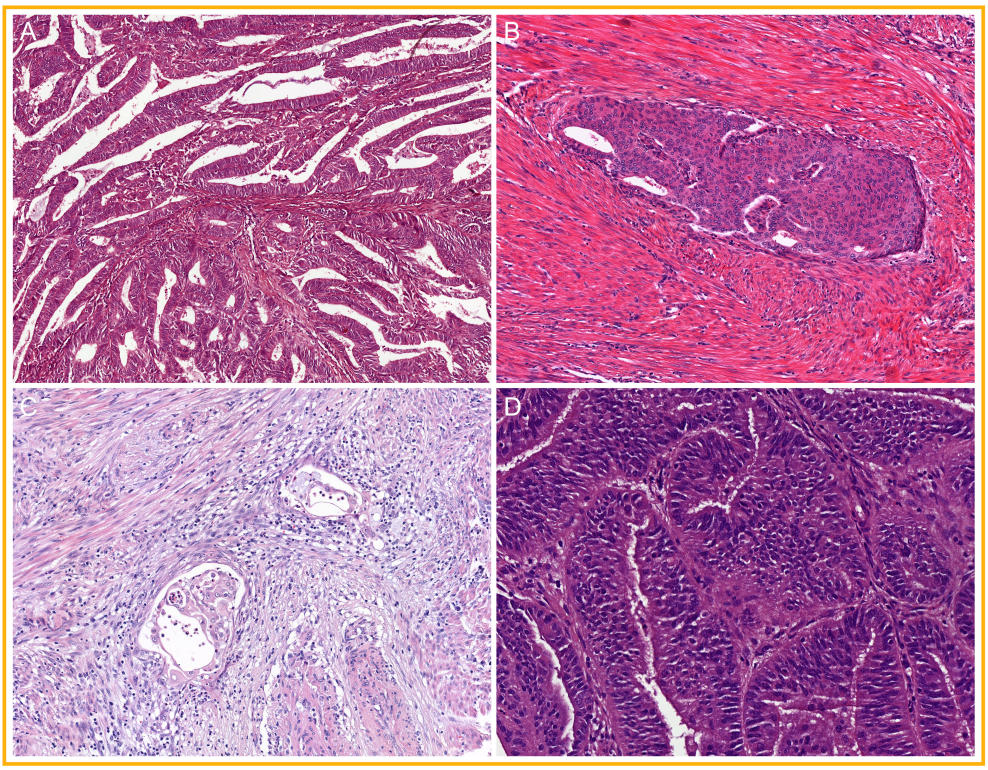

FIGURE 6
A selection of prototypical morphological features found in non-specific molecular profile endometrial cancer (NSMP EC): **(A)** glands with smooth luminal borders; **(B)** squamous differentiation; **(C)** microcystic elongated and fragmented (MELF) type of invasion; and **(D)** mild nuclear atypia.

molecular subgroups. For instance, evidence showed that a broad range of histological subtypes and grades can be found in *POLE*mut EC and p53abn EC while carrying distinct genomic alterations and having excellent and poor prognosis, respectively (5, 14, 15, 17–20). Likewise, the prognostic relevance of other morphological features is under investigation such as the presence of LVSI within *POLE*mut EC and MMRd EC (15, 66, 77) and the lymphocyte density in NSMP EC and p53abn EC (69, 70, 72).

Implementation of the molecular EC classification is a step forward, but it is questionable whether histological features have become completely obsolete. Morphological features may still contain pertinent information beyond the molecular classification such as additional prognostic indicators. It is, however, becoming increasingly complex for pathologists to distinguish relevant morphological subtleties in EC diagnostics. In this context, DL models may be capable of learning relevant morphological features in association with molecular alterations on digitized H&E-stained EC tumor slides. DL-based research may show that further refining of the EC classification is possible by accurately combining histological and molecular data (Figure 2).

Deep learning can recognize phenotypes of mutations on H&E tumor slides

Landmark studies have recently provided the proof of principle for the prediction of genetic mutations from H&E whole slide images by DL in several types of cancer (28–52, 54, 55), albeit more frequently in colorectal cancer (29–37) and breast cancer (35, 38–44). For example, the feasibility of predicting *TP53* mutation status has been explored across breast, colorectal, lung, stomach, pan-gastrointestinal, bladder, and liver cancer (36, 48, 51, 52, 55). In breast cancer, prediction of hormone receptor status (38, 39, 42) and homologous recombination deficiency (35, 40) has also been investigated. A common task for DL has also been the prediction of microsatellite instability, particularly in colorectal cancer (29, 31–36) and gastrointestinal cancer (40, 45, 46). Across all these studies, encouraging performance was measured with the area under the receiver operating characteristic (ROC) curve (AUC) on some external test sets above 0.80 (29, 29, 32, 34, 36–39, 45, 48). Although sensitivity and specificity may be, at this date, insufficient for end-to-end clinical implementations, this is a

proof of concept that genotype–phenotype correlations can be identified using DL on H&E-stained tumor slides. Sirinukunwattana et al. (37) tackled a more complex task: a DL model predicted the four-class consensus molecular subtypes (CMS) of colorectal cancer, obtaining a 0.84 AUC on the TCGA external test set (N=431 slides from 430 patients). Among these DL-based studies, one research angle that has got some particular interests is explaining the DL predictions, frequently referred to as a "black box" and deriving human-interpretable features (31, 36, 37, 44). For instance, this can be done by extracting subregions of the input slide image that were assigned strong weights by the DL model to molecularly classify a given case. The visual assessment of these regions of interest can be used to reveal relevant morpho-molecular correlates, although this may not always provide human-interpretable visual information. Another approach can be to correlate the DL predictions with clinicopathological data.

Similar DL performance and interpretability still need to be shown in large cohorts of EC. To date, only Wang et al. (53) and Hong et al. (54) have trained supervised binary classification models from H&E-stained EC tumor slides and labels publicly available from the TCGA and Clinical Proteomic Tumour Analysis Consortium (CPTAC) datasets. Wang et al. (53) limited the predictions to high microsatellite instability (MSI) on the TCGA (N=516 of which 128 MSI patients) and obtained an AUC of 0.73 on 25% hold-out internal test set. Hong et al. (54) predicted various mutations and each of the four TCGA-derived molecular EC classes separately. To this end, they reached an AUC of 0.66 for *POLE* mutation (N=7 *POLE*-mutated patients), 0.76 for MSI (N=25 MSI patients), 0.87 for copy-number high (N=20 copy-number high patients), and 0.65 for copy-number low (N=43 copy-number low patients) on the CPTAC external test set. Additionally, they obtained an AUC of 0.77 for the prediction of the *TP53* mutational status (N=30 *TP53*-mutated patients). Although both studies represent first proofs of concept of predicting genetic mutations from H&E slide images that can be expanded to EC, the test sets remained relatively small and did not reflect the heterogeneity of histological subtypes and FIGO grades known to be present in each molecular class. Specifically, with a few non-endometroid samples included in the TCGA (5) and CPTAC (96), the applicability to large cohorts of non-endometrioid EC remains unknown. In addition, the authors limited the scope of mutation prediction to binary classification tasks. Thus, to date, leveraging DL to predict the four-class molecular EC classification and deriving human-interpretable morpho-molecular correlates, have yet to be explored. Finally, the DL models used in both studies first divided the slide images into tiles, which is a standard computational method in the field, and then classified each tile individually with labels assigned at the slide-level. The tile-level classification may introduce training noise because morphological information in a given tile does not always correlate with the given true slide-level label. Hong et al. (54) have also acknowledged this architectural limitation, reporting that non-tumor tiles were often given inconclusive prediction scores. The discrepancy between tile and slide-level classification labels remains a well-known challenge in the field, which has started transitioning to more state-of-the-art DL architectures promising better performance (97–100).

Integrating deep learning into the current molecular EC classification

The gynecopathological community has started exploring how morphology-based information could be used to aid the molecular EC classification for an optimized risk-stratification strategy. Assistance to accurately weigh both histopathological and molecular variables would be welcome, as the number of relevant variables is steadily increasing (101). The innate strength of DL technology for the analysis of multi-modal datasets including both image and molecular information suggests that DL could aid in the refinement of the current morpho-molecular classification of EC.

EC-specific DL tasks could range from predicting one specific molecular alteration to predicting the complete four-class molecular classification from standard H&E images or from a combination of H&E images and special stains. To achieve this, the input data for DL models are digitized whole slide histopathology images of EC with the associated EC molecular classes. Importantly, such models achieve an incrementally increasing performance with the size and quality of the available datasets, ground-truth annotations, and advances in DL technology. Furthermore, such models can be purposely designed to generalize to previously unseen datasets and can be run in a cloud environment, potentially enabling broad access to AI-guided classification in future pathology.

This remarkable technological paradigm chance could support an increase in the fidelity of EC patient diagnosis, prognostic, and predictive classification impacting the whole diagnostic process and treatment decision-making. Theoretically, if a DL model predicts the four-class molecular EC classification at near perfect high specificity and sensitivity, then one could envision that DNA sequencing and immunohistochemistry would only be required for confirmatory testing, if at all. If such a DL model is also shown to be generalizable to external cohorts, EC patients could be molecularly classified using only digitized H&E-stained tumor slides. In this scenario, this automated tool would be clinically relevant by (i) providing a cost-effective alternative to expensive molecular testing without the need of additional tissue and (ii) speeding the diagnosis process up and advancing treatment initiation, which, in a real-world practice, can be delayed by weeks with next-generation sequencing. Until a clinical-grade accuracy of such model has been achieved, alternative and less complex DL tasks can be taken forward to support EC patient management. In

fact, a binary predictive model trained toward one single specific molecular alteration may yield a higher sensitivity and specificity than a four-class model and could subsequently serve as a pre-screening tool. In the field of EC, pre-identifying p53abn EC in a population of low-risk EC may potentially be used to identify those few patients with a poor prognosis for confirmatory testing (15). Similarly, it may be possible to identify the aggressive subset of NSMP EC that lack hormone receptor expression (94). Furthermore, preselecting cases that would further require *POLE* testing given MMR-IHC and p53-IHC would be particularly supportive and cost saving in high-risk ECs, as treatment de-escalation of *POLE*mut EC with good prognosis is currently being explored (5, 14–17, 19, 65, 70).

An avenue by which DL has currently probably the biggest role is as a research tool combined with gynecopathological expertise. Several studies showed (36, 37, 44) that after training an EC-specific DL model, image-based information associated with EC molecular alterations could be visually extracted and reviewed by gynecopathologists. From there on, the morpho-molecular correlates that are outlined in this review may be confirmed, but the DL model may also reveal morpho-molecular features that have so far not struck the attention of human observers. Increasing knowledge about the morphology of the molecular classes will help to understand the biological processes and dynamics of tumor–host interaction in the tumor microenvironment. The prognostic value of the identified morpho-molecular features can be subsequently explored, which may open new doors to prognostic refinement in EC.

With increasing availability of digitalization aids such as cloud computing and resources, DL-driven diagnostic tools could be made available worldwide as an additional inexpensive, if not free, resource without the need of local hardware or knowledge (102). Particularly, users without access to scanners would only need to generate slide images using microscope cameras or even existing mobile phones for diagnostic classification in a central expert center. However, high-quality slide images may remain a limitation to the applicability in low-income countries and country-specific regulations on patient data transfer.

Discussion

In the past four decades, the classification of EC has evolved from a histology-based to a molecular system. The recent integration into guidelines indicates the increasing prognostic value of the four-class molecular classification over morphology in EC (1, 2), yet the integrated management with former histopathological variables is still a challenge in the diagnostic routine (9). As a result, questions have been raised about the relevance of these histopathological features and the role of morphology beyond the molecular EC classification. Now, given the four molecular EC classes, a number of studies have described some distinct morphological characteristics, but they remain insufficient to achieve accurate classification (66–70, 72, 73, 76, 83), and the pathologist's eyes are not sufficiently trained to spotlight them. In fact, this review stresses the difficulty in weighing image-based information in relation to the current four molecular EC classes. First, each molecular subclass shows heterogeneity for histological subtype, FIGO grade, and associated microscopic features. Second, many microscopic features appear to be non-exclusive, for instance the presence of high levels of immune cells between *POLE*mut *EC* and MMRd EC (69, 70). Lastly, some morphological traits are detectable at different magnifications and growth patterns, and nuclear atypia within p53abn EC is one example.

In the quickly progressing research domain of computer science, DL has demonstrated a well-known capability to work with high-dimensional and multi-modal datasets, up to learn phenotype–genotype correlates from highly complex and extra-large digitized tumor slides (29–52, 54, 55). Hence, future DL-based breakthroughs have legitimate potential to resolve the current dilemma between molecular and histopathological variables or even support EC patient management for pre-screening and decision-making on treatment, ultimately impacting EC diagnostics and patient care as a whole. As for today, an urgent assignment given to DL technology in combination with gynecopathological expertise is bringing to the surface the clinical relevance of each morphological feature associated with the four molecular EC subclasses, while improving morphological and biological understanding of the genomic EC alterations. Combining the strengths of molecular-, clinical-, and DL-based information may refine the EC classification to reach optimal prognostication and prediction for our future EC patients.

Author contributions

SF wrote sections of the review. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

VK: Research grants from Swiss Federal Institute of Technology Strategic Focus Area: Personalized Health and Related Technologies PHRT, the Swiss National Science Foundation and Promedica unrelated to the current works.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza M, Marnitz S, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer* (2021) 31(1):12–39. doi: 10.1136/ijgc-2020-002230

2. Herrington CS, (ed.), Editorial Board WHOCOT. *WHO Classification of Tumours Female Genital Tumours. 5th* ed. International Agency for Research on Cancer (2020).

3. Bokhman J. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol* (1983) 15(1):10–7. doi: 10.1016/0090-8258(83)90111-7

4. Amant F, Mirza MR, Koskas M, Creutzberg CL. Cancer of the corpus uteri. *Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet* (2018) 143 (Suppl 2):37–50. doi: 10.1002/ijgo.12612

5. Cancer Genome Atlas Research Network, Kandoth C, Schultz N, Cherniack A, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. *Nature* (2013) 497(7447):67–73. doi: 10.1038/nature12325

6. Singh N, Piskorz A, Bosse T, Jimenez-Linan M, Rous B, Brenton J, et al. p53 immunohistochemistry is an accurate surrogate for TP53 mutational analysis in endometrial carcinoma biopsies. *J Pathol* (2020) 250(3):336–45. doi: 10.1002/path.5375

7. Vermij L, Léon-Castillo A, Singh N, Powell ME, Edmondson RJ, Genestie C. TransPORTEC consortium. p53 immunohistochemistry in endometrial cancer: clinical and molecular correlates in the PORTEC-3 tria. In: *Mod Pathol* (2022). doi: 10.1038/s41379-022-01102-x.

8. León-Castillo A, Gilvazquez E, Nout R, Smit V, McAlpine J, McConechy M, et al. Clinicopathological and molecular characterisation of "multiple-classifier" endometrial carcinomas. *J Pathol* (2020) 250:312–22. doi: 10.1002/path.5373

9. Vermij L, Smit V, Nout R, Bosse T. Incorporation of molecular characteristics into endometrial cancer management. *Histopathology* (2020) 76:52–63. doi: 10.1111/his.14015

10. Hoang L, Kinloch M, Leo J, Grondin K, Lee C, Ewanowich C, et al. Interobserver agreement in endometrial carcinoma histotype diagnosis varies depending on the cancer genome atlas (TCGA)-based molecular subgroup. *Am J Surg Pathol* (2017) 41:245–52. doi: 10.1097/PAS.0000000000000764

11. Boennelycke M, Peters EEM, Léon-Castillo A, Smit VTHBM, Bosse T, Christensen IJ, et al. Prognostic impact of histological review of high-grade endometrial carcinomas in a large Danish cohort. *Virchows Arch Int J Pathol* (2021) 479(3):507–14. doi: 10.1007/s00428-021-03133-2

12. de Boer SM, Wortman BG, Bosse T, Powell ME, Singh N, Hollema H, et al. Clinical consequences of upfront pathology review in the randomised PORTEC-3 trial for high-risk endometrial cancer. *Ann Oncol* (2018) 29(2):424–30. doi: 10.1093/annonc/mdx753

13. Perrone E, Felice FD, Capasso I, Distefano E, Lorusso D, Nero C, et al. The immunohistochemical molecular risk classification in endometrial cancer: A pragmatic and high-reproducibility method. *Gynecol Oncol* (2022) 165(3):585–93. doi: 10.1016/j.ygyno.2022.03.009

14. León-Castillo A, de Boer S, Powel M, Mileshkin L, Mackay H, Leary A, et al. Molecular classification of the PORTEC-3 trial for high-risk endometrial cancer: Impact on prognosis and benefit from adjuvant therapy. *J Clin Oncol* (2020) 38:3388–97. doi: 10.1200/JCO.20.00549

15. Stelloo E, Nout R, Osse E, Jürgenliemk-Schulz I, Jobsen J, Lutgens L, et al. Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer–combined analysis of the PORTEC cohorts. *Clin Cancer Res* (2016) 22:4215–24. doi: 10.1158/1078-0432.CCR-15-2878

16. Bosse T, Nout RA, McAlpine JN, McConechy MK, Britton H, Hussein YR, et al. Molecular classification of grade 3 endometrioid endometrial cancers identifies distinct prognostic subgroups. *Am J Surg Pathol* (2018) 42(5):561–8. doi: 10.1097/PAS.0000000000001020

17. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, et al. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. *Ann Oncol Off J Eur Soc Med Oncol* (2018) 29 (5):1180–8. doi: 10.1093/annonc/mdy058

18. Leon-Castillo A, Horeweg N, Peters E, Rutten T, Smit V, Kroon D, et al. Prognostic relevance of the molecular classification in high-grade endometrial cancer for patients staged by lymphadenectomy and without adjuvant treatment. *Gynecol Oncol* (2022) 164(3):577–586. doi: 10.1016/j.ygyno.2022.01.00

19. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: A simple, genomics-based clinical classifier for endometrial cancer. *Cancer* (2017) 123(5):802–13. doi: 10.1002/cncr.30496

20. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A clinically applicable molecular-based classification for endometrial cancers. *Br J Cancer* (2015) 113(2):299–310. doi: 10.1038/bjc.2015.190

21. Alexa M, Hasenburg A, Battista MJ. The TCGA molecular classification of endometrial cancer and its possible impact on adjuvant treatment decisions. *Cancers (Basel)* (2021) 13(6):1478. doi: 10.3390/cancers13061478

22. van den Heerik ASVM, Horeweg N, Nout RA, Lutgens LCHW, van der Steen-Banasik EM, Westerveld GH, et al. PORTEC-4a: international randomized trial of molecular profile-based adjuvant treatment for women with high-intermediate risk endometrial cancer. *Int J Gynecol Cancer Off J Int Gynecol Cancer Soc* (2020) 30(12):2002–7. doi: 10.1136/ijgc-2020-001929

23. Bosse T, Powell M, Crosbie E, Leary A, Kroep J, Han K, et al. 595 implementation of collaborative translational research (TransPORTEC) findings in an international endometrial cancer clinical trials program (RAINBO). *Int J Gynecol Cancer* (2021) 31(Suppl 3):A108. doi: 10.1136/ijgc-2021-ESGO.171

24. Jamieson A, Bosse T, McAlpine JN. The emerging role of molecular pathology in directing the systemic treatment of endometrial cancer. *Ther Adv Med Oncol* (2021) 13:17588359211035960. doi: 10.1177/17588359211035959

25. Krizhevsky A, Sutskever I, Hinton GE. ImageNet classification with deep convolutional neural networks. In: F Pereira, CJC Burges, L Bottou and KQ Weinberger, editors. *Proceedings of the 25th International Conference on Neural Information Processing Systems - Volume 1*. Red Hook, NY,USA: Curran Associates Inc (2012) . p. 1097–105. (NIPS'12).

26. Litjens G, Bandi P, Ehteshami Bejnordi B, Geessink O, Balkenhol M, Bult P, et al. 1399 H&E-stained sentinel lymph node sections of breast cancer patients: the CAMELYON dataset. *GigaScience* (2018) 7(6):giy065. doi: 10.1093/gigascience/giy065

27. Russakovsky O, Deng J, Su H, Krause J, Satheesh S, Ma S, et al. ImageNet Large scale visual recognition challenge. *Int J Comput Vis IJCV* (2015) 115(3):211–52. doi: 10.1007/s11263-015-0816-y

28. Cifci D, Foersch S, Kather JN. Artificial intelligence to identify genetic alterations in conventional histopathology. *J Pathol* (2022) 257(4):430–444. doi: 10.1002/path.5898

29. Echle A, Grabsch HI, Quirke P, van den Brandt PA, West NP, Hutchins GGA, et al. Clinical-grade detection of microsatellite instability in colorectal tumors by deep learning. *Gastroenterology* (2020) 159(4):1406–1416.e11. doi: 10.1053/j.gastro.2020.06.021

30. Jang HJ, Lee A, Kang J, Song IH, Lee SH. Prediction of clinically actionable genetic alterations from colorectal cancer histopathology images using deep learning. *World J Gastroenterol* (2020) 26(40):6207–23. doi: 10.3748/wjg.v26.i40.6207

31. Yamashita R, Long J, Longacre T, Peng L, Berry G, Martin B, et al. Deep learning model for the prediction of microsatellite instability in colorectal cancer: a diagnostic study. *Lancet Oncol* (2021) 22(1):132–41. doi: 10.1016/S1470-2045(20)30535-0

32. Lee SH, Song IH, Jang HJ. Feasibility of deep learning-based fully automated classification of microsatellite instability in tissue slides of colorectal cancer. *Int J Cancer* (2021) 149(3):728–40. doi: 10.1002/ijc.33599

33. Cao R, Yang F, Ma SC, Liu L, Zhao Y, Li Y, et al. Development and interpretation of a pathomics-based model for the prediction of microsatellite instability in colorectal cancer. *Theranostics* (2020) 10(24):11080–91. doi: 10.7150/thno.49864

34. Schrammen PL, Ghaffari Laleh N, Echle A, Truhn D, Schulz V, Brinker TJ, et al. Weakly supervised annotation-free cancer detection and prediction of genotype in routine histopathology. *J Pathol* (2022) 256(1):50–60. doi: 10.1002/path.5800

35. Schirris Y, Gavves E, Nederlof I, Horlings HM, Teuwen J. DeepSMILE: Contrastive selfsupervised pre-training benefits MSI and HRD classification directly from H&E wholeslide images in colorectal and breast cancer. *Med Image Anal* (2022) 79:102464. doi: 10.1016/j.media.2022.102464

36. Bilal M, Raza SEA, Azam A, Graham S, Ilyas M, Cree IA, et al. Development and validation of a weakly supervised deep learning framework to predict the status of molecular pathways and key mutations in colorectal cancer from routine histology images: a retrospective study. *Lancet Digit Health* (2021) 3(12):e763–e772. doi: 10.1016/S2589-7500(21)00180-1

37. Sirinukunwattana K, Domingo E, Richman S, Redmond K, Blake A, Verrill C, et al. Image-based consensus molecular subtype (imCMS) classification of colorectal cancer using deep learning. *Gut* (2021) 70:544–54. doi: 10.1136/gutjnl-2019-319866

38. Naik N, Madani A, Esteva A, Keskar NS, Press MF, Ruderman D, et al. Deep learning-enabled breast cancer hormonal receptor status determination from base-level H&E stains. *Nat Commun* (2020) 11(1):5727. doi: 10.1038/s41467-020-19334-3

39. Rawat RR, Ortega I, Roy P, Sha F, Shibata D, Ruderman D, et al. Deep learned tissue "fingerprints" classify breast cancers by ER/PR/Her2 status from H&E images. *Sci Rep* (2020) 10(1):7275. doi: 10.1038/s41598-020-64156-4.

40. Valieris R, Amaro L, Osório CAB de T, Bueno AP, Rosales Mitrowsky RA, Carraro DM, et al. Deep learning predicts underlying features on pathology images with therapeutic relevance for breast and gastric cancer. *Cancers (Basel)* (2020) 12 (12):3687. doi: 10.3390/cancers12123687

41. Qu H, Zhou M, Yan Z, Wang H, Rustgi VK, Zhang S, et al. Genetic mutation and biological pathway prediction based on whole slide images in breast carcinoma using deep learning. *NPJ Precis Oncol* (2021) 5(1):87. doi: 10.1038/s41698-021-00225-9

42. Farahmand S, Fernandez AI, Ahmed FS, Rimm DL, Chuang JH, Reisenbichler E, et al. Deep learning trained on hematoxylin and eosin tumor region of interest predicts HER2 status and trastuzumab treatment response in HER2+ breast cancer. *Mod Pathol Off J U S Can Acad Pathol Inc* (2022) 35(1):44–51. doi: 10.1038/s41379-021-00911-w

43. Wang X, Zou C, Zhang Y, Li X, Wang C, Ke F, et al. Prediction of BRCA gene mutation in breast cancer based on deep learning and histopathology images. *Front Genet [Internet]* (2021) 12:661109. doi: 10.3389/fgene.2021.661109

44. Diao JA, Wang JK, Chui WF, Mountain V, Gullapally SC, Srinivasan R, et al. Human-interpretable image features derived from densely mapped cancer pathology slides predict diverse molecular phenotypes. *Nat Commun* (2021) 12 (1):1613. doi: 10.1038/s41467-021-21896-9

45. Kather J, Pearson A, Halama N, Jäger D, Krause J, Loosen S, et al. Deep learning can predict microsatellite instability directly from histology in gastrointestinal cancer. *Nat Med* (2019) 25:1054–6. doi: 10.1038/s41591-019-0462-y

46. Muti HS, Heij LR, Keller G, Kohlruss M, Langer R, Dislich B, et al. Development and validation of deep learning classifiers to detect Epstein-Barr virus and microsatellite instability status in gastric cancer: a retrospective multicentre cohort study. *Lancet Digit Health* (2021) 3(10):e654–64. doi: 10.1016/S2589-7500(21)00133-3

47. Kather JN, Heij LR, Grabsch HI, Loeffler C, Echle A, Muti HS, et al. Pan-cancer image-based detection of clinically actionable genetic alterations. *Nat Cancer* (2020) 1(8):789–99. doi: 10.1038/s43018-020-0087-6

48. Chen M, Zhang B, Topatana W, Cao J, Zhu H, Juengpanich S, et al. Classification and mutation prediction based on histopathology H&E images in liver cancer using deep learning. *NPJ Precis Oncol* (2020) 4(1):14. doi: 10.1038/s41698-020-0120-3

49. Coudray N, Ocampo PS, Sakellaropoulos T, Narula N, Snuderl M, Fenyö D, et al. Classification and mutation prediction from non-small cell lung cancer histopathology images using deep learning. *Nat Med* (2018) 24(10):1559–67. doi: 10.1038/s41591-018-0177-5

50. Schmauch B, Romagnoni A, Pronier E, Saillard C, Maillé P, Calderaro J, et al. A deep learning model to predict RNA-seq expression of tumours from whole slide images. *Nat Commun* (2020) 11(1):3877. doi: 10.1038/s41467-020-17678-4

51. Noorbakhsh J, Farahmand S, Foroughi Pour A, Namburi S, Caruana D, Rimm D, et al. Deep learning-based cross-classifications reveal conserved spatial behaviors within tumor histological images. *Nat Commun* (2020) 11(1):6367. doi: 10.1038/s41467-020-20030-5

52. Fu Y, Jung AW, Torne RV, Gonzalez S, Vöhringer H, Shmatko A, et al. Pan-cancer computational histopathology reveals mutations, tumor composition and prognosis. *Nat Cancer* (2020) 1(8):800–10. doi: 10.1038/s43018-020-0085-8

53. Wang T, Lu W, Yang F, Liu L, Dong Z, Tang W, et al. Microsatellite instability prediction of uterine corpus endometrial carcinoma based on H&E histology whole-slide imaging. In: *2020 IEEE 17th international symposium on biomedical imaging (ISBI)* (2020) IEEE. p. 1289–92.

54. Hong R, Liu W, DeLair D, Razavian N, Fenyö D. Predicting endometrial cancer subtypes and molecular features from histopathology images using multi-resolution deep learning models. *Cell Rep Med* (2021) 2(9):100400. doi: 10.1016/j.xcrm.2021.100400

55. Jang HJ, Lee A, Kang J, Song IH, Lee SH. Prediction of genetic alterations from gastric cancer histopathology images using a fully automated deep learning approach. *World J Gastroenterol* (2021) 27:7687–704. doi: 10.3748/wjg.v27.i44.7687

56. Vale-Silva LA, Rohr K. Long-term cancer survival prediction using multimodal deep learning. *Sci Rep* (2021) 11(1):13505. doi: 10.1038/s41598-021-92799-4

57. Schulz S, Woerl AC, Jungmann F, Glasner C, Stenzel P, Strobl S, et al. Multimodal deep learning for prognosis prediction in renal cancer. *Front Oncol* (2021) 11:788740. doi: 10.3389/fonc.2021.788740

58. Courtiol P, Maussion C, Moarii M, Pronier E, Pilcer S, Sefta M, et al. Deep learning-based classification of mesothelioma improves prediction of patient outcome. *Nat Med* (2019) 25(10):1519–25. doi: 10.1038/s41591-019-0583-3

59. Yamashita R, Long J, Saleem A, Rubin DL, Shen J. Deep learning predicts postsurgical recurrence of hepatocellular carcinoma from digital histopathologic images. *Sci Rep* (2021) 11(1):2047. doi: 10.1038/s41598-021-81506-y

60. Wang X, Chen Y, Gao Y, Zhang H, Guan Z, Dong Z, et al. Predicting gastric cancer outcome from resected lymph node histopathology images using deep learning. *Nat Commun* (2021) 12(1):1637. doi: 10.1038/s41467-021-21674-7

61. Church DN, Briggs SEW, Palles C, Domingo E, Kearsey SJ, Grimes JM, et al. DNA Polymerase ϵ and δ exonuclease domain mutations in endometrial cancer. *Hum Mol Genet* (2013) 22(14):2820–8. doi: 10.1093/hmg/ddt131

62. Conlon N, Da Cruz Paula A, Ashley C, Segura S, De Brot L, da Silva E, et al. Endometrial carcinomas with a "Serous" component in young women are enriched for DNA mismatch repair deficiency, lynch syndrome, and POLE exonuclease domain mutations. *Am J Surg Pathol* (2020) 44:641–8. doi: 10.1097/PAS.0000000000001461

63. Kim S, Cloutier B, Leung S, Cochrane D, Britton H, Pina A, et al. Molecular subtypes of clear cell carcinoma of the endometrium: Opportunities for prognostic and predictive stratification. *Gynecol Oncol* (2020) 158:3–11. doi: 10.1016/j.ygyno.2020.04.043

64. Gotoh O, Sugiyama Y, Takazawa Y, Kato K, Tanaka N, Omatsu K, et al. Clinically relevant molecular subtypes and genomic alteration-independent differentiation in gynecologic carcinosarcoma. *Nat Commun* (2019) 10:4965. doi: 10.1038/s41467-019-12985-x

65. Imboden S, Nastic D, Ghaderi M, Rydberg F, Rau T, Mueller M, et al. Phenotype of POLE-mutated endometrial cancer. *PLoS One* (2019) 14:e0214318. doi: 10.1371/journal.pone.0214318

66. Van Gool I, Ubachs J, Stelloo E, de Kroon C, Goeman J, Smit V, et al. Blinded histopathological characterisation of POLE exonuclease domain-mutant endometrial cancers: sheep in wolf's clothing. *Histopathology* (2018) 72:248–58. doi: 10.1111/his.13338

67. Bakhsh S, Kinloch M, Hoang L, Soslow R, Köbel M, Lee C, et al. Histopathological features of endometrial carcinomas associated with POLE mutations: implications for decisions about adjuvant therapy. *Histopathology* (2016) 68:916–24. doi: 10.1111/his.12878

68. Hussein Y, Weigelt B, Levine D, Schoolmeester J, Dao L, Balzer B, et al. Clinicopathological analysis of endometrial carcinomas harboring somatic POLE exonuclease domain mutations. *Mod Pathol* (2015) 28:505–14. doi: 10.1038/modpathol.2014.143

69. Horeweg N, de Bruyn M, Nout R, Stelloo E, Kedziersza K, León-Castillo A, et al. Prognostic integrated image-based immune and molecular profiling in early-stage endometrial cancer. *Cancer Immunol Res* (2020) 8:1508–19. doi: 10.1158/2326-6066.CIR-20-0149

70. Talhouk A, Derocher H, Schmidt P, Leung S, Milne K, Gilks C, et al. Molecular subtype not immune response drives outcomes in endometrial carcinoma. *Clin Cancer Res* (2019) 25:2537–48. doi: 10.1158/1078-0432.CCR-18-3241

71. Raffone A, Travaglino A, Raimondo D, Boccellino M, Maletta M, Borghese G, et al. Tumor-infiltrating lymphocytes and POLE mutation in endometrial carcinoma. *Gynecol Oncol* (2021) 161:621–8. doi: 10.1016/j.ygyno.2021.02.030

72. Horeweg N, Workel H, Church D, Vermij L, León-Castillo A, Krog R, et al. Tertiary lymphoid structures critical for prognosis in endometrial cancer patients. (2021) 13(1):1373. doi: 10.1038/s41467-022-29040-x

73. Lin Z, Huang L, Li S, Gu J, Cui X, Zhou Y. Pan-cancer analysis of genomic properties and clinical outcome associated with tumor tertiary lymphoid structure. *Sci Rep* (2020) 10(1):21530. doi: 10.1038/s41598-020-78560-3

74. Shimizu Y, Suzuki S, Ukai M, Hattori S, Yoshikawa N, Kajiyama H. The prognostic significance of peritumoral lymphocytes' band-like structure in type II endometrial cancer. *Anticancer Res* (2021) 41(1):249–58. doi: 10.21873/anticanres.14771

75. Kim K, Hwangbo S, Kim H, Kim YB, No JH, Suh DH, et al. Clinicopathologic and protein markers distinguishing the "polymerase epsilon exonuclease" from the "copy number low" subtype of endometrial cancer. *J Gynecol Oncol* (2022) 33(3):e27. doi: 10.3802/jgo.2022.33.e27

76. Howitt B, Shukla S, Sholl L, Ritterhouse L, Watkins J, Rodig S, et al. Association of polymerase e-mutated and microsatellite-instable endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and expression of PD-1 and PD-L1. *JAMA Oncol* (2015) 1:1319–23. doi: 10.1001/jamaoncol.2015.2151

77. Kim SR, Pina A, Albert A, McAlpine J, Wolber R, Blake Gilks C, et al. Does MMR status in endometrial cancer influence response to adjuvant therapy? *Gynecol Oncol* (2018) 151(1):76–81. doi: 10.1016/j.ygyno.2018.08.020

78. Devereaux KA, Weiel JJ, Pors J, Steiner DF, Ho C, Charu V, et al. Prospective molecular classification of endometrial carcinomas: institutional implementation, practice, and clinical experience. *Mod Pathol Off J U S Can Acad Pathol Inc* (2021) 35(5):688–696. doi: 10.1038/s41379-021-00963-y

79. Segura SE, Pedra Nobre S, Hussein YR, Abu-Rustum NR, Weigelt B, Soslow RA, et al. DNA Mismatch repair-deficient endometrial carcinosarcomas portend distinct clinical, morphologic, and molecular features compared with traditional carcinosarcomas. *Am J Surg Pathol* (2020) 44(11):1573–9. doi: 10.1097/PAS.0000000000001561

80. Post C, Stelloo E, Smit V, Ruano D, Tops C, Vermij L, et al. Prevalence and prognosis of lynch syndrome and sporadic mismatch repair deficiency in endometrial cancer. *J Natl Cancer Inst* (2021) 113:1212–20. doi: 10.1093/jnci/djab029

81. Moukarzel L, Da Cruz Paula A, Ferrando L, Hoang T, Sebastiao A, Pareja F, et al. Clonal relationship and directionality of progression of synchronous endometrial and ovarian carcinomas in patients with DNA mismatch repair-deficiency associated syndrome. *Mod Pathol* (2021) 34:994–1007. doi: 10.1038/s41379-020-00721-6

82. Zhang K, Liu Y, Liu X, Du J, Wang Y, Yang J, et al. Clinicopathological significance of multiple molecular features in undifferentiated and dedifferentiated endometrial carcinomas. *Pathol (Phila)* (2021) 53(2):179–86. doi: 10.1016/j.pathol.2020.07.015

83. Kang E, Wiebe N, Aubrey C, Lee C, Anglesio M, Tilley D, et al. Selection of endometrial carcinomas for p53 immunohistochemistry based on nuclear features. *J Pathol Clin Res* (2022) 8:19–32. doi: 10.1002/cjp2.243

84. Momeni-Boroujeni A, Dahoud W, Vanderbilt CM, Chiang S, Murali R, Rios-Doria EV, et al. Clinicopathologic and genomic analysis of TP53-mutated endometrial carcinomas. *Clin Cancer Res Off J Am Assoc Cancer Res* (2021) 27(9):2613–23. doi: 10.1158/1078-0432

85. Brett MA, Atenafu EG, Singh N, Ghatage P, Clarke BA, Nelson GS, et al. Equivalent survival of p53 mutated endometrial endometrioid carcinoma grade 3 and endometrial serous carcinoma. *Int J Gynecol Pathol Off J Int Soc Gynecol Pathol* (2021) 40(2):116–23. doi: 10.1097/PGP.0000000000000674

86. Ross DS, Devereaux KA, Jin C, Lin DY, Zhang Y, Marra A, et al. Histopathologic features and molecular genetic landscape of HER2-amplified endometrial carcinomas. *Mod Pathol Off J U S Can Acad Pathol Inc* (2022) 35(7):962–971. doi: 10.1038/s41379-021-00997-2

87. Costigan D, Dong F, Nucci M, Howitt B. Clinicopathologic and immunohistochemical correlates of CTNNB1 mutated endometrial endometrioid carcinoma. *Int J Gynecol Pathol* (2020) 39:119–27. doi: 10.1097/PGP.0000000000000583

88. Stelloo E, Bosse T, Nout RA, MacKay HJ, Church DN, Nijman HW, et al. Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative. *Mod Pathol Off J U S Can Acad Pathol Inc* (2015) 28(6):836–44. doi: 10.1038/modpathol.2015.43

89. Ashihara K, Saito T, Mizumoto H, Adachi K, Tanaka R, Nishimura M, et al. Loss of γ-catenin expression in squamous differentiation in endometrial carcinomas. *Int J Gynecol Pathol* (2002) 21(3):246–54. doi: 10.1097/00004347-200207000-00007

90. De Leo A, de Biase D, Lenzi J, Barbero G, Turchetti D, Grillini M, et al. ARID1A and CTNNB1/β-catenin molecular status affects the clinicopathologic features and prognosis of endometrial carcinoma: Implications for an improved surrogate molecular classification. *Cancers [Internet]* (2021) 13(5):950. doi: 10.3390/cancers13050950

91. Stewart CJR, Amanuel B, Grieu F, Carrello A, Iacopetta B. KRAS mutation and microsatellite instability in endometrial adenocarcinomas showing MELF-type myometrial invasion. *J Clin Pathol* (2010) 63(7):604–8. doi: 10.1136/jcp.2009.069500

92. He D, Wang H, Dong Y, Zhang Y, Zhao J, Lv C, et al. POLE mutation combined with microcystic, elongated and fragmented (MELF) pattern invasion in endometrial carcinomas might be associated with poor survival in Chinese women. *Gynecol Oncol* (2020) 159(1):36–42. doi: 10.1016/j.ygyno.2020.07.102

93. an den Heerik ASVM, Aiyer KTS, Stelloo E, Jürgenliemk-Schulz IM, Lutgens LCHW, Jobsen JJ, et al. Microcystic elongated and fragmented (MELF) pattern of invasion: Molecular features and prognostic significance in the PORTEC-1 and -2 trials. *Gynecol Oncol* (2022) S0090-8258(22):00431–0. doi: 10.1016/j.ygyno.2022.06.027

94. Vermij L. Molecular profiling of NSMP high-risk endometrial cancers of the PORTEC-3 trial - prognostic refinement and druggable targets. In: *22nd European congress on gynaecological oncology*. Prague, Czech Republic (2021).

95. Vermij L. Prognostic relevance of FIGO grading is limited to NSMP endometrial carcinomas. In: *USCAP 111th annual meeting;*. Los Angeles, CA (2022).

96. Dou Y, Kawaler EA, Zhou DC, Gritsenko MA, Huang C, Blumenberg L, et al. Proteogenomic characterization of endometrial carcinoma. *Cell* (2020) 180(4):729–48.e26. doi: 10.1016/j.cell.2020.01.026

97. Campanella G, Hanna MG, Geneslaw L, Miraflor A, Werneck Krauss Silva V, Busam KJ, et al. Clinical-grade computational pathology using weakly supervised deep learning on whole slide images. *Nat Med* (2019) 25(8):1301–9. doi: 10.1038/s41591-019-0508-1

98. Lu MY, Williamson DFK, Chen TY, Chen RJ, Barbieri M, Mahmood F. Data-efficient and weakly supervised computational pathology on whole-slide images. *Nat BioMed Eng* (2021) 5(6):555–70. doi: 10.1038/s41551-020-00682-w

99. Lu MY, Chen TY, Williamson DFK, Zhao M, Shady M, Lipkova J, et al. AI-Based pathology predicts origins for cancers of unknown primary. *Nature* (2021) 594(7861):106–10. doi: 10.1038/s41586-021-03512-4

100. Lipkova J, Chen TY, Lu MY, Chen RJ, Shady M, Williams M, et al. Deep learning-enabled assessment of cardiac allograft rejection from endomyocardial biopsies. *Nat Med* (2022) 28(3):575–82. doi: 10.1038/s41591-022-01709-2

101. Wu J, Horeweg N, de Bruyn M, Nout RA, Jürgenliemk-Schulz IM, Lutgens LCHW, et al. Automated causal inference in application to randomized controlled clinical trials. *Nat Mach Intell* (2022) 4:436–44. doi: 10.1038/s42256-022-00470-y

102. Schubert M. Erasing pathology's borders. how even the simplest outreach can change the lives of pathologists and patients with few resources. *Lab Digit Comput Pathol Precis Med Technol Innov Softw Hardw* (2019). Available at: https://thepathologist.com/inside-the-lab/erasing-pathologys-borders

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EDITED BY
Andrzej Semczuk,
Medical University of Lublin, Poland

REVIEWED BY
Olugbenga Akindele Silas,
University of Jos, Nigeria
Federica Perelli,
Santa Maria Annunziata Hospital, Italy

*CORRESPONDENCE
Ziling Liu
ziling@jlu.edu.cn

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Endometrial clear cell carcinoma: A population-based study

Pengfei Cui, Xiaofeng Cong, Youhao Zhang, Huimin Zhang and Ziling Liu*

Cancer Center, The First Hospital of Jilin University, Changchun, China

Background: A systematic analysis of prognostic factors concerning endometrial clear cell carcinoma (ECCC) is lacking. The current study aimed to construct nomograms predicting the overall survival (OS) of ECCC patients.

Methods: We performed a retrospective study, and predicted nomograms for 3-, 5-, and 10-year OS were established. The nomograms were verified with the consistency index (C-index), calibration curve, and decision curve analysis (DCA).

Results: A total of 1778 ECCC patients, 991 from FIGO stage I/II and 787 from FIGO stage III/IV, were included in this study. The age at diagnosis, marital status, T stage, tumor size, and surgery-independent prognostic factors in FIGO stage I/II, and the age at diagnosis, T stage, lymph node involvement, distant metastasis, tumor size, surgery, radiotherapy, and chemotherapy in FIGO stage III/IV were independent prognostic factors. The C-indexes of the training and validation group were 0.766 and 0.697 for FIGO stage I/II and 0.721 and 0.708 for FIGO stage III/IV, respectively. The calibration curve revealed good agreement between nomogram-predicted and actual observation values. The DCA established that nomograms had better clinical benefits than the traditional FIGO stage.

Conclusions: The predicted nomograms showed good accuracy, excellent discrimination ability, and clinical benefits, depicting their usage in clinical practice.

KEYWORDS

endometrial clear cell carcinoma, prognosis, nomogram, FIGO stage, risk classification system

Introduction

Endometrial carcinoma (EC) is the sixth most diagnosed cancer among women (1). An estimated 66,570 new cases of uterine corpus cancer and 12,940 deaths were reported in the United States in 2021 (2). EC is usually diagnosed during stage I, and patients have a good prognosis as it induces symptoms from an early stage (3). Postmenopausal vaginal

bleeding is the most common symptom of EC (3, 4). Additionally, tumor invasion of the cervix can lead to blood or pus in the uterine cavity, causing abdominal swelling and cramping pain. Patients having advanced disease may suffer pelvic and lumbosacral pain due to the invasion of the tumor within the surrounding tissues or nerves (3, 4). EC is classified as type I and type II based on Bokhman's dualist model[5]. Type I EC is estrogen-dependent and accounts for nearly 80% of all EC. Its pathological type is primarily endometrioid carcinoma (3–5). Endometrial clear cell carcinoma (ECCC) is a type II EC accounting for approximately 2–4% of the total EC, and is more common in older women (6). ECCC is an estrogen-independent tumor whose onset has no apparent relationship with estrogen (4, 5, 7). ECCC is more aggressive and prone to early metastasis than endometrioid carcinoma (4, 5, 7). Many studies report a 5-year survival rate of less than 50%, irrespective of the ECCC clinical stage. However, all these studies included small samples having limited persuasion (4). Only a few small retrospective cohort studies and some case reports have explored the prognostic factors in ECCC due to its low incidence. Notably, there are no systematic analyses of ECCC from a large population sample. Therefore, the current study aimed to perform a comprehensive retrospective analysis depending on the Surveillance, Epidemiology, and End Results (SEER) database to evaluate the survival and prognostic risk factors for ECCC. Moreover, it establishes definitive individualized prognostic prediction models to predict the 3-, 5- and 10-year overall survival (OS) in ECCC patients. The findings contribute to developing appropriate treatment and follow-up strategies for ECCC.

Methods

Data source and patient selection

The present study recruited ECCC patients from 18 registries of the SEER database between 2000 and 2018 using the SEER* Stat software (version 8.3.9). The National Cancer Institute established the SEER database in 1973, covering approximately 28% of the U.S. population. It includes age, sex, race, and year of diagnosis (8). All the data for this study were retrieved from the SEER database.

The inclusion criteria for this study were: (1) Primary site-labeled: C54.1-Endometrium. (2) ICD-O-3 Hist/behave: 8310/2: Clear cell adenocarcinoma *in situ*, 8310/3: Clear cell adenocarcinoma, NOS. (3) Year of diagnosis: 2000-2018. (4) Diagnosis confirmed based on histology or cytology. (5) Single primary cancer.

The exclusion criteria were: (1) The survival time was 0 and unknown. (2) The T stage was T0. (3) Unknown race. (4) Unknown AJCC stage.

Variable selection

Each of the following variables was considered for every patient: age at diagnosis, marital status (married, divorced, separated, unmarried, widowed, or unknown), race (white, black, or other), T stage (T1, T2, T3, T4, or TX), lymph node involvement (no, yes, or unknown), distant metastasis (no, yes, or unknown), tumor size (< 4.5 cm, 4.5–6.1 cm, > 6.1 cm or unknown), grade (I: well differentiated, II: moderately differentiated, III: poorly differentiated, IV: undifferentiated, or unknown), the International Federation of Gynecology and Obstetrics (FIGO) stage (I, II, III, or IV), surgery (partial hysterectomy, or total hysterectomy), radiotherapy (no or yes), chemotherapy (no/unknown, or yes), vital status (dead or alive), and time of survival (length in months). The FIGO stage of the patients was obtained based on the TNM staging system since no data on the FIGO stage was available in the SEER database. The endpoint of this study was OS, defined as the time from diagnosis to death or from the last follow-up (patients lost to follow-up).

Statistical analysis

The Chi-square test and Cox regression analysis were performed with the SPSS software. In contrast, the R software performed the C-index, calibration curve, DCA, and Log-rank tests. The Chi-square test determined the potential statistical differences in the demographic clinicopathological features and treatment patterns among patients with early and advanced ECCC. Then, patients having ECCC in FIGO stages I/II and III/IV were randomly assigned to the training and validation cohort at a 7:3 ratio, respectively. Univariate and multivariate Cox regression analyses were performed in the FIGO I/II and III/IV training cohorts to identify the independent OS risk factors. Predictive nomograms were established depending on the results of the Cox regression analysis for OS to predict the 3-, 5- and 10-year OS. The accuracy of the nomograms was validated with the consistency index (C-index). Moreover, the calibration curves were developed to compare the consistency between the OS predicted by the nomogram at 3, 5, and 10 years and their actual values. The clinical benefit of the nomograms and classical FIGO staging system was compared through a decision curve analysis (DCA). The survival curve of patients from different risk groups was analyzed with the Log-rank tests. The significance threshold had been set at $P < 0.05$.

Results

Demographics and clinical characteristics

A total of 1,778 patients diagnosed with ECCC were enrolled in this study between 2000 and 2018, depending on the inclusion and

exclusion criteria. They were divided into 991 (55.7%) patients from FIGO stage I/II and 787 (44.3%) from FIGO stage III/IV. The demographic features of patients with ECCC are listed in Table 1. The median age of the patients was 68 years. Most patients were white (72.5%) and had been subjected to total hysterectomy (87.3%). Almost one-third of the patients (29.3%) had a tumor < 4.5 cm in size, 45.7% were married, 47.6% were in pathological grade III, 42.5% received radiotherapy, and 46.2% received chemotherapy. There were statistical differences between the FIGO stage I/II and FIGO Stage III/IV patients in marital status, race, tumor size, grade, surgery, radiotherapy, and chemotherapy (all $P < 0.05$). The number of patients with tumor size < 4.5 cm and pathological grade I in FIGO stage I/II was more than that in FIGO stage III/IV (36.2% vs. 20.6% and 1.9% vs. 0.8%, respectively) respectively). In contrast, the number of patients with tumor size > 6.1cm and pathological grade III in FIGO Stage III/IV was more than that in FIGO stage I/II (23.1% *vs.* 8.2% and 51.1% *vs.* 44.9%, respectively). Total hysterectomy and radiotherapy rates in the FIGO stage I/II were 92.1% and 46.9%, respectively, higher than the FIGO stage III/IV (81.2% and 36.8%).

Independent risk factors for OS

Univariate and multivariate cox analyses indicated that age at diagnosis, marital status, T stage, tumor size, and surgery were independent risk factors for OS among patients with FIGO stage

TABLE 1 Basic characteristics of ECCC patients from the total population, FIGO stage I/II, and FIGO stage III/IV cohorts.

Variables	Total population		FIGO stage I/II		FIGO stage III/IV		P value
	N=1778		N=991		N=787		
Age (years)	68 (27~96)		68 (31~96)		68 (27~95)		0.165
Marital status							0.018
Married	813	45.7	479	48.3	334	42.4	
Divorced	192	10.8	99	10.0	93	11.8	
Separated	22	1.2	11	1.1	11	1.4	
Unmarried	275	15.5	133	13.4	142	18.0	
Widowed	392	22.0	215	21.7	177	22.5	
Unknown	84	4.7	54	5.4	30	3.8	
Race							0.002
White	1289	72.5	739	74.6	550	69.9	
Black	312	17.5	146	14.7	166	21.1	
Other	177	10.0	106	10.7	71	9.0	
Tumor size (cm)							<0.001
<4.5	521	29.3	359	36.2	162	20.6	
4.5-6.1	226	12.7	106	10.7	120	15.2	
>6.1	263	14.8	81	8.2	182	23.1	
Unknown	768	43.2	445	44.9	323	41.0	
Grade							0.006
I	25	1.4	19	1.9	6	0.8	
II	95	5.3	65	6.6	30	3.8	
III	847	47.6	445	44.9	402	51.1	
IV	331	18.6	189	19.1	142	18.0	
Unknown	480	27.0	273	27.5	207	26.3	
Surgery							<0.001
Partial hysterectomy	226	12.7	78	7.9	148	18.8	
Total hysterectomy	1552	87.3	913	92.1	639	81.2	
Radiotherapy							<0.001
No/Unknown	1023	57.5	526	53.1	497	63.2	
Yes	755	42.5	465	46.9	290	36.8	
Chemotherapy							<0.001
No/Unknown	957	53.8	697	70.3	260	33.0	
Yes	821	46.2	294	29.7	527	67.0	
Median OS (m)	34		56 (1-227)		21 (1-225)		<0.001
Cases of dead	926		366	39.5	560	60.5	

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TABLE 2 Univariate and multivariate COX analyses of OS in the FIGO stage I/II training cohort.

Variables	Univariate analysis				Multivariate analysis			
	HR	95%CI		P value	HR	95%CI		P value
		Lower	Upper			Lower	Upper	
Age (years)	1.078	1.064	1.091	<0.001	1.071	1.056	1.086	<0.001
Marital status								
Divorced vs Married	1.450	0.933	2.252	0.098	1.183	0.758	1.846	0.460
Separated vs Married	1.513	0.478	4.786	0.481	3.892	1.194	12.687	0.024
Unmarried vs Married	1.170	0.772	1.772	0.460	1.423	0.922	2.198	0.111
Widowed vs Married	2.954	2.204	3.959	<0.001	1.453	1.054	2.003	0.023
Unknown vs Married	1.479	0.828	2.640	0.186	1.031	0.572	1.856	0.920
Race								
Black vs White	1.055	0.748	1.489	0.759	0.713	0.494	1.028	0.070
Other vs White	0.558	0.348	0.894	0.015	0.795	0.492	1.285	0.349
T								
T2 vs T1	2.029	1.553	2.650	<0.001	1.700	1.283	2.252	<0.001
Tumor size (cm)								
4.5~6.1 vs <4.5	0.739	0.428	1.274	0.276	0.632	0.364	1.096	0.102
>6.1 vs <4.5	1.998	1.244	3.211	0.004	1.884	1.140	3.112	0.013
Unknown vs <4.5	1.434	1.070	1.922	0.016	1.261	0.926	1.716	0.141
Grade								
II vs I	1.511	0.567	4.028	0.409	–	–	–	–
III vs I	1.564	0.639	3.829	0.328	–	–	–	–
IV vs I	1.385	0.548	3.501	0.491	–	–	–	–
Unknown vs I	1.456	0.586	3.621	0.419	–	–	–	–
Surgery								
Total hysterectomy vs Partial hysterectomy	0.175	0.126	0.243	<0.001	0.230	0.158	0.336	<0.001
Radiotherapy								
Yes vs No/Unknown	1.062	0.829	1.360	0.633	–	–	–	–
Chemotherapy								
Yes vs No/Unknown	0.692	0.507	0.945	0.021	1.147	0.824	1.598	0.417

I/II (all $P < 0.05$) (Table 2). Additionally, age at diagnosis, T stage, lymph node involvement, distant metastasis, tumor size, surgery, radiotherapy, and chemotherapy were independent predictors for OS stage III/IV patients ($P < 0.05$) (Table 3).

Nomograms for the prediction of OS

Predictive nomograms were developed depending on the independent risk variables to predict the 3-, 5- and 10-year OS (Figure 1). Surgery (31 scores) had the most prognostic impact on FIGO stage I/II among the categorical variables. It was followed by marital status (28 scores), tumor size (24 scores), and T stage (13 scores) (Table 4). Surgery (52 scores) was also the most important factor in the FIGO stage III/IV among the categorical variables, followed by distant metastasis (48 scores), T stage (38 scores), tumor size (25 scores), chemotherapy (23 scores), lymph node involvement (20 scores), and radiotherapy (14 scores) (Table 5). The internal and external validations of the nomograms were performed in the training and validation cohorts, respectively. The C-indexes of the training and validation groups from the FIGO stage I/II and FIGO stage III/IV were 0.766 (95% CI: 0.750-0.782) and 0.697 (95% CI: 0.640–0.754), and 0.721 (95% CI: 0.708–0.734) and 0.708 (95% CI: 0.667–0.749), respectively. These values depicted that the constructed nomograms showed a good predictive performance. In addition, the calibration curves of the two groups had a good agreement between the nomogram-predicted and the real observation values (Figures 2 and 3). The DCA models revealed that the nomograms outperformed the FIGO staging system in clinical benefit (Figures 4 and 5), suggesting that nomograms showed more predictive power than the traditional staging system.

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TABLE 3 Univariate and multivariate COX analyses of OS in the FIGO stage III/IV training cohort.

Variables	Univariate analysis				Multivariate analysis			
	HR	95%CI		P value	HR	95%CI		P value
		Lower	Upper			Lower	Upper	
Age (years)	1.028	1.017	1.038	<0.001	1.020	1.008	1.032	0.001
Marital status								
Divorced vs Married	1.472	1.050	2.062	0.025	1.097	0.775	1.553	0.603
Separated vs Married	1.508	0.618	3.679	0.367	1.245	0.500	3.101	0.637
Unmarried vs Married	1.232	0.928	1.636	0.149	1.071	0.794	1.444	0.655
Widowed vs Married	1.831	1.427	2.350	<0.001	1.172	0.889	1.544	0.261
Unknown vs Married	0.896	0.518	1.550	0.694	0.598	0.338	1.060	0.079
Race								
Black vs White	1.444	1.130	1.845	0.003	1.275	0.986	1.649	0.064
Other vs White	0.828	0.574	1.194	0.311	0.866	0.595	1.260	0.452
T stage								
T2 vs T1	1.376	0.898	2.108	0.142	1.288	0.830	1.997	0.259
T3 vs T1	1.886	1.365	2.606	<0.001	2.037	1.452	2.858	<0.001
T4 vs T1	2.984	1.992	4.469	<0.001	2.297	1.492	3.536	<0.001
TX vs T1	3.543	2.364	5.309	<0.001	1.880	1.188	2.974	0.007
Lymph nodes involvement								
Yes vs No	0.905	0.729	1.124	0.367	1.430	1.129	1.812	0.003
Unknown vs No	1.925	1.417	2.617	<0.001	1.476	1.052	2.070	0.024
Distant metastasis								
Yes vs No	2.441	1.993	2.989	<0.001	1.970	1.538	2.524	<0.001
Unknown vs No	5.839	0.813	41.928	0.079	1.325	0.173	10.153	0.787
Tumor size (cm)								
4.5~6.1 vs <4.5	1.465	1.037	2.068	0.030	1.482	1.039	2.114	0.030
>6.1 vs <4.5	1.275	0.924	1.759	0.140	1.180	0.842	1.655	0.337
Unknown vs <4.5	1.596	1.208	2.109	0.001	1.053	0.763	1.452	0.754
Grade								
II vs I	0.71	0.226	2.233	0.558	–	–	–	–
III vs I	0.897	0.333	2.414	0.830	–	–	–	–
IV vs I	0.753	0.274	2.069	0.583	–	–	–	–
Unknown vs I	0.888	0.327	2.414	0.816	–	–	–	–
Surgery								
Total hysterectomy vs Partial hysterectomy	0.337	0.264	0.430	<0.001	0.372	0.283	0.490	<0.001
Radiotherapy								
Yes vs No/Unknown	0.550	0.444	0.682	<0.001	0.680	0.537	0.861	0.001
Chemotherapy								
Yes vs No/Unknown	0.568	0.462	0.698	<0.001	0.582	0.461	0.734	<0.001

The novel risk-stratification system

The ECCC patients were divided into low-risk, medium-risk, and high-risk groups based on the total scores from each variable. The median survival time in the high, medium, and low-risk groups for the FIGO stage I/II were 14.5, 40, and 69 months (Figure 6A), respectively, and 7, 18, and 33 months, respectively (Figure 6B) for the FIGO stage III/IV. The log-rank tests revealed that the survival times for the three risk groups differed significantly (both $P < 0.001$).

Discussion

Unlike EC, most ECCC patients have a tumor negative for the estrogen and progesterone receptors. However, positive for hepatocyte nuclear factor 1β and Napsin A. Notably, *TP53* is the most commonly mutated gene in ECCC (4, 9–11). The abnormal p53 expression is considered a poor prognostic factor for EC (11). Previous studies observed that the mutation rate of the *TP53* gene in POLE wild-type ECCC is 46%, while that of non-POLE endometrioid carcinoma is only 11% (11). ECCC patients

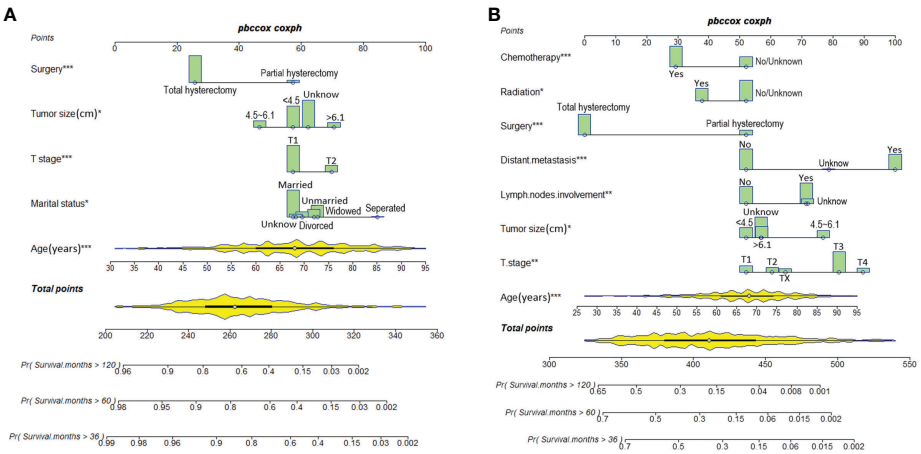

FIGURE 1
Nomograms for predicting 3-, 5-, and 10-year OS among patients with FIGO stage I/II **(A)** and FIGO stage III/IV **(B)** ECCC.

are accompanied by high-risk factors for poor prognoses, including advanced clinical stage, deep muscular infiltration, lymphovascular space involvement, and distant metastasis, with a high recurrence rate, high mortality, and poor prognosis than in type I EC (3, 4, 7). Currently, The Cancer Genome Atlas (TCGA) classification is the most authoritative classification system of EC. However, it does not include ECCC patients. Therefore, it is essential to analyze the demographic and clinicopathological characteristics of ECCC patients independently. Moreover, we must comprehensively evaluate their prognosis to develop an adequate treatment guide for ECCC patients.

TABLE 4 Nomogram scores of each independent prognostic factor in the FIGO stage I/II ECCC patients.

Variables	Scores
Age (years)	1.56*Age-48.71
Marital status	
Married	57
Divorced	60
Separated	85
Unmarried	64
Widowed	65
Unknown	58
T stage	
T1	57
T2	70
Tumor size (cm)	
<4.5	57
4.5~6.1	47
>6.1	71
Unknown	62
Surgery	
Partial hysterectomy	57
Total hysterectomy	26

Our study identified that age was an essential prognostic factor among ECCC patients, positively correlating with the risk of death. The findings of this study concerning the relationship between age and prognosis in EC patients were consistent with previous studies. A retrospective study found that patients aged ≤ 40 include more favorable prognostic factors, such as a higher proportion of non-invasive carcinoma, a lower proportion in the uterine segment involvement, and less invasion of the lymphatic vascular space than in EC patients aged 40-60 years (12). Furthermore, EC patients aged ≤ 40 years had a lower probability of mismatch repair defects due to MLH1 methylation, a mutation associated with poor prognosis, than patients aged 41-60 years (12). Another study also found that ECCC patients aged ≥ 70 had worse progression-free survival time and OS independent of the treatment modality they were subjected to (13). An investigation on the influence of marital status on the diagnosis and prognosis of EC revealed that marriage was a protective prognostic factor for OS and cancer-specific survival among EC patients. Unmarried, divorced/separated, and widowed patients showed a higher risk of death than married patients (14). This phenomenon was because married patients were more likely to be diagnosed early, possibly due to the stability of the endogenous hormone levels in women associated with emotional benefits (14). In this study, separated and widowed patients having early ECCC had a higher risk of death than married patients at the same stage. However, marital status had no significant effect on the prognosis of patients with advanced ECCC. Previous studies have evaluated the relationship between tumor diameter, myometrium invasion

TABLE 5 Nomogram scores of each independent prognostic factor in the FIGO stage III/IV ECCC patients.

Variables	Scores
Age (years)	1.29*age-34.59
T stage	
T1	52
T2	60
T3	82
T4	90
TX	65
Lymph nodes involvement	
No	52
Yes	71
Unknown	72
Distant metastasis	
No	52
Yes	100
Unknown	79
Tumor size (cm)	
<4.4	52
4.5~6.1	77
>6.1	57
Unknown	57
Surgery	
Partial hysterectomy	52
Total hysterectomy	0
Radiotherapy	
No/Unknown	52
Yes	38
Chemotherapy	
No/Unknown	52
Yes	29

depth, and prognosis of EC patients. Nilufer et al. observed that more than half of the ECCC patients with a tumor diameter > 2 cm were prone to myometrial invasion (15). Kohei et al. identified that large tumor size is associated with deeper myometrial infiltration and lymph node metastasis among EC patients (16). In this study, ECCC patients with large tumor sizes and late T stages significantly enhanced the risk of death. These findings were consistent with a retrospective study that inferred that large tumor size and deep muscle invasion could be associated with poor prognosis among ECCC patients (17). Lymphatic metastasis is the main route of EC metastasis. The survival time of patients is significantly shortened once they develop lymph node metastasis, indicating disease progression (18). This study also depicted that OS is significantly decreased in ECCC patients with lymph node involvement.

A study revealed that black patients with EC were more likely to develop invasive and non-endometrioid cancer than white EC patients from America (19). However, this study did not observe a correlation between race and prognosis in ECCC patients. The degree of differentiation was not an independent prognostic factor for ECCC. Therefore, we hypothesized that the prognosis was poor irrespective of the degree of differentiation due to the high invasiveness of ECCC and thus had no significant effect on the prognosis of ECCC patients.

The preferred treatment method to cure ECCC is extensive staging surgery, including total uterine and bilateral adnexectomy, pelvic and para-aortic lymph node dissection, more significant omentum biopsy, and examination of the peritoneal washing fluid (20–22). The advantage of surgery is that the tumor stage is more accurately identified, facilitating the subsequent selection of the appropriate adjuvant treatment. Our results revealed that total hysterectomy was a favorable factor for a good prognosis. The risk of death after total hysterectomy was lower than after partial hysterectomy in both early and advanced stages. Therefore, active surgical treatment was recommended for ECCC patients. Patients who cannot undergo radical surgery should also be treated with tumor-reducing surgery, depending on their physical condition. Adjuvant radiotherapy and chemotherapy are fundamental approaches in treating ECCC. Numerous studies underline that the choice of adjuvant radiotherapy and chemotherapy is associated with the stage of ECCC (23). The adjuvant therapy in patients with early ECCC should be chosen based on prognosis-related factors, such as age and the depth of myometrial invasion. Although our results depicted that radiotherapy and chemotherapy had no role in improving the prognosis of patients with early ECCC, this factor did not hinder patients with early ECCC from benefiting from radiotherapy and chemotherapy. Our results were attributed to the SEER database limitations, which did not allow us to know the adjuvant treatment regimen and course, thus preventing the specific stratification study of the enrolled patients.

The FIGO stage of EC represents the pathological surgical stage, which includes factors related to patient prognoses, such as depth of muscular invasion, nodal metastasis, and distant metastasis. It is the primary tool clinicians use to evaluate the prognosis of EC patients. However, the FIGO stage does not include other factors associated with the survival of patients, such as age, marital status, and treatment methods. At the same time, the nomograms contain the demographic, clinical characteristics, and therapeutic approaches of the ECCC patients. Additionally, the DCA curves applied to ECCC patients established that nomograms had better clinical benefits than traditional FIGO stages in stages I/II and III/IV. Therefore, the nomograms had a significant practical value due to their good accuracy, excellent discrimination ability, and clinical benefits.

Compared to the existing prognostic classification, our predictive model demonstrated several strengths. All the

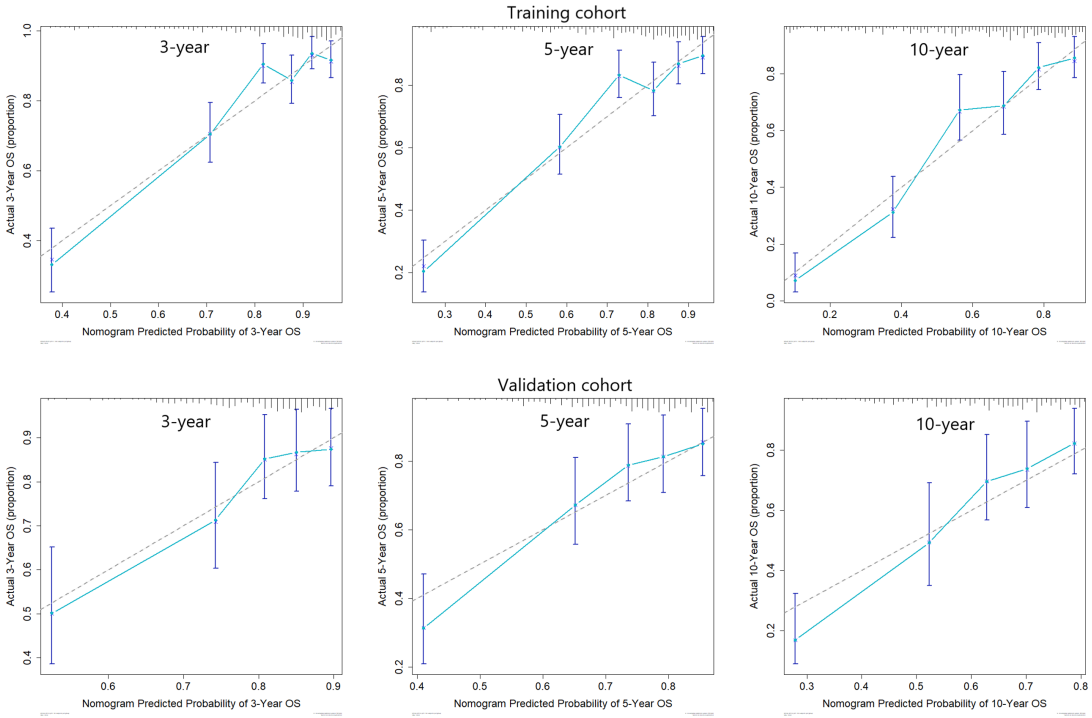

FIGURE 2
Calibration curves for 3-, 5-, and 10-year OS among patients with FIGO stage I/II ECCC within the training and validation cohorts.

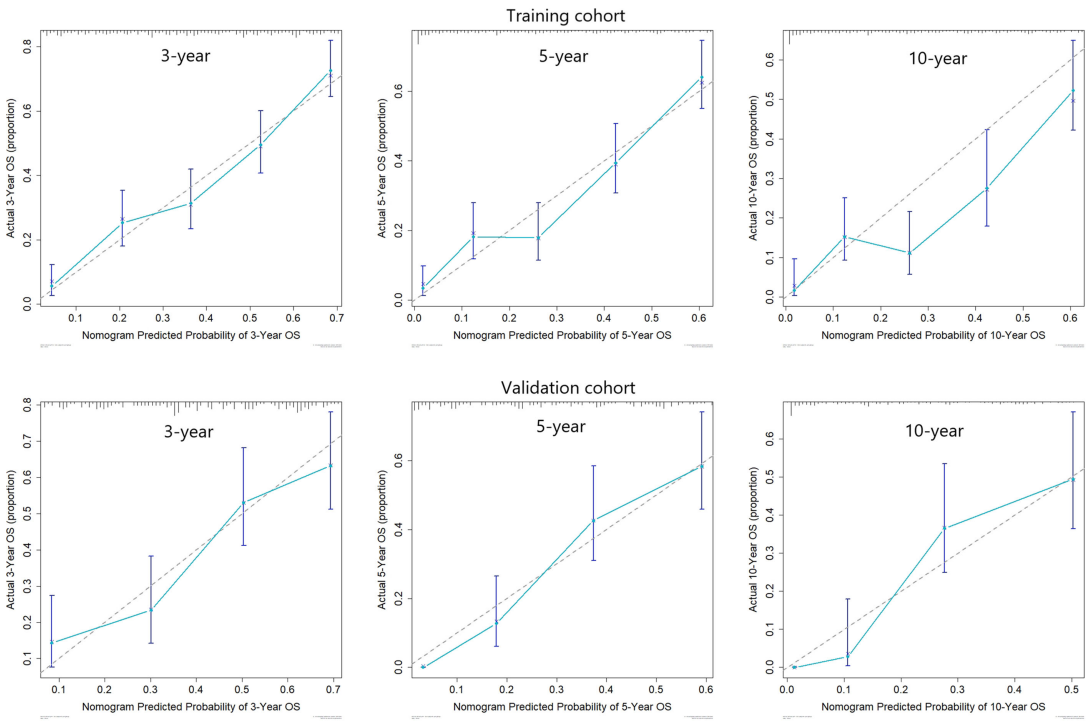

FIGURE 3
Calibration curves for 3-, 5-, and 10-year OS among patients with FIGO stage III/IV ECCC within the training and validation cohorts.

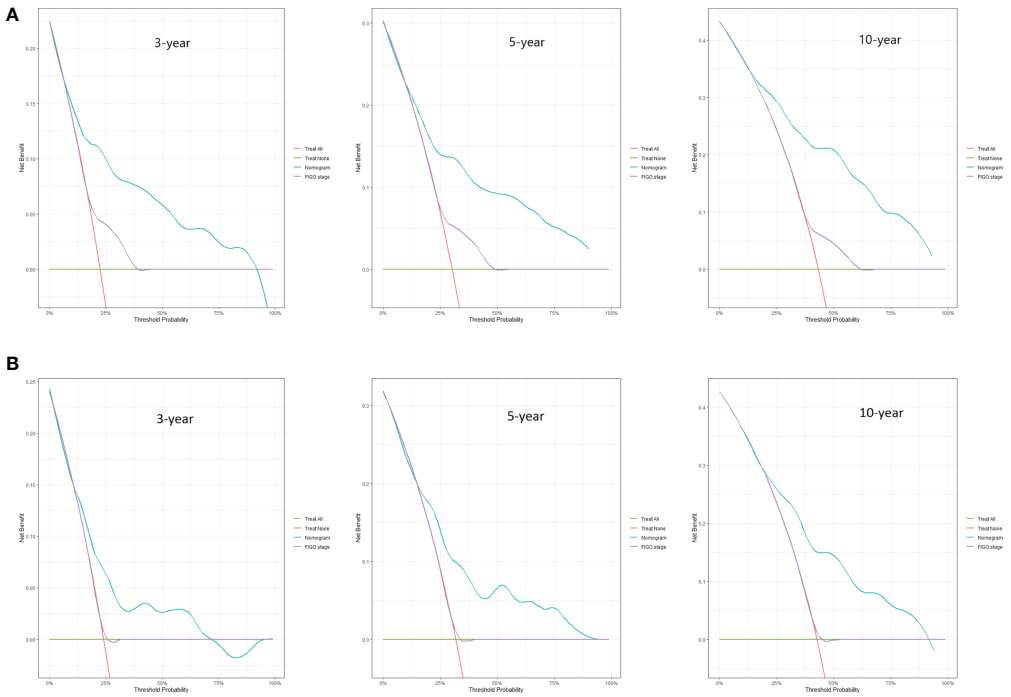

FIGURE 4
DCA curves for 3-, 5-, and 10-year OS among ECCC patients with FIGO stage I/II within the training cohort **(A)** and validation cohort **(B)**.

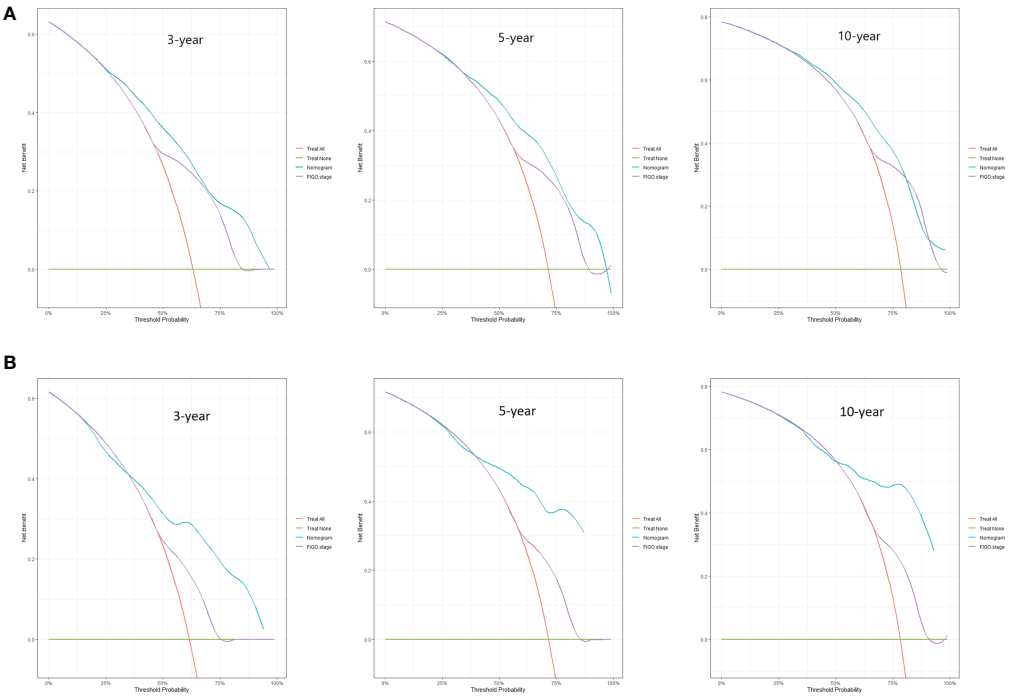

FIGURE 5
DCA curves for 3-, 5-, and 10-year OS among ECCC patients with FIGO stage III/IV within the training cohort **(A)** and validation cohort **(B)**.

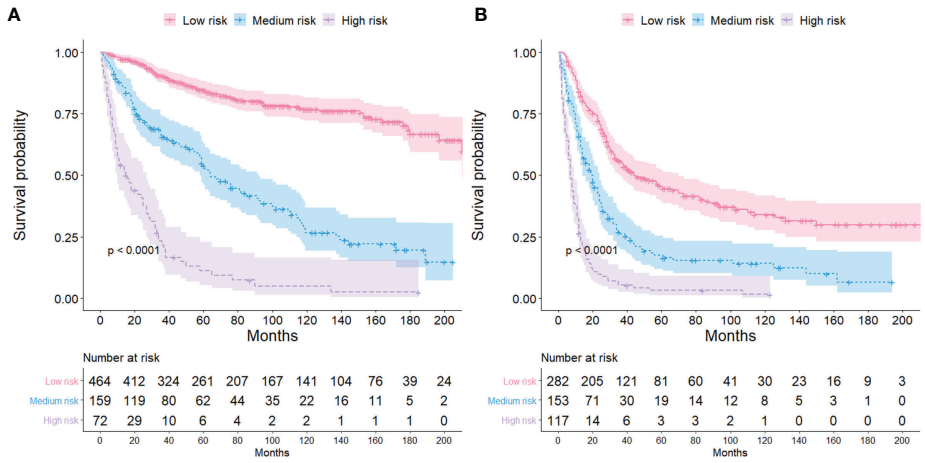

FIGURE 6
Kaplan-Meier survival curves for different risk groups among ECCC patients with FIGO stage I/II **(A)** and FIGO stage III/IV **(B)**.

clinical variables included in the survival prediction model were easily accessible. This study enrolled ECCC patients; thus, our nomogram was more applicable to ECCC patients than other classification systems. Moreover, the nomogram intuitively and clearly showed 3-, 5- and 8-year survival rates, which is convenient for clinicians. However, this study had several limitations. Firstly, all the variables selected in our study were clinical characteristics. Several genetic and epigenetic features, including Non-Coding RNAs, identified as predictors of EC patients in previous studies (24, 25), were not included in this study due to the limitations of the SEER database. The absence of these new molecular characteristics deteriorated the practicability of the nomogram model. Secondly, this was a retrospective study; thus, the bias significantly affected the results because the information about the patients was partially missing. For instance, the tumor size of 43.2% of the patients was unknown, which significantly reduced the accuracy of the prediction model. Finally, it was unclear whether the patients received neoadjuvant therapy, and the specific information based on surgery, radiotherapy, chemotherapy, and a potential targeted therapy was unknown.

Conclusions

Nomograms for predicting 3-, 5-, and 10-year OS in ECCC patients were successfully constructed. Moreover, new risk stratification systems were further built to stratify patients into different risk groups. These predictive models may be valuable tools to aid ECCC management and treatment in clinical practice.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

ZL, XC, and PC conceived and designed the study. YZ and HZ helped in data collection. PC and YZ performed the analysis and wrote the manuscript. All the authors have reviewed the final draft of the manuscript and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654

3. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. *Lancet* (2016) 387(10023):1094–108. doi: 10.1016/S0140-6736(15)00130-0

4. Kurman RJ, Carcangiu ML, Herrington CS, Young RH eds. *World health organization classification of tumours of female reproductive organs*. Lyon: IARC Press (2014).

5. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol* (1983) 15(1):10–7. doi: 10.1016/0090-8258(83)90111-7

6. Bogani G, Ray-Coquard I, Concin N, Ngoi NYL, Morice P, Enomoto T, et al. Uterine serous carcinoma. *Gynecol Oncol* (2021) 162(1):226–34. doi: 10.1016/j.ygyno.2021.04.029

7. Nieto K, Adams W, Pham N, Block AM, Grover S, Small WJr, et al. Adjuvant therapy in patients with clear cell endometrial carcinoma: An analysis of the national cancer database. *Gynecol Oncol* (2018) 148(1):147–53. doi: 10.1016/j.ygyno.2017.11.010

8. Wang S, Chen L, Feng Y, Swinnen JV, Jonscher C, Van Ongeval C, et al. Heterogeneity of synchronous lung metastasis calls for risk stratification and prognostic classification: Evidence from a population-based database. *Cancers (Basel)* (2022) 14(7):1608. doi: 10.3390/cancers14071608

9. Bogani G, Ray-Coquard I, Concin N, Ngoi NYL, Morice P, Enomoto T, et al. Clear cell carcinoma of the endometrium. *Gynecol Oncol* (2022) 164(3):658–66. doi: 10.1016/j.ygyno.2022.01.012

10. Kim SR, Cloutier BT, Leung S, Cochrane D, Britton H, Pina A, et al. Molecular subtypes of clear cell carcinoma of the endometrium: Opportunities for prognostic and predictive stratification. *Gynecol Oncol* (2020) 158(1):3–11. doi: 10.1016/j.ygyno.2020.04.043

11. DeLair DF, Burke KA, Selenica P, Lim RS, Scott SN, Middha S, et al. The genetic landscape of endometrial clear cell carcinomas. *J Pathol* (2017) 243(2):230–41. doi: 10.1002/path.4947

12. Son J, Carr C, Yao M, Radeva M, Priyadarshini A, Marquard J, et al. Endometrial cancer in young women: prognostic factors and treatment outcomes in women aged ≤40 years. *Int J Gynecol Cancer* (2020) 30(5):631–9. doi: 10.1136/ijgc-2019-001105

13. Armbruster SD, Previs R, Soliman PT, Westin SN, Fellman B, Jhingran A, et al. Clinicopathologic features and treatment in patients with early stage uterine clear cell carcinoma: A 16-year experience. *Gynecol Oncol* (2019) 154(2):328–32. doi: 10.1016/j.ygyno.2019.06.001

14. Dong J, Dai Q, Zhang F. The effect of marital status on endometrial cancer-related diagnosis and prognosis: a surveillance epidemiology and end results database analysis. *Future Oncol* (2019) 15(34):3963–76. doi: 10.2217/fon-2019-0241

15. Cetinkaya N, Selcuk İ, Ozdal B, Meydanli MM, Gungor T. Prognostic factors in endometrial clear cell carcinoma. *Arch Gynecol Obstet* (2017) 295 (1):189–95. doi: 10.1007/s00404-016-4183-x

16. Nakamura K, Nakayama K, Ishikawa N, Minamoto T, Ishibashi T, Ohnishi K, et al. Preoperative tumor size is associated with deep myometrial invasion and lymph node metastases and is a negative prognostic indicator for patients with endometrial carcinoma. *Oncotarget* (2018) 9(33):23164–72. doi: 10.18632/oncotarget.25248

17. Zhang Z, Gao P, Bao Z, Zeng L, Yao J, Chai D, et al. Clear cell carcinoma of the endometrium: Evaluation of prognostic parameters in 27 cases. *Front Oncol* (2021) 11:732782. doi: 10.3389/fonc.2021.732782

18. AlHilli MM, Mariani A. The role of para-aortic lymphadenectomy in endometrial cancer. *Int J Clin Oncol* (2013) 18(2):193–9. doi: 10.1007/s10147-013-0528-7

19. Crosbie EJ, Kitson SJ, McAlpine JN, Mukhopadhyay A, Powell ME, Singh N. Endometrial cancer. *Lancet* (2022) 399(10333):1412–28. doi: 10.1016/S0140-6736(22)00323-3

20. Sarı ME, Meydanlı MM, Türkmen O, Cömert GK, Turan AT, Karalök A, et al. Prognostic factors and treatment outcomes in surgically-staged non-invasive uterine clear cell carcinoma: a Turkish gynecologic oncology group study. *J Gynecol Oncol* (2017) 28(4):e49. doi: 10.3802/jgo.2017.28.e49

21. Mahdi H, Lockhart D, Moselmi-Kebria M. Prognostic impact of lymphadenectomy in uterine clear cell carcinoma. *J Gynecol Oncol* (2015) 26 (2):134–40. doi: 10.3802/jgo.2015.26.2.134

22. Sugiyama T, Takeuchi S, Itamochi H. Surgical management of non-invasive uterine clear cell carcinoma. *J Gynecol Oncol* (2017) 28(4):e55. doi: 10.3802/jgo.2017.28.e55

23. Olawaiye AB, Leath CA3rd. Contemporary management of uterine clear cell carcinoma: A society of gynecologic oncology (SGO) review and recommendation. *Gynecol Oncol* (2019) 155(2):365–73. doi: 10.1016/j.ygyno.2019.08.031

24. Cavaliere AF, Perelli F, Zaami S, Piergentili R, Mattei A, Vizzielli G, et al. Towards personalized medicine: Non-coding RNAs and endometrial cancer. *Healthcare (Basel)* (2021) 9(8):965. doi: 10.3390/healthcare9080965

25. Piergentili R, Zaami S, Cavaliere AF, Signore F, Scambia G, Mattei A, et al. Non-coding RNAs as prognostic markers for endometrial cancer. *Int J Mol Sci* (2021) 22(6):3151. doi: 10.3390/ijms22063151

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EDITED BY
Janina Tokarz,
Helmholtz Association of German Research Centres (HZ), Germany

REVIEWED BY
Eswari Dodagatta-Marri,
University of California, San Francisco, United States
Carlo Ronsini,
Università degli Studi della Campania "Luigi Vanvitelli", Italy
Jin Gao,
Washington State University, United States
Li-Xing Yang,
National Cheng Kung University, Taiwan
Qian Chen, Guangxi Medical University Cancer Hospital, China

*CORRESPONDENCE
Zhiqi Wang
wangzqnet@sina.com

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Tumor immune microenvironment in endometrial cancer of different molecular subtypes: evidence from a retrospective observational study

Yibo Dai[1], Luyang Zhao[1], Dingchao Hua[2], Lina Cui[2], Xiaobo Zhang[3], Nan Kang[3], Linlin Qu[3], Liwei Li[1], He Li[1], Danhua Shen[3], Zhiqi Wang[1]* and Jianliu Wang[1]

[1]Department of Obstetrics and Gynecology, Peking University People's Hospital, Beijing, China, [2]Department of Medical Affairs, 3D Medicines Inc., Shanghai, China, [3]Department of Pathology, Peking University People's Hospital, Beijing, China

Objective: Tumor immune microenvironmental features may predict survival and guide treatment. This study aimed to comprehensively decipher the immunological features of different molecular subtypes of endometrial cancer.

Methods: In this retrospective study, 26 patients with primary endometrial cancer and four with recurrent disease treated in our center from December 2018 to November 2021 were included. Next-generation sequencing was performed on tumor samples. Patients were classified into four subtypes, including *POLE* mutant, microsatellite instability high (MSI-H), no specific molecular profile (NSMP) and *TP53* mutant subtypes. Tumor-infiltrating immune cells were quantified using multiplex immunofluorescence assays.

Results: Of the 26 primary endometrial cancer cases, three were *POLE* mutant, six were MSI-H, eight were NSMP and nine were *TP53* mutant. Of the four recurrent cases, two belonged to the NSMP subtype and two belonged to the *TP53* mutant subtype. The tumor mutation burden (TMB) levels of *POLE* mutant and MSI-H cases were significantly higher than that of the other two subtypes ($p < 0.001$). We combined *POLE* mutant and MSI-H subtypes into the TMB high (TMB-H) subtype. The TMB-H subtype showed a high degree of infiltration of $CD8^+$ T cells. In the NSMP subtype, the overall degree of intra-tumoral infiltrating immune cells was low. In the *TP53* mutant subtype, the densities of both PD-$L1^+$ macrophages ($p = 0.047$) and PD-1^+ T cells ($p = 0.034$) in tumor parenchyma were the highest among the four subtypes.

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Conclusion: Endometrial cancer of TMB-H, NSMP and *TP53* mutant subtypes displayed phenotypes of normal immune response, absence of immune infiltration, and suppressed immune response, respectively. These features may provide mechanistic explanations for the differences in patients' prognosis and efficacy of immune checkpoint blockade therapies among different endometrial cancer subtypes.

KEYWORDS

uterine neoplasms (MeSH), molecular subtype, tumor immune microenvironment, prognosis, immunotherapy

Introduction

In the past decade, the development of high-throughput sequencing technologies and computational algorithms has facilitated the understanding of cancer genomics. In endometrial cancer (EC), the establishment of molecular subtypes by the Cancer Genome Atlas (TCGA) consortium (1), on the one hand, has affected patient stratification, promoting individualized clinical management. In 2021, the National Comprehensive Cancer Network (NCCN) guidelines for uterine neoplasms recommended molecular subtyping in EC diagnosis (2). In addition, the European Society of Gynecologic Oncology (ESGO)/European Society for Radiotherapy and Oncology (ESTRO)/European Society of Pathology (ESP) guideline for EC further incorporated molecular subtypes into the risk stratification system for guiding postoperative adjuvant therapies (3).

On the other hand, molecular subtypes, to some extent, also indicated possibly different routes of EC development and differences in cancer microenvironmental features. Specifically, immune components in the cancer microenvironment have attracted increasing attention in recent years due to their potential roles in predicting patients' prognosis and guiding immune checkpoint blockade therapies (4, 5). Improvements in methodologies, including single-cell and spatial transcriptomics, immune deconvolution algorithms (6) and multiplex immunofluorescence assays (7), have significantly promoted research in cancer immune microenvironment. In 2018, European researchers, for the first time, established an immune risk score based on tumor-infiltrating T cells in colon cancer tissue and validated its effectiveness in predicting recurrence in a large retrospective cohort (8). In EC, previous findings have indicated the prognostic value of immune-related gene signatures (9–11). However, most previous studies on the immune microenvironmental features of EC were only based on data of next-generation sequencing. Furthermore, extensive data regarding the association of tumor-infiltrating immune cells with patients' molecular features are still needed to establish incorporated risk stratification systems for clinical applications.

In this study, we aimed to analyze the tumor immune microenvironmental features in different molecular subtypes of EC using multiplex immunofluorescence method, so as to provide a better understanding of the mechanisms underlying the differences in prognosis and immunotherapeutic efficacy among different EC subtypes.

Materials and methods

Study population and data collection

This retrospective study included 30 EC cases treated at Peking University People's Hospital from December 2018 to November 2021. The cases were consecutively included on the condition that for each case the genetic testing and PD-L1 immunohistochemical assays were performed on fresh surgical specimens. We avoided using archived pathological specimens for the above assays so as to guarantee the accuracy of the testing results. Among all the eligible patients, 26 were primary cases and 4 were recurrent cases. All surgeries were conducted by experienced gynecologic oncologists in our center. For all early-stage primary EC cases, surgical staging was conducted, including total hysterectomy + bilateral salphingoophrectomy ± pelvic lymphnectomy ± paraaortic lymphnectomy ± omentectomy. Hysterectomy was performed through either open or laparoscopic approaches, following the routine procedures (12). Pelvic washing was collected during surgeries and sent for cytology testing. For advanced-stage primary EC cases, cytoreductive surgery was conducted. Postoperative adjuvant therapies, including chemotherapies and radiotherapies, were delivered based on patients' clinicopathological risk factors. For recurrent cases, surgery was performed in an individualized manner, and postoperative chemotherapies were delivered. All pathological reviews were finished in the Department of Pathology of Peking University People's Hospital by two independent gynecologic pathologists. When disconcordance occurred in pathological diagnosis, the case was sent to the expert committee of the

Department of Pathology for a final diagnosis. Patients' clinical data, including age, height and weight at diagnosis, disease history, disease stage, and pathological information were retrieved from the electronic medical record system of the hospital. The staging was determined according to the International Federation of Gynecology and Obstetrics (FIGO) 2009 staging system (13). Histopathological classification was performed according to the World Health Organization (WHO) 2014 classification system (14). The grading of tumors was in accordance with the FIGO criteria (15). The study was approved by the Institutional Review Board of Peking University People's Hospital (2022PHB097-001).

Genetic testing

(1) Sample processing and DNA extraction: Formalin-fixed paraffin-embedded (FFPE) tissue sections were stained with hematoxylin and eosin (H&E) to evaluate tumor cell content. Samples with a tumor content of ≥ 20% were used for subsequent analyses. After deparaffinization, the samples were incubated together with lysis buffer and proteinase K at 56°C overnight until completely digested. Then the lysate was incubated at 80°C for 4 hours to reverse formaldehyde crosslinks. Genomic DNA was extracted with the ReliaPrepTM FFPE gDNA Miniprep System (Promega) and then quantified using the QubitTM dsDNA HS Assay Kit (Thermo Fisher Scientific). For each sample subject to the following steps, a final concentration of DNA ≥ 0.6 ng/μL was needed, and the total content of DNA was required to be ≥ 30 ng.

(2) Library preparation and targeted capture: DNA extracts were fragmented by an S220 focused ultrasonicator (Covaris). Then, we prepared libraries using the KAPA Hyper Prep Kit (KAPA Biosystems). For each library, the concentration and size distribution of DNA fragments were quantified using a Qubit 3.0 fluorometer (Thermo Fisher Scientific) and a LabChip GX Touch HT Analyzer (PerkinElmer) respectively. The DNA concentration was approximately 50-80 ng/μL, and the length of the DNA fragments was approximately 390 bp. The library was then subjected to hybridization with probes targeting 733 cancer-related genes. The probe baits were individually synthesized 5′ biotinylated 120 bp DNA oligonucleotides (IDT). Repetitive elements were filtered out from intronic baits according to the annotation by UCSC Genome RepeatMasker (16). The xGen® Hybridization and Wash Kit (IDT) was used for hybridization enrichment. The concentration and fragment size distribution of the final library were quantified with a Qubit 3.0 fluorometer (Thermo Fisher Scientific) and a LabChip GX Touch HT Analyzer (PerkinElmer) respectively.

(3) DNA sequencing and data processing: The final libraries were loaded onto a NovaSeq 6000 platform (Illumina) for paired-end sequencing with a mean sequencing depth of 800-1000×. Raw sequencing data were then mapped to the reference human genome hg19 with the Burrows–Wheeler Aligner (v0.7.12) (17). PCR duplicate reads were removed with Picard (v1.130), and sequence metrics were collected with SAMtools (v1.1.19). Single nucleotide variants and indels were then analyzed. Variants were filtered by their unique supporting read depth, strand bias and base quality based on the method in a previous study (18). Single-nucleotide polymorphism (SNPs) were annotated by ANNOVAR against the databases dbSNP (v138), 1000Genome and ESP6500 (population frequency > 0.015). Finally, only missense, silent, nonsense, frameshift and non-frameshift indel mutations were kept.

(4) Determination of microsatellite status: In this study, microsatellite status was determined according to the previously described algorithm (19). We examined 100 microsatellite loci, and the top 30 loci with the best coverage were used for microsatellite-instability (MSI) score calculation. The model for determining the stability of each locus is as follows:

$$P(X = n_i) = C_{N_i}^{n_i} p_i^{n_i} (1 - p_i)^{N_i - n_i}$$

In the model, i is the locus being examined, p_i is the cumulative percentage at the cut-point repeat length of the microsatellite-stable (MSS) subtype, n_i represents the number of unstable reads, and N_i represents the total number of reads for that locus. A locus was considered unstable if $P(X \geq n_i) \leq 0.001$. An MSI score was defined as the percentage of unstable loci. Any sample with an MSI score of ≥ 0.4 was classified as MSI high (MSI-H).

(5) Calculation of tumor mutation burden (TMB): TMB was defined as the number of somatic mutations per 1 Mb in examined coding regions, excluding driver mutations. Tumor somatic mutations include missense, silent, nonsense, frameshift and non-frameshift indel mutations in coding regions.

Molecular classification of EC cases

The molecular subtype of each EC case was determined according to *POLE* gene status, microsatellite status, and *TP53* gene status. The pipeline for subtyping was designed in accordance with the transPORTEC classification system (20), as shown in Figure 1. Four molecular subtypes (*POLE* mutant, MSI-H, no specific molecular profile [NSMP], and *TP53* mutant) were identified accordingly.

PD-L1 immunohistochemical testing

PD-L1 expression levels of each sample were tested with a PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies). The expression level of PD-L1 was quantified using tumor proportion score (TPS), which is defined as the percentage of viable tumor cells with partial or complete membrane PD-L1 staining at any intensity. In this study, positive PD-L1 expression was defined as TPS ≥ 1% (21).

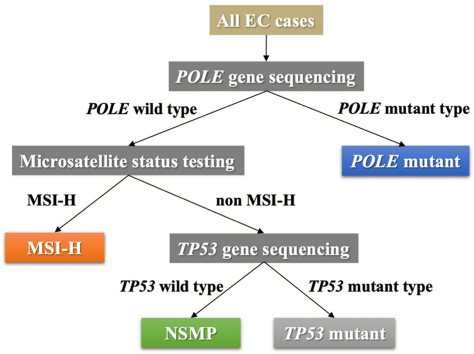

FIGURE 1
The pipeline of molecular subtyping of EC. EC, endometrial cancer; MSI-H, microsatellite instability high; NSMP, no specific molecular profile.

Testing of tumor infiltrating immune cells

For each sample, infiltrating immune cells were examined using multiplex immunofluorescence staining, which was conducted with the Akoya OPAL Polaris 7-Color Automation IHC kit (NEL871001KT), following the manufacturer's guide. FFPE tissue slides were first deparaffinized in a BOND RX system (Leica Biosystems), which was followed by epitope retrieval. Then, the slides were incubated with primary antibodies in two panels. In panel 1, the primary antibodies against CD163 (Abcam, ab182422, 1:500), CD8 (Abcam, ab178089, 1:200), CD68 (Abcam, ab213363, 1:1000), PD-1 (CST, D4W2J, 86163S, 1:200), PD-L1 (CST, E1L3N, 13684S, 1:400) and pan-CK (Abcam, ab7753, 1:100) were added sequentially. In panel 2, the primary antibodies against CD20 (DAKO, L26, IR604, 1:1), CD3 (DAKO, A0452, 1:1), CD56 (Abcam, ab75813, 1:1000), CD4 (Abcam, ab133616, 1:100), FOXP3 (Abcam, ab20034, 1:100) and pan-CK (Abcam, ab7753, 1:100) were added sequentially. After incubating with each primary antibody, the samples were incubated with secondary antibodies and the corresponding reactive Opal fluorophores (see Table S1 for details). Nuclei acids were stained with DAPI. Slides bound with primary and secondary antibodies but without fluorophores were used as negative controls. The tissue slides were scanned by the Vectra Polaris Quantitative Pathology Imaging System (Akoya Biosciences) at 20 nm wavelength intervals from 440 nm to 780 nm, with a fixed exposure time and an absolute magnification of ×200. All scans were then superimposed to obtain a single image for each slide. The cellular phenotype identification was performed as described previously (22). Briefly, the images were imported into inForm v.2.4.8 (Akoya Biosciences) for image analysis, and deconvolution was performed based on a multinomial logistic regression model, according to the manufacturer's guideline. The files generated were then imported into HALO® (Indica Labs) for cellular quantifications. For each case, the entire tissue section was used for analysis. Tumor parenchyma and mesenchyme were differentiated according to pan-CK staining, and were also verified by pathological review of H&E stained slides. The percentage of a certain immune cell type was defined as the percentage of positively stained cells among all nucleated cells. We calculated the fraction of $CD8^+$ T cells, regulatory T cells (T_{reg} cells, $CD3^+$ $CD4^+$ $FOXP3^+$), M1 macrophages ($CD68^+$ $CD163^-$), M2 macrophages ($CD68^+$ $CD163^+$), CD56 dimly stained natural killer ($CD56^{dim}$ NK) cells, PD-$L1^+$ $CD68^+$ cells and $CD8^+$ PD-1^+ cells in the tumor parenchyma and mesenchyme accordingly.

Statistical analysis

In this study, all intergroup comparisons were performed based on the data obtained from tissue sections of multiple samples in each group. For categorical variables, Fisher's exact test was used to compare the differences among groups. For continuous variables, the normality of the data distribution was tested. Variables in accordance with normal distribution were described with the mean value and standard deviation (SD), and intergroup comparisons were conducted with one-way analysis of variance (ANOVA). Variables not in accordance with normal distribution were described with the median value and interquartile range (IQR), and the Kruskal–Wallis test was conducted to compare the differences among groups. All statistical analyses were performed using SPSS 26.0 (IBM Corporation, Armonk, NY, USA) and R 4.1.0 (https://www.r-project.org/). In all tests, two-sided p values were used. Statistically significant differences were considered when $p< 0.05$.

Results

Clinicopathological and molecular features of EC cases

In this study, 26 primary EC cases were included, including three of the *POLE* mutant subtype, six of the MSI-H subtype, eight of the NSMP subtype, and nine of the *TP53* mutant subtype (see Figure S1 for the mutational profiles of all patients). The mean age of all patients was 62.38 years. Compared with the other three subtypes, the *TP53* mutant subtype showed numerically higher age at diagnosis (66.56 ± 10.90 y) and tended towards a larger proportion of postmenopausal patients (88.9%), although the differences were not significant. Body mass index (BMI) and disease history were similar across the four molecular subtypes. Three patients had other malignancies, including one case of the *TP53* mutant subtype with cooccurring ovarian cancer, and two cases of the MSI-H subtype with a history of colon cancer. One of the MSI-H cases was later diagnosed as Lynch syndrome. (Table 1)

We found significant differences in pathological types across different molecular subtypes ($p< 0.001$). All seven patients with non-endometrioid EC had *TP53* mutations, and of these seven patients, one had carcinosarcoma and six had uterine serous carcinoma. The percentage of advanced-stage cases in the *TP53* mutant subtype was the highest among all the molecular subtypes (66.7%, $p = 0.028$). Deep myometrial invasion, cervical stromal invasion, lymphovascular space invasion, adnexal involvement, and lymph node metastasis were more common in the *TP53* mutant subtype than in other subtypes, but the differences were not statistically significant, possibly due to the relatively small sample size. For all primary cases, no patient received neoadjuvant therapies. The proportion of patients receiving open surgery ($p = 0.024$) and postoperative chemotherapies ($p = 0.005$) was significantly higher in the *TP53* mutant subtype than in the other subtypes (Table 1)

TABLE 1 Clinicopathological characteristics of primary EC cases[a].

Characteristics	Total (n = 26)	*POLE* mutant (n= 3)	MSI-H (n = 6)	NSMP (n = 8)	*TP53* mutant (n = 9)	*p* value
Age, y, mean (SD)	62.38 (8.79)	60.33 (11.02)	59.33 (8.60)	60.75 (4.20)	66.56 (10.90)	0.381[b]
BMI, kg/m^2, mean (SD)	26.04 (4.15)	25.07 (2.88)	24.49 (1.99)	28.09 (6.45)	25.57 (2.47)	0.399[b]
Postmenopause, No. (%)						0.208
No	6 (23.1)	2 (66.7)	2 (33.3)	1 (12.5)	1 (11.1)	
Yes	20 (76.9)	1 (33.3)	4 (66.7)	7 (87.5)	8 (88.9)	
Hypertension, No. (%)						0.817
No	13 (50.0)	1 (33.3)	4 (66.7)	4 (50.0)	4 (44.4)	
Yes	13 (50.0)	2 (66.7)	2 (33.3)	4 (50.0)	5 (55.6)	
Diabetes, No. (%)						0.931
No	19 (73.1)	2 (66.7)	5 (83.3)	6 (75.0)	6 (66.7)	
Yes	7 (26.9)	1 (33.3)	1 (16.7)	2 (25.0)	3 (33.3)	
Other malignancies, No. (%)						0.278
No	23 (88.5)	3 (100.0)	4 (66.7)	8 (100.0)	8 (88.9)	
Yes	3 (11.5)	0	2 (33.3)	0	1 (11.1)	
Pathological type, No. (%)						<0.001
Endometrioid	19 (73.1)	3 (100.0)	6 (100.0)	8 (100.0)	2 (22.7)	
Non-endometrioid	7 (26.9)	0	0	0	7 (77.8)	
FIGO stage, No. (%)						0.028
Early (stage I - II)	18 (69.2)	3 (100.0)	6 (100.0)	6 (75.0)	3 (33.3)	
Advanced (stage III - IV)	8 (30.8)	0	0	2 (25.0)	6 (66.7)	
Grade[c], No. (%)						0.415
Low (grade 1-2)	17 (65.4)	2 (66.7)	5 (83.3)	8 (100.0)	2 (100.0)	
High (grade 3)	2 (7.7)	1 (33.3)	1 (16.7)	0	0	
Depth of myometrial invasion, No. (%)						0.269
<50%	14 (53.8)	3 (100.0)	3 (50.0)	5 (62.5)	3 (33.3)	
≥50%	12 (46.2)	0	3 (50.0)	3 (37.5)	6 (66.7)	
Cervical stromal invasion, No. (%)						1.000
No	22 (84.6)	3 (100.0)	5 (83.3)	7 (87.5)	7 (77.8)	
Yes	4 (15.4)	0	1 (16.7)	1 (12.5)	2 (22.2)	
Lymphovascular space invasion, No. (%)						0.175

(Continued)

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TABLE 1 Continued

Characteristics	Total (n = 26)	*POLE* mutant (n= 3)	MSI-H (n = 6)	NSMP (n = 8)	*TP53* mutant (n = 9)	*p* value
No	17 (68.0)	3 (100.0)	5 (83.3)	6 (75.0)	3 (37.5)	
Yes	8 (32.0)	0	1 (16.7)	2 (25.0)	5 (62.5)	
Adnexal involvement, No. (%)						0.223
No	20 (76.9)	3 (100.0)	6 (100.0)	6 (75.0)	5 (55.6)	
Yes	6 (23.1)	0	0	2 (25.0)	4 (44.4)	
Lymph node metastasis[d], No. (%)						0.178
No	20 (76.9)	3 (100.0)	6 (100.0)	7 (87.5)	4 (57.1)	
Yes	4 (15.4)	0	0	1 (12.5)	3 (42.9)	
Peritoneal cytology, No. (%)						0.283
Negative	22 (91.7)	2 (66.7)	6 (100.0)	8 (100.0)	6 (85.7)	
Positive	2 (8.3)	1 (33.3)	0	0	1 (14.3)	
Surgical approach, No. (%)						0.024
Open	9 (34.6)	1 (33.3)	2 (33.3)	0	6 (66.7)	
Minimally invasive	17 (65.4)	2 (66.7)	4 (66.7)	8 (100.0)	3 (33.3)	
Postoperative chemotherapy, No. (%)						0.005
No	10 (38.5)	3 (100.0)	3 (50.0)	4 (50.0)	0	
Yes	16 (61.5)	0	3 (50.0)	4 (50.0)	9 (100.0)	
Postoperative radiotherapy, No. (%)						0.619
No	17 (65.4)	3 (100.0)	3 (50.0)	5 (62.5)	6 (66.7)	
Yes	9 (34.6)	0	3 (50.0)	3 (37.5)	3 (33.3)	

[a] For some characteristics, the number of cases did not sum up to the heading totals due to missing data.
[b] One-way ANOVA test, all others were by Fisher's exact test.
[c] Grade was only determined and calculated in endometrioid EC.
[d] Only cases receiving lymph node resections were included in the calculation.
MSI-H, microsatellite instability high; NSMP, no specific molecular profile; BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics; EC, endometrial cancer.

We also included four recurrent EC cases in this study, with two of the NSMP subtype and two of the *TP53* mutant subtype. Detailed clinicopathological and molecular genetic information on the recurrent cases are shown in Table 2 and Figure S1.

TMB levels of different molecular subtypes of EC

We analyzed the tumor immune microenvironmental features of the 30 EC cases, including TMB, infiltration of antitumor-related immune cells and negatively regulatory immune cells, and the expression of immune checkpoint molecules. Consistent with TCGA data (1), patients with *POLE* mutations showed the highest level of TMB, followed by the MSI-H subtype, NSMP, and *TP53* mutant subtypes (Figure 2). Recent studies have indicated that TMB is highly associated with tumor-infiltrating immune cells, PD-L1 expression, and patients' prognosis in both endometrial cancer and other cancer types (23–25). Additionally, considering the relatively small sample size of the *POLE* mutant and MSI-H subtypes, we combined the two subtypes into the TMB high (TMB-H) subtype in the following analysis.

Infiltration of immune cell subsets in different molecular subtypes of EC

We examined tumor-infiltrating immune cells in the three molecular subtypes using multiplex immunofluorescence assays (Figure 3 and Figure S2). The fractions of $CD8^+$ T cells in both the tumor parenchyma and the tumor mesenchyme were higher in the TMB-H and *TP53* mutant subtypes than in the NSMP subtype, although the differences were not statistically significant (p = 0.094 for tumor parenchyma, p = 0.215 for tumor mesenchyme). The infiltration of M1 macrophages and $CD56^{dim}$ NK cells did not differ significantly among the three subtypes. (Figure 4 and Table S2)

With regard to negatively regulatory immune cells, a trend of a higher degree of infiltration of M2 macrophages was observed in tumors of the *TP53* mutant subtype comparing with tumors of the other two subtypes (Figures 5A, B). Similar trends were also observed in the ratios of M2 macrophage fractions to M1 macrophage fractions (Figures 5C, D). The percentage of T_{reg} cells and the ratio of T_{reg} cell fractions to $CD8^+$ T cell fractions in the tumor parenchyma were the highest in the *TP53* mutant subtype among the three molecular subtypes, but the differences were not significant. Interestingly, we noticed that the fraction of

TABLE 2 Clinicopathological and molecular features of recurrent EC cases.

Characteristics	Patient A	Patient B	Patient C	Patient D
Age at recurrence, y	65	55	67	68
Time of recurrence	Second time	First time	First time	First time
Disease-free interval, months	42	15	14	7
Site of recurrence	Abdominal wall	Chest wall	Ilium	Paraaortic lymph node
Pathological type of recurrent tumor	Endometrioid	Endometrioid	Endometrioid	Clear cell
Grade[a]	Grade 3	Grade 3	Grade 3	–
Molecular subytpe	*TP53* mutant	NSMP	NSMP	*TP53* mutant
Chemotherapy before sampling	Yes	Yes	Yes	Yes
Radiotherapy before sampling	Yes	No	No	No
Targeted therapy before sampling	No	No	No	No

[a]Grade was only determined in endometrioid EC. EC, endometrial cancer; NSMP, no specific molecular profile.

T_{reg} cells in the tumor mesenchyme was the highest in the TMB-H subtype ($p = 0.008$), but the difference in the ratio of T_{reg} cell fractions to $CD8^+$ T cell fractions in the tumor mesenchyme was not significant across the three subtypes (Figures 5E–H and Table S3).

Expression of immune checkpoint molecules in different molecular subtypes of EC

We also analyzed the expression of PD-L1 and PD-1 in tumor samples of the three molecular subtypes. The TMB-H subtype showed the highest rate of positive PD-L1 expression in tumor cells (33.3%), although the difference was not significant (Figure 6A and Table S4). The *TP53* mutant subtype had the largest fraction of PD-$L1^+$ $CD68^+$ macrophages in both the tumor parenchyma and mesenchyme ($p = 0.047$ and 0.025, respectively). In tumor parenchymal regions, $CD8^+$ PD-1^+ T cell infiltrations were the highest in the *TP53* mutant subtype among all three molecular subtypes ($p = 0.034$). In the tumor mesenchyme, the fraction of $CD8^+$ PD-1^+ T cells was higher in the TMB-H and *TP53* mutant subtypes compared with that in the NSMP subtype ($p = 0.004$), yet the proportion of $CD8^+$ PD-1^+ T cells in all $CD8^+$ T cells was the highest in the *TP53* mutant subtype among all three molecular subtypes, although not significant enough ($p = 0.084$). (Figure 6 and Table S4)

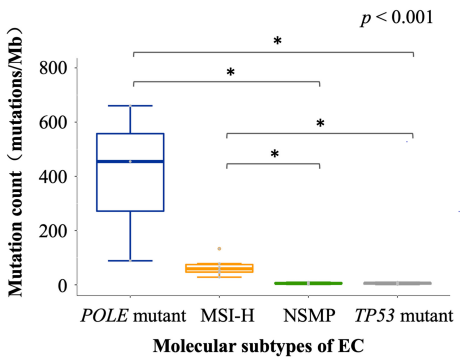

FIGURE 2
TMB of different molecular subtypes of EC. The *p* value of Kruskal-Wallis test for overall comparison is given, and significant levels in pairwise comparisons are shown in the figure. In the comparisons, n = 3 for *POLE* mutant, 6 for MSI-H, 10 for NSMP, 11 for *TP53* mutant. The dot above the boxplot indicates an outlier. MSI-H, microsatellite instability high; NSMP, no specific molecular profile; EC, endometrial cancer; TMB, tumor mutation burden. *$p < 0.05$.

Discussion

EC is one of the most common gynecologic malignancies worldwide (26). Personalized treatment strategies for EC are essential both for better precision care and for reducing treatment-related health economic burdens. With the emerging trend of applying immunotherapies in EC treatment, and the increasing evidence indicating the potential role of immunological features in predicting treatment responses (27–29), understanding tumor immune microenvironmental features and the associations with molecular features of cancer is necessary to guide immunotherapy design and predict patients' prognosis. In this study, we compared the clinicopathological features of different molecular subtypes of primary EC. Additionally we systemically analyzed the association of EC molecular subtypes with tumor immune microenvironmental features using experimental approaches. The information provided here could be informative for the design of relevant basic and clinical studies in the future.

Our data revealed that *TP53* mutant EC was associated with non-endometrioid histology, advanced stage, and multiple negative prognostic factors, indicating compromised survival outcomes. These results were in accordance with previous

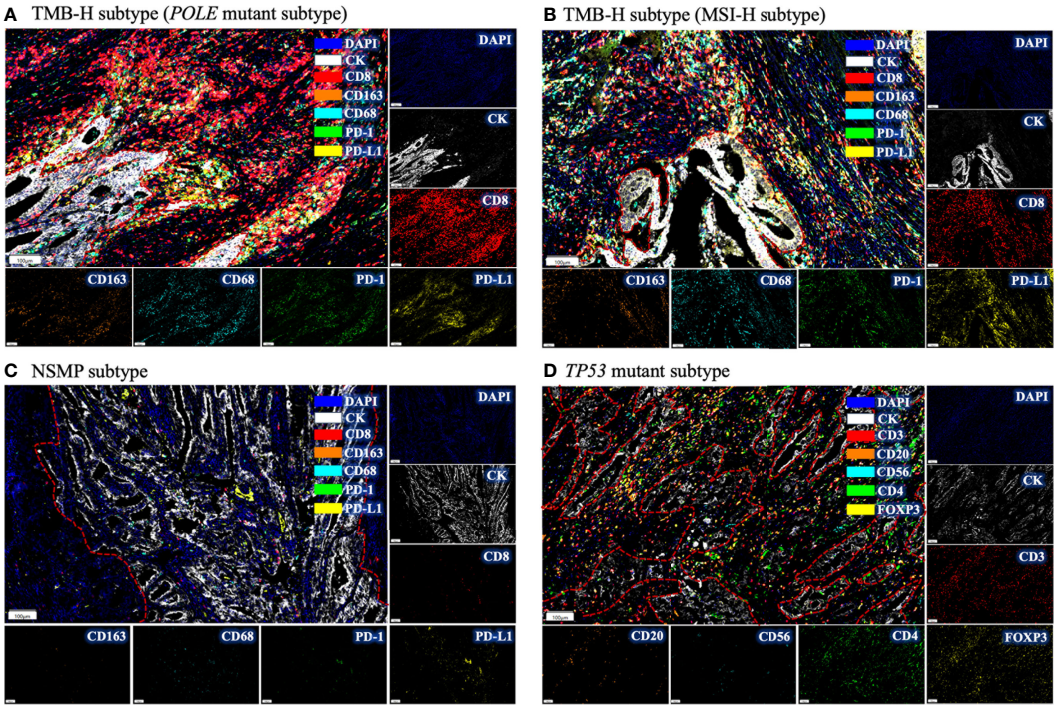

FIGURE 3
Immune infiltration in different molecular subtypes of EC by multiplex immunofluorescence. **(A)** The immune infiltrations in EC of *POLE* mutant subtype. Intense red fluorescence indicates large amount of $CD8^+$ cell infiltration. **(B)** The immune infiltration in EC of MSI-H subtype. **(C)** The immune infiltration in NSMP subtype. Few fluorescence signals could be observed, indicating absence of immune infiltration. **(D)** The immune infiltration in *TP53* mutant subtype. Intense yellow fluorescence indicates the infiltration of $FOXP3^+$ cells. For each subtype, a representative filed was selected, and the major tumor regions are outlined. TMB-H, high tumor mutation burden; MSI-H, microsatellite instability high; NSMP, no specific molecular profile; EC, endometrial cancer.

studies (20, 30–33). The following analysis of the tumor immune microenvironment could, to some extent, provide explanations for this. Indeed, in recent years, studies based on cancer genomics have indicated that immunological subtypes could be used to predict patient prognosis (5). There are also available models for predicting clinical response to immune checkpoint blockade therapies (34, 35). Nevertheless, current models are still not sufficiently accurate, and further explorations are warranted.

Previous studies have indicated that increased infiltration of intratumoral $CD8^+$ T cells is associated with a better prognosis in different cancer types (36–38), and a recent study based on multiplex immunofluorescent assays further supported the prognostic value of tumor infiltrating T cells in early-stage endometrial cancer (39). In this study, we also observed relatively high percentages of $CD8^+$ T cells in samples of TMB-H EC, which is believed to have a favorable survival outcome (23). However, studies have shown that the functional status of $CD8^+$ T cells changes with tumor progression, and different stages of dysfunctional T cells, characterized by the expression of specific immune checkpoint molecules, are thought to be associated with distinct response rates to immune checkpoint inhibitors (40). In this study, we noticed that *TP53* mutant EC showed abundant infiltration of $CD8^+$ $PD\text{-}1^+$ T cells in the tumor parenchyma and a high proportion of $CD8^+$ T cells with PD-1 expression in the tumor mesenchyme, indicating unfavorable clinical outcomes of *TP53* mutant EC.

Innate immune cells, including macrophages and NK cells, could also modulate the tumor immune microenvironment and regulate antitumor responses. Macrophages can be further divided into M1 macrophages and M2 macrophages based on cell surface markers and functions. M1 macrophages mainly show antitumoral functions, while M2 macrophages promote tumor progression *via* stimulating tumor cell proliferation, angiogenesis, and epithelial-mesenchymal transitions (41). According to our data, a trend of higher fractions of M2 macrophages in *TP53* mutant EC relative to the other molecular subtypes was observed. This could possibly help explain the higher rate of advanced-stage diseases in this subtype as a result of tumor progression. Besides, NK cells could also be divided into two types, $CD56^{dim}$ and $CD56^{bright}$ (CD56 brightly stained NK cells). $CD56^{bright}$ NK cells are mainly responsible for secreting cytokines, while $CD56^{dim}$ NK cells show more potent cytotoxic effects (42). One recent study indicated that low NK cell infiltration in the tumor was associated with worse survival (43). However, based on our data, the fraction of

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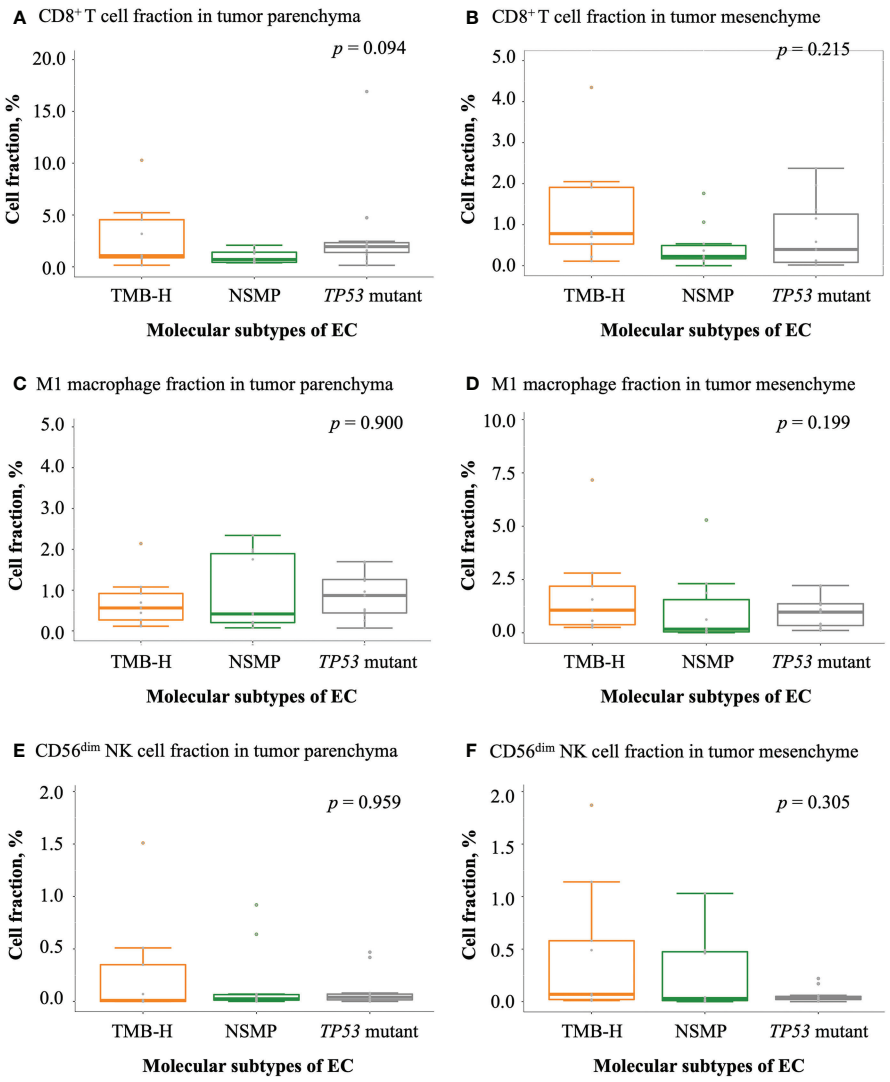

FIGURE 4
Infiltration of antitumor-related immune cells in EC. **(A, B)** $CD8^+$ T cell fractions in tumor parenchyma and mesenchyme. **(C, D)** M1 macrophage fractions in tumor parenchyma and mesenchyme. **(E, F)** $CD56^{dim}$ NK cell fractions in tumor parenchyma and mesenchyme. For **(A-F)**, the *p* values of Kruskal-Wallis test for overall comparisons are given. In all panels, n = 9 for TMB-H, 10 for NSMP, 11 for *TP53* mutant. The dots above the boxplots indicate outliers. TMB-H, high tumor mutation burden; NSMP, no specific molecular profile; EC, endometrial cancer; $CD56^{dim}$ NK cell, CD56 dimly stained natural killer cell.

$CD56^{dim}$ NK cells was not significantly different among different EC molecular subtypes, possibly due to the limited sample size.

T_{reg} cells are another vital cell type with prognostic significance. Evidence has shown that high T_{reg} cell infiltration level is associated with compromised survival and hyperprogression of disease following immune checkpoint blockade therapy (44, 45). But interestingly enough, in this study, a higher proportion of T_{reg} cells was observed in the tumor mesenchyme of the TMB-H subtype, instead of the *TP53* mutant subtype, which are commonly thought to have poor survival. According to previous studies, T_{reg} cells responsible for modulating immune responses typically express the same transcription factors, which are also expressed in the cells that they regulate; moreover, in antitumor immune responses, T_{reg} cells could be triggered by the same chemotaxis molecules that also recruit $CD8^+$ T cells to the tumor site (46). Therefore, the higher proportion of $CD8^+$ T cells in the tumor mesenchyme in TMB-H tumors may have contributed to the higher proportion of T_{reg} cells. Furthermore, the similar ratio of T_{reg} cells to $CD8^+$ T cells in the three molecular subtypes also supported the above hypothesis.

In summary, EC patients with high TMB showed abundant tumor-infiltrating $CD8^+$ T cells and relatively high levels of PD-L1 expression in tumor cells, which is consistent with data from

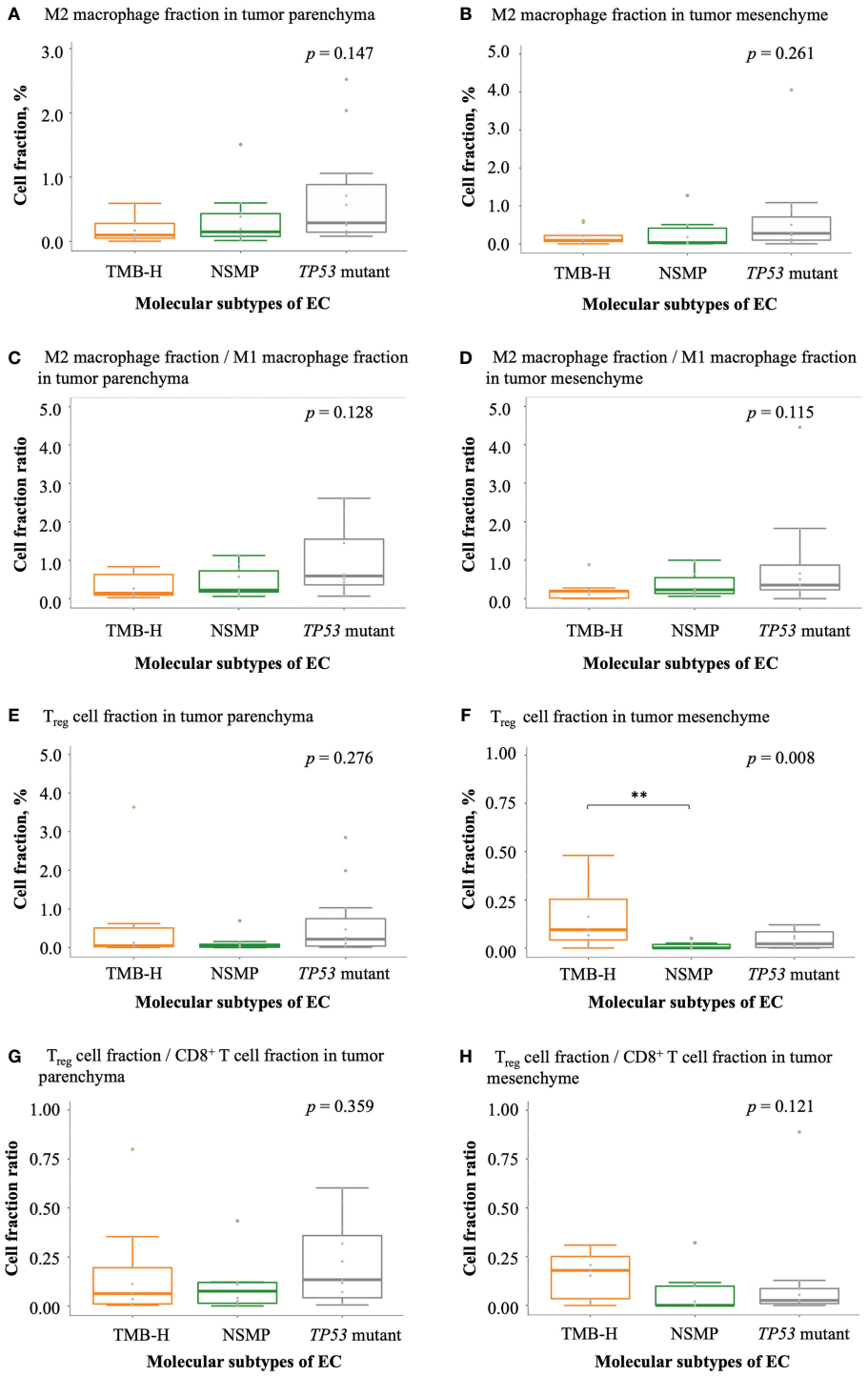

FIGURE 5
Infiltration of negatively regulatory immune cells in EC. **(A, B)** M2 macrophage fractions in tumor parenchyma and mesenchyme. **(C, D)** The ratio of M2 macrophage fractions to M1 macrophage fractions in tumor parenchyma and mesenchyme. **(E, F)** T_{reg} cell fractions in tumor parenchyma and mesenchyme. **(G, H)** The ratio of T_{reg} cell fractions to $CD8^+$ T cell fractions in tumor parenchyma and mesenchyme. The *p* values of Kruskal-Wallis test for overall comparisons are given, and significant levels in pairwise comparisons are shown in the figure. In all panels, n = 9 for TMB-H, 10 for NSMP, 11 for *TP53* mutant. The dots above the boxplots indicate outliers. TMB-H, high tumor mutation burden; NSMP, no specific molecular profile; EC, endometrial cancer; T_{reg} cell, regulatory T cell. $^{**}p < 0.01$.

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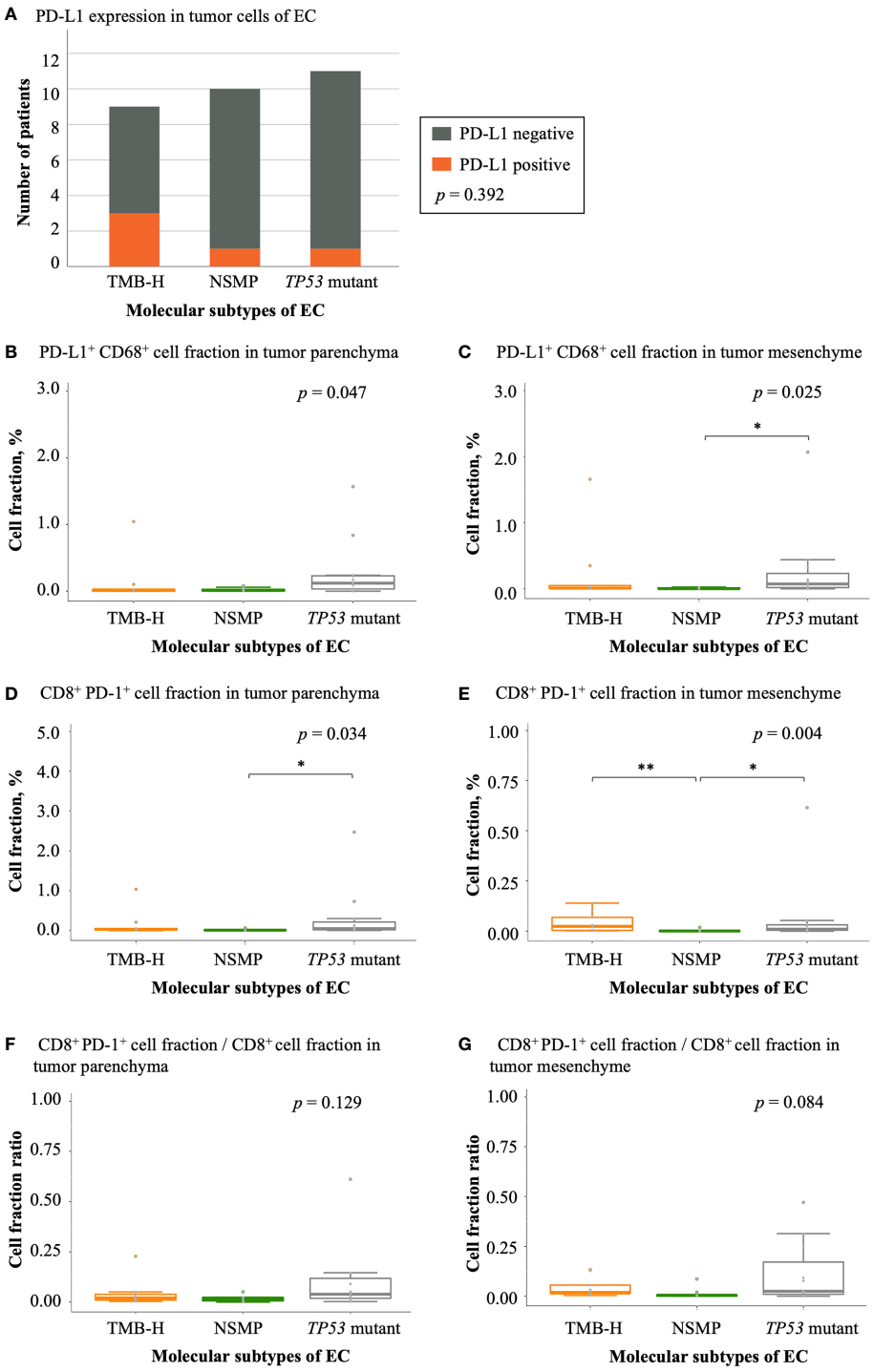

FIGURE 6
Expression of immune checkpoint molecules in EC. **(A)** PD-L1 expression in different EC molecular subtypes. The *p* value of Fisher's exact test for overall comparison is given. **(B, C)** PD-L1$^+$ CD68$^+$ cell fractions in tumor parenchyma and mesenchyme. **(D, E)** CD8$^+$ PD-1$^+$ cell fractions in tumor parenchyma and mesenchyme. **(F, G)** The ratio of CD8$^+$ PD-1$^+$ cell fractions to CD8$^+$ T cell fractions in tumor parenchyma and mesenchyme. For **(B-G)**, the *p* values of Kruskal-Wallis test for overall comparisons are given, and significant levels in pairwise comparisons are shown in the figure. In all panels, n = 9 for TMB-H, 10 for NSMP, 11 for *TP53* mutant. The dots above the boxplots indicate outliers. TMB-H, high tumor mutation burden; NSMP, no specific molecular profile; EC, endometrial cancer. *$p < 0.05$, **$p < 0.01$.

previous studies on *POLE* mutant and MSI-H EC (47–49). The above indicates that there are strong antitumor immune responses in TMB-H tumors and that this subtype is potentially suitable for immune checkpoint blockade therapies. In the NSMP subtype, the TMB, the proportions of multiple tumor-infiltrating immune cells and the expression levels of immune checkpoint molecules were low, indicating a lack of effective antitumor immune responses. In the *TP53* mutant subtype, the TMB level was low. However, the proportions of T_{reg} cells, M2 macrophages, PD-L1$^+$ CD68$^+$ macrophages and CD8$^+$ PD-1$^+$ T cells were relatively high, indicating a strong immune suppressive microenvironment in this molecular subtype. Based on the immune microenvironmental features analyzed above, we summarize the immune phenotype of the three molecular subtypes as normal immune response, absence of immune infiltration, and suppressed immune response.

The distribution pattern of immune cells in the tumor tissue could also indicate differences in their functions. Recently, Keren et al. (50) proposed a model for describing immune infiltrative patterns in the tumor: compartmentalized pattern, which suggested that tumor cells and immune cells form relatively independent regions; cold pattern, which indicated low levels of tumor infiltrating immune cells; and mixed pattern, which implied the highly mixed distribution of tumor and immune cells. Among these, patients with the compartmentalized pattern showed better survival than those with the mixed pattern (50). In this study, we also analyzed the distribution of immune cells in the tumor parenchyma and mesenchyme. Our study showed that CD8$^+$ T cells were distributed in both tumor parenchymal and mesenchymal regions in TMB-H EC. The fraction of CD8$^+$ T cells in the tumor mesenchyme was the highest in the TMB-H subtype, which means that a part of T cells could form a relatively isolated region adjacent to the tumor cells. Among the three molecular subtypes, the NSMP subtype displayed the lowest degree of CD8$^+$ T cell infiltration. In the *TP53* mutant subtype, CD8$^+$ T cells were mostly distributed in tumor parenchyma, with a relatively low median level of infiltration in tumor mesenchyme. The features of CD8$^+$ T cell distribution in the three molecular subtypes, to some extent, resemble that of the model mentioned above (50). Besides, *TP53* mutant EC typically shows the worst survival (1, 20, 30–33) across all subtypes, which is also consistent with the survival features revealed by the above model (50). Another recent study analyzed the interactions of cellular neighborhoods in colorectal cancer with distinct immune infiltrative features (51). In brief, in tumors with numerous tertiary lymphoid structures, T cell exchange between the T cell cluster and tumor invasive front could help enhance the antitumor immune response; while in tumors with diffuse inflammatory infiltration, the immune suppressive macrophage cluster showed strong contact with the tumor invasive front and inhibited effective immune responses (51). The mechanisms described above might also explain the distinct survival features in EC of different molecular subtypes and immune infiltrative patterns.

Notably, in this study, both the TMB-H subtype and *TP53* mutant subtype showed high levels of immune checkpoint molecule expression. However, in the TMB-H subtype, PD-L1 was mainly expressed in tumor cells, while in the *TP53* mutant subtype, there were high levels of PD-L1 expression in macrophages and high levels of PD-1 expression in T cells. These results indicate that the cellular distribution of immune checkpoint molecules could also provide information on the immune response status of the patient and differences in patients' responses to immune checkpoint blockade therapies. Further studies with larger sample sizes are needed to confirm the findings.

In this study, we quantitively analyzed tumor immune microenvironmental features in different EC molecular subtypes and provided information about the spatial distributions of multiple immune cell types in the tumor tissue. A key strength is that we adopted experimental methods for *in situ* visualization of the cells, which showed direct evidence for tumor immune infiltrations. In this regard, this study could provide a vital supplement to previous studies based on bulk tissue sequencing and computational deconvolution. By systemically analyzing the TMB, tumor infiltrating immune cells and immune checkpoint molecules, we summarized the immunophenotypes of different EC molecular subtypes, thus providing clues for understanding their distinct survival features and treatment responses. However, there are also some limitations. First, the sample size of the study was relatively small, which limited the statistical power in some analyses. Studies with larger sample sizes are needed to further validate our findings. Second, since most cases in this study were treated recently and follow-ups are on-going, survival information is still lacking. Long-term follow-up is necessary to analyze the associations of tumor immune microenvironmental features with patients' recurrence, survival and responses to multiple treatment modalities. Finally, as an explorative study, the panel we used for testing tumor-infiltrating immune cells included multiple cellular markers. Further explorations are needed to refine the testing strategies and develop clinically feasible panels for better practical applications.

Currently, with the deep clinical influence of TCGA molecular subtyping and its surrogate methods (1, 20, 31), the diagnosis and treatment of EC are becoming increasingly more comprehensive and individualized. Molecular markers provide vital information and rationality for applying targeted drugs and immune checkpoint blockade therapies in specific patient groups (52). Nevertheless, heterogeneity in prognosis and treatment response could still be seen even within the same pathological and molecular subtype, which urges further refinement of the current risk stratification system (3). During this effort, barriers still exist that the cellular architectures of the tumor tissues and the biological behaviors of malignant and surrounding stromal and immune cells are far less understood in EC. As indicated above (50, 51), this information may also be highly associated with clinical outcomes. The results from this study, on the one hand, established the connection between molecular subtypes and the immune microenvironmental features of EC. On the other hand, it paved the way for further designing related studies. We encourage more efforts using multiplex imaging methods to establish a prognostic or treatment-related classification system based on

immunological markers. Based on these efforts, incorporating effective immunological features into current EC patients' risk stratification systems would be another vital step for better individualized treatment.

Data availability statement

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (53) in National Genomics Data Center (54), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA003458) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human. The raw data of multiplex immunofluorescence assays have been deposited in the OMIX, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (https://ngdc.cncb.ac.cn/omix: accession no. PRJCA013221).

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of Peking University People's Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YD and ZW contributed to study conception and design. Development of methodology was performed by YD, DH, LC, LZ and ZW. Acquisition of data was conducted by YD, LZ, XZ, NK, LQ, LL and HL. Data analysis was performed by YD, DH and LZ. The first draft of the manuscript was written by YD, and the manuscript was reviewed and revised by LC, JW and ZW. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Authors DH and LC were employed by 3D Medicines Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1035616/full#supplementary-material

References

1. The Cancer Genome Atlas Research Network. Integrated genomic characterization of endometrial carcinoma. *Nature* (2013) 497:67–73. doi: 10.1038/nature12113

2. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: uterine neoplasms. Available at: https://www.nccn.org2021.

3. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer.* (2021) 31(1):12–39. doi: 10.1136/ijgc-2020-002230

4. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf Anna C, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity.* (2013) 39(4):782–95. doi: 10.1016/j.immuni.2013.10.003

5. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang T-H, et al. The immune landscape of cancer. *Immunity.* (2018) 48(4):812–30. doi: 10.1016/j.immuni.2018.03.023

6. Sturm G, Finotello F, Petitprez F, Zhang JD, Baumbach J, Fridman WH, et al. Comprehensive evaluation of transcriptome-based cell-type quantification methods for immuno-oncology. *Bioinformatics* (2019) 35(14):i436–45. doi: 10.1093/bioinformatics/btz363

7. Tan WCC, Nerurkar SN, Cai HY, Ng HHM, Wu D, Wee YTF, et al. Overview of multiplex immunohistochemistry/immunofluorescence techniques in the era of

cancer immunotherapy. *Cancer Commun (Lond).* (2020) 40(4):135–53. doi: 10.1002/cac2.12023

8. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, et al. International validation of the consensus immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet.* (2018) 391(10135):2128–39. doi: 10.1016/S0140-6736(18)30789-X

9. Ni Y, Soliman A, Joehlin-Price A, Abdul-Karim F, Rose PG, Mahdi H. Immune cells and signatures characterize tumor microenvironment and predict outcome in ovarian and endometrial cancers. *Immunotherapy.* (2021) 13 (14):1179–92. doi: 10.2217/imt-2021-0052

10. Li BL, Wan XP. Prognostic significance of immune landscape in tumour microenvironment of endometrial cancer. *J Cell Mol Med* (2020) 24(14):7767–77. doi: 10.1111/jcmm.15408

11. Guo C, Tang Y, Zhang Y, Li G. Mining TCGA data for key biomarkers related to immune microenvironment in endometrial cancer by immune score and weighted correlation network analysis. *Front Mol Biosci* (2021) 8:645388. doi: 10.3389/fmolb.2021.645388

12. Gueli Alletti S, Restaino S, Finelli A, Ronsini C, Lucidi A, Scambia G, et al. Step by step total laparoscopic hysterectomy with uterine arteries ligation at the origin. *J Minim Invasive Gynecol.* (2020) 27(1):22–3. doi: 10.1016/j.jmig.2019.06.001

13. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynaecol Obstet.* (2009) 105(2):103–4. doi: 10.1016/j.ijgo.2009.02.012

14. Kurman R, Carcangiu M, Herrington C, Young R. Tumours of the uterine corpus. In: *WHO classification of tumours of female reproductive organs, 4th ed.* Lyon: International Agency for Research on Cancer (2014). p. 121–54.

15. Soslow RA, Tornos C, Park KJ, Malpica A, Matias-Guiu X, Oliva E, et al. Endometrial carcinoma diagnosis: Use of FIGO grading and genomic subcategories in clinical practice: Recommendations of the international society of gynecological pathologists. *Int J Gynecol Pathol* (2019) 38(1):S64–74. doi: 10.1097/PGP.0000000000000518

16. Karolchik D. The UCSC table browser data retrieval tool. *Nucleic Acids Res* (2004) 32(90001):D493–6. doi: 10.1093/nar/gkh103

17. Li H, Durbin R. Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics* (2009) 25(14):1754–60. doi: 10.1093/bioinformatics/btp324

18. Su D, Zhang D, Chen K, Lu J, Wu J, Cao X, et al. High performance of targeted next generation sequencing on variance detection in clinical tumor specimens in comparison with current conventional methods. *J Exp Clin Cancer Res* (2017) 36(1):121. doi: 10.1186/s13046-017-0591-4

19. Xiao J, Li W, Huang Y, Huang M, Li S, Zhai X, et al. A next-generation sequencing-based strategy combining microsatellite instability and tumor mutation burden for comprehensive molecular diagnosis of advanced colorectal cancer. *BMC Cancer* (2021) 21(1):282. doi: 10.1186/s12885-021-07942-1

20. Stelloo E, Bosse T, Nout RA, MacKay HJ, Church DN, Nijman HW, et al. Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative. *Mod Pathol* (2015) 28(6):836–44. doi: 10.1038/modpathol.2015.43

21. Paver EC, Cooper WA, Colebatch AJ, Ferguson PM, Hill SK, Lum T, et al. Programmed death ligand-1 (PD-L1) as a predictive marker for immunotherapy in solid tumours: a guide to immunohistochemistry implementation and interpretation. *Pathology* (2021) 53(2):141–56. doi: 10.1016/j.pathol.2020.10.007

22. Italiano A, Bessede A, Pulido M, Bompas E, Piperno-Neumann S, Chevreau C, et al. Pembrolizumab in soft-tissue sarcomas with tertiary lymphoid structures: a phase 2 PEMBROSARC trial cohort. *Nat Med* (2022) 28(6):1199–206. doi: 10.1038/s41591-022-01821-3

23. Zhang J, An L, Zhou X, Shi R, Wang H. Analysis of tumor mutation burden combined with immune infiltrates in endometrial cancer. *Ann Transl Med* (2021) 9 (7):551. doi: 10.21037/atm-20-6049

24. Picard E, Verschoor CP, Ma GW, Pawelec G. Relationships between immune landscapes, genetic subtypes and responses to immunotherapy in colorectal cancer. *Front Immunol* (2020) 11:369. doi: 10.3389/fimmu.2020.00369

25. Huang RSP, Haberberger J, Severson E, Duncan DL, Hemmerich A, Edgerly C, et al. A pan-cancer analysis of PD-L1 immunohistochemistry and gene amplification, tumor mutation burden and microsatellite instability in 48,782 cases. *Mod Pathol* (2021) 34(2):252–63. doi: 10.1038/s41379-020-00664-y

26. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

27. Ott PA, Bang Y-J, Piha-Paul SA, Razak ARA, Bennouna J, Soria J-C, et al. T-cell–inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. *J Clin Oncol* (2019) 37(4):318–27. doi: 10.1200/JCO.2018.78.2276

28. du Rusquec P, de Calbiac O, Robert M, Campone M, Frenel JS. Clinical utility of pembrolizumab in the management of advanced solid tumors: an evidence-based review on the emerging new data. *Cancer Manag Res* (2019) 11:4297–312. doi: 10.2147/CMAR.S151023

29. Makker V, Colombo N, Casado Herráez A, Santin AD, Colomba E, Miller DS, et al. Lenvatinib plus pembrolizumab for advanced endometrial cancer. *N Engl J Med* (2022) 386(5):437–48. doi: 10.1056/NEJMoa2108330

30. Stelloo E, Nout RA, Osse EM, Jürgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC. Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the PORTEC cohorts. *Clin Cancer Res* (2016) 22(16):4215–24. doi: 10.1158/1078-0432.CCR-15-2878

31. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A clinically applicable molecular-based classification for endometrial cancers. *Br J Cancer* (2015) 113(2):299–310. doi: 10.1038/bjc.2015.190

32. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: A simple, genomics-based clinical classifier for endometrial cancer. *Cancer* (2017) 123(5):802–13. doi: 10.1002/cncr.30496

33. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, et al. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. *Ann Oncol* (2018) 29(5):1180–8. doi: 10.1093/annonc/mdy058

34. Chen DS, Mellman I. Elements of cancer immunity and the cancer–immune set point. *Nature* (2017) 541(7637):321–30. doi: 10.1038/nature21349

35. Sanmamed MF, Chen L. A paradigm shift in cancer immunotherapy: From enhancement to normalization. *Cell* (2018) 175(2):313–26. doi: 10.1016/j.cell.2018.09.035

36. Rousset-Rouviere S, Rochigneux P, Chrétien AS, Fattori S, Gorvel L, Provansal M, et al. Endometrial carcinoma: Immune microenvironment and emerging treatments in immuno-oncology. *Biomedicines* (2021) 9(6):632. doi: 10.3390/biomedicines9060632

37. Oshi M, Asaoka M, Tokumaru Y, Yan L, Matsuyama R, Ishikawa T, et al. CD8 T cell score as a prognostic biomarker for triple negative breast cancer. *Int J Mol Sci* (2020) 21(18):6968. doi: 10.3390/ijms21186968

38. Yang Y, Attwood K, Bshara W, Mohler JL, Guru K, Xu B, et al. High intratumoral $CD8^+$ T-cell infiltration is associated with improved survival in prostate cancer patients undergoing radical prostatectomy. *Prostate* (2021) 81 (1):20–8. doi: 10.1002/pros.24068

39. Mendiola M, Pellinen T, Ramon-Patino JL, Berjon A, Bruck O, Heredia-Soto V, et al. Prognostic implications of tumor-infiltrating T cells in early-stage endometrial cancer. *Mod Pathol* (2022) 35(2):256–65. doi: 10.1038/s41379-021-00930-7

40. Van Der Leun AM, Thommen DS, Schumacher TN. $CD8^+$ T cell states in human cancer: insights from single-cell analysis. *Nat Rev Cancer* (2020) 20(4):218–32. doi: 10.1038/s41568-019-0235-4

41. Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. *Cancer Res* (2019) 79(18):4557–66. doi: 10.1158/0008-5472.CAN-18-3962

42. Myers JA, Miller JS. Exploring the NK cell platform for cancer immunotherapy. *Nat Rev Clin Oncol* (2021) 18(2):85–100. doi: 10.1038/s41571-020-0426-7

43. Stangl S, Tontcheva N, Sievert W, Shevtsov M, Niu M, Schmid TE, et al. Heat shock protein 70 and tumor-infiltrating NK cells as prognostic indicators for patients with squamous cell carcinoma of the head and neck after radiochemotherapy: A multicentre retrospective study of the German cancer consortium radiation oncology group (DKTK-ROG). *Int J Cancer* (2018) 142 (9):1911–25. doi: 10.1002/ijc.31213

44. Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Cell Res* (2017) 27(1):109–18. doi: 10.1038/cr.2016.151

45. Kamada T, Togashi Y, Tay C, Ha D, Sasaki A, Nakamura Y, et al. PD-1^+ regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. *Proc Natl Acad Sci U.S.A.* (2019) 116(20):9999–10008. doi: 10.1073/pnas.1822001116

46. Savage PA, Malchow S, Leventhal DS. Basic principles of tumor-associated regulatory T cell biology. *Trends Immunol* (2013) 34(1):33–40. doi: 10.1016/j.it.2012.08.005

47. Temko D, Van Gool IC, Rayner E, Glaire M, Makino S, Brown M, et al. Somatic *POLE* exonuclease domain mutations are early events in sporadic endometrial and colorectal carcinogenesis, determining driver mutational landscape, clonal neoantigen burden and immune response. *J Pathol* (2018) 245 (3):283–96. doi: 10.1002/path.5081

48. Pakish JB, Zhang Q, Chen Z, Liang H, Chisholm GB, Yuan Y, et al. Immune microenvironment in microsatellite-instable endometrial cancers: Hereditary or sporadic origin matters. *Clin Cancer Res* (2017) 23(15):4473–81. doi: 10.1158/1078-0432.CCR-16-2655

49. Song Y, Gu Y, Hu X, Wang M, He Q, Li Y. Endometrial tumors with MSI-h and dMMR share a similar tumor immune microenvironment. *Onco Targets Ther* (2021) 14:4485–97. doi: 10.2147/OTT.S324641

50. Keren L, Bosse M, Marquez D, Angoshtari R, Jain S, Varma S, et al. A structured tumor-immune microenvironment in triple negative breast cancer revealed by multiplexed ion beam imaging. *Cell* (2018) 174(6):1373–87. doi: 10.1016/j.cell.2018.08.039

51. Schürch CM, Bhate SS, Barlow GL, Phillips DJ, Noti L, Zlobec I, et al. Coordinated cellular neighborhoods orchestrate antitumoral immunity at the colorectal cancer invasive front. *Cell* (2020) 182(5):1341–59. doi: 10.1016/j.cell.2020.07.005

52. Yen TT, Wang TL, Fader AN, Shih IM, Gaillard S. Molecular classification and emerging targeted therapy in endometrial cancer. *Int J Gynecol Pathol* (2020) 39(1):26–35. doi: 10.1097/PGP.0000000000000585

53. The genome sequence archive family: Toward explosive data growth and diverse data types. *Genomics Proteomics Bioinformatics* (2021) 19(4):578–83.

54. Database resources of the National Genomics Data Center. China National Center for Bioinformation in 2022. *Nucleic Acids Res* (2022) 50(D1):D27–38.

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EDITED BY
Kelechi Njoku,
The University of Manchester,
United Kingdom

REVIEWED BY
Bethany Geary,
University of Dundee, United Kingdom
Victor Sánchez-Margalet,
Virgen Macarena University Hospital,
Spain
Neal Chris Ramchander,
Manchester University NHS
Foundation Trust (MFT),
United Kingdom

*CORRESPONDENCE
Špela Smrkolj
spela.smrkolj@mf.uni-lj.si
Tea Lanišnik Rižner
tea.lanisnik-rizner@mf.uni-lj.si

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Models including preoperative plasma levels of angiogenic factors, leptin and IL-8 as potential biomarkers of endometrial cancer

Luka Roškar[1,2], Maja Pušić[3], Irena Roškar[3], Marko Kokol[4,5], Boštjan Pirš[6], Špela Smrkolj[1,6*] and Tea Lanišnik Rižner[3*]

[1]Department of Gynaecology and Obstetrics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Division of Gynaecology and Obstetrics, General Hospital Murska Sobota, Murska Sobota, Slovenia, [3]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [4]Faculty of Electrical Engineering and Computer Science, University of Maribor, Maribor, Slovenia, [5]Semantika Research, Semantika d.o.o., Maribor, Slovenia, [6]Division of Gynaecology and Obstetrics, University Medical Centre, Ljubljana, Slovenia

Background: The diversity of endometrial cancer (EC) dictates the need for precise early diagnosis and pre-operative stratification to select treatment options appropriately. Non-invasive biomarkers invaluably assist clinicians in managing patients in daily clinical practice. Currently, there are no validated diagnostic or prognostic biomarkers for EC that could accurately predict the presence and extent of the disease.

Methods: Our study analyzed 202 patients, of whom 91 were diagnosed with EC and 111 were control patients with the benign gynecological disease. Using Luminex xMAP™ multiplexing technology, we measured the pre-operative plasma concentrations of six previously selected angiogenic factors – leptin, IL-8, sTie-2, follistatin, neuropilin-1, and G-CSF. Besides basic statistical methods, we used a machine-learning algorithm to create a robust diagnostic model based on the plasma concentration of tested angiogenic factors.

Results: The plasma levels of leptin were significantly higher in EC patients than in control patients. Leptin was higher in type 1 EC patients versus control patients, and IL-8 was higher in type 2 EC versus control patients, particularly in poorly differentiated endometrioid EC grade 3. IL-8 plasma levels were significantly higher in EC patients with lymphovascular or myometrial invasion. Among univariate models, the model based on leptin reached the best results on both training and test datasets. A combination of age, IL-8, leptin and G-CSF was determined as the most important feature for the multivariate model, with ROC AUC 0.94 on training and 0.81 on the test dataset. The model utilizing a combination of all six AFs, BMI and age reached a ROC AUC of 0.89 on both the training and test dataset, strongly indicating the capability for predicting the risk of EC even on unseen data.

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Conclusion: According to our results, measuring plasma concentrations of angiogenic factors could, provided they are confirmed in a multicentre validation study, represent an important supplementary diagnostic tool for early detection and prognostic characterization of EC, which could guide the decision-making regarding the extent of treatment.

KEYWORDS

angiogenic factors, endometrial cancer, diagnostic biomarkers, angiogenesis, machine learning models, leptin, IL-8, sTie-2

1 Introduction

Endometrial cancer (EC) is the most frequent gynecological malignancy in developed countries, with an increasing incidence rate (1, 2). The diversity of EC dictates the need for precise early diagnosis and pre-operative stratification to appropriately select the extent of surgery and lower both the recurrence rate and risk for overtreatment. A dualistic model, built mainly on the histological findings and prognosis, was introduced by Bokhman back in 1983 (3–5). Recent advances based on molecular classification stratify EC into four risk categories: POLE ultramutated, microsatellite instability hypermutated, copy-number low, and copy-number high (6, 7). This classification of endometrial cancer has been validated and incorporated in the ESMO/ESTRO risk stratification and is currently used in clinical practice to guide management decisions. However, refinements of the current classification with additional biomarkers are likely to further improve and de-escalate treatment in certain subtypes of EC (8).

Biomarkers represent a noninvasive approach for more precise stratification of various malignant diseases (9). No single serum/plasma biomarker alone has yet been classified for clinical use in the diagnostics of EC (10). Emerging findings in genomics, transcriptomics, and proteomics may present a pivotal role for noninvasive early diagnostic options (11).

Angiogenesis is the process of the formation of new vessels from the preexisting vasculature. The process may be activated due to ischemia and tissue trauma as part of normal tissue healing, or it can be one of the key processes in cancerogenesis, allowing fast growth of cancerous tissue and spreading to distant organs. Tumor tissue receives oxygen and nutrition at its very early stage *via* diffusion independently of the vascular network. When the tumor size exceeds 1–2 mm^3, the existing capillary network becomes insufficient, causing hypoxia in solid tumors. The resulting shortage of cellular oxygen and nutrients causes the production of angiogenic factors (AFs), mostly cytokine molecules, which are secreted into the surrounding tissue and provoke the growth of new vessels (12–16). This angiogenic switch makes AFs potential biomarker candidates for early EC detection and assessment of prognosis (12, 17, 18).

In our previous research, we investigated 37 AFs as potential biomarkers for EC. AFs' concentrations in preoperative plasma samples of patients with endometrioid EC (n = 38) and control patients with benign gynecological conditions (e.g., prolapsed uterus or myoma; n = 38) were measured using Luminex xMAP™ multiplexing technology. Our discovery study demonstrated significant differences in the plasma levels of six AFs: sTie-2, G-CSF, and leptin were present in different concentrations in EC versus control patients, and IL-8, neuropilin-1 and follistatin differed among different EC subgroups (19). The roles of these AFs have been described in detail in our recent review paper (12). In the present study, we aimed to validate our previous findings in a larger group of patients.

Recently, machine learning algorithms, a part of artificial intelligence, have evolved to help develop cancer risk stratification systems with great precision. With the aim to differentiate between patient groups, the machine learning approach simultaneously consider multiple disease-specific risk factors, which would otherwise present an impossible statistical obstacle, especially in the heterogeneous type of disease like EC (20, 21). In our study, besides basic statistical methods, we used machine learning algorithms to create a robust diagnostic model based on the plasma concentration of tested AFs.

2 Materials and methods

2.1 Patient enrollment

Patient enrolment took place between June 2012 and October 2021 at the Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, Slovenia. We included 215 consecutive eligible women who underwent surgical treatment, including a group of histologically confirmed EC patients (n = 98) and a control group of women with a

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prolapsed uterus or myoma (n = 117). 13 women were excluded from data analysis (7 EC patients and 6 control patients) due to the presence of other malignancies, withdrawal of consent or when surgery was subsequently cancelled for any other reason.

The patients were recruited by senior gynecologists with the help of study nurses. All histological analysis was performed in the University Medical Centre Ljubljana, Department of Pathology. Each sample was consecutively analysed by two pathologists and their consensus report was logged into study case report form. None of the included patients received drugs with known anti-angiogenic effects, and no neoadjuvant chemotherapy was used. One day to one week prior to surgery, morning blood samples were collected, and additional information was obtained regarding patients' lifestyle, medications used, and gynecological and clinical status (Table 1). For sample collection and processing, strict and

TABLE 1 Detailed clinical characteristics of the study participants.

	Control patients n = 111 (100%)	EC patients n = 91 (100%)	p[a] values
Age category			
<50 years	33 (29.7)	8 (8.8)	
50–59.9 years	38 (34.2)	22 (24.2)	<0.001
60–69.9 years	25 (22.5)	40 (44.0)	
70-79.9 years	14 (12.6)	16 (17.6)	
>80 years	1 (0.9)	5 (5.5)	
Body mass index (kg/m^2)			
<18.5 (underweight)	1 (0.9)	0 (0)	
18.5–24.9 (normal weight)	40 (36.0)	19 (20.9)	
25–29.9 (overweight)	41 (36.9)	25 (27.5)	
30–34.9 (class I obesity)	23 (20.7)	22 (24.2)	<0.001
35–39.9 (class II obesity)	5 (4.5)	13 (14.3)	
40–49.9 (class III obesity)	1 (0.9)	9 (9.9)	
> 50.0 (class IV obesity)	0 (0)	1 (1.1)	
Missing data	0 (0)	2 (2.2)	
Smoking status			
Nonsmoker	68 (61.3)	63 (69.2)	
Smoker	21 (18.9)	11 (12.1)	ns
Former smoker	19 (17.1)	15 (16.5)	
Missing data	3 (2.7)	2 (2.2)	
Hormonal therapy in the past			
No	67 (60.4)	56 (61.5)	
Yes	9 (8.1)	6 (6.6)	ns
Missing	35 (31.5)	29 (31.9)	
Peroral contraception in the past			
No	60 (54.1)	41 (45.1)	
Yes	26 (23.4)	21 (23.1)	ns
Missing	25 (22.5)	29 (31.9)	
Diabetes			
No	94 (84.7)	70 (76.9)	
Yes	15 (13.5)	21 (23.1)	ns
Missing data	2 (1.8)	0 (0)	
Arterial hypertension			
No	86 (77.5)	49 (53.8)	
Yes	24 (21.6)	42 (46.2)	<0.001
Missing data	1 (0.9)	0 (0)	
Menopausal status			
No	46 (41.4)	15 (16.5)	
Yes	62 (55.9)	75 (82.4)	<0.001
Missing data	3 (2.7)	1 (1.1)	

[a]p values were calculated using non-parametric Mann–Whitney test for continuous variables and chi-squared test for categorical variables; ns = not significant

detailed standard operating procedures were followed. Briefly, 6 ml of blood was collected from each patient by venipuncture using BD vacutainer K2 EDTA tubes (Cat. No#: 367864, BD Medical, New Jersey, USA). Immediately after collection, tubes were inverted 10 times to assure sufficient mixing of blood with anticoagulant. Collected blood samples were centrifuged within 1 hour of collection at 1400 x g for 10 minutes at 4°C. Obtained plasma was transferred to a 5 ml polypropylene tube (Cat. No#: 352063, BD Medical) and mixed several times using a disposable plastic Pasteur pipette. Finally, plasma samples were divided into 200 µl aliquotes and stored in cryogenic tubes (Cat. No#: 375418, Thermo Scientific, Waltham, Massachusetts, USA) at – 80°C until further analysis.

This study was approved by the National Medical Ethics Committee of the Republic of Slovenia (No. 0120-515/2017/4 and 0120-541/2019/7). All participants signed written informed consent before participating in this study.

2.2 Measurements of AFs

All samples were anonymized, and the person performing the assays was blind to the identity of the samples. Plasma samples were tested for 6 circulating angiogenesis biomarkers using Luminex xMAP multiplexing technology with two Milliplex® MAP Human Angiogenesis/Growth Factor Magnetic Bead Panels: HANG2MAG-12K and HAGP1MAG-12K (Merck Millipore, Burlington, Massachusetts, USA, LOT#3601567 and LOT#3601566, respectively). All tests were performed according to the manufacturer's protocol. Briefly, 10 µl of each plasma sample was used for the conduction of assays. Samples were diluted 1:3 (4 AFs- Bead Panel 1) and 1:5 (2 AFs-Bead Panel 2) using the Assay Buffer provided in the manufacturer's kit. Samples were mixed with 5.6 µm polystyrene beads on 96 well plates and incubated overnight at 4°C with shaking. Each bead was coated with a specific captured antibody and labelled with two different fluorescent dyes at different ratios assigned for each individual antibody. Plates were washed 3 times and incubated with detection antibody at room temperature (RT) for 1h. Following incubation with streptavidin-phycoerythrin for 30 minutes, plates were washed again, and Drive Fluid was added to all wells. Reading was performed on a MagPix® instrument (Luminex, Austin, Texas, USA). Bio-Plex Manager Software (Bio-Rad Laboratories, Hercules, California, USA) and five-parameter logistic regression modelling were used to calculate the final concentrations.

2.3 Statistics

To assess the normality of the distributions, a Shapiro-Wilk test was used. For univariate statistical analysis, the parametric t-test or non-parametric Mann-Whitney U test was used to assess the statistical significance of the difference in plasma concentrations of 6 AF between EC patients and control patients and between different subgroups of EC patients. The non-parametric Kruskal–Wallis test with Dunn's multiple comparison corrections as *post-hoc* tests was used to compare more than two groups. Fisher's exact and Chi-square tests were used for comparison of categorical variables. Statistical significance was set at $p < 0.05$. Results of the descriptive analysis (i.e. patient's clinical data) were presented as mean ± standard deviation (SD), while the concentrations of the measured proteins were presented as median and range (Tables 1, 2, respectively). Before further analysis, we excluded measurements with reported out-of-range concentrations. Outliers were detected and excluded from further analysis, using the ROUT method (22) with cut off set at 0,1%.

2.4 Machine learning based classification

The case/control classification models were created using the dataset of the 202 women described in the Patient Enrolment section using the scikit-learn library version 1.0.1 (23) for model training and evaluation. Based on well-performing algorithms for small datasets (24), the ml-jar library version 0.11.2 (25, 26) was chosen to automate the model selection and perform the hyper-parameters tuning and design an ensemble-based classification model.

Preprocessing included the following steps: all out-of-range values below the detection threshold (11.1 pg/ml for follistatin, 5.4 pg/ml for G-CSF, 0.2 pg/ml for IL-8, 42.8 pg/ml for leptin, 12.2 pg/ml for sTie-2 and 54.9 pg/ml for neuropilin-1) were replaced with 0, all out-of-range values above the detection threshold were defined as missing, and finally, missing data imputation was performed. Imputation was done using the mean method for interval/ratio level data and the mode method for categorical data (27).

The dataset was then split into training and test datasets using the scikit-learn library (23), using the built-in train/test split function in an 80% to 20% ratio. All variables were then compared using the Mann-Whitney U statistics to confirm no significant differences between the training and the test datasets. Finally, data were imported into Python using the Pandas library (28, 29), and finally, input and output column selection was performed for each of the hypotheses tested.

The models were trained by optimising the area under the curve (AUC) for the Receiver Operating Characteristic (ROC) curve using the mljar built-in roc_auc metric. The training was performed for 20 minutes for multivariate models and for 15 minutes for univariate models, with a 5-minute limit per individual run. The model selection during training was performed using the k-fold cross-validation method with 20

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TABLE 2 Plasma leptin and IL-8 levels in patients with endometrial cancer and control patients.

Patient group	n (%)	Leptin		p	IL-8		p
		Median	Range	(adj. p)[a]	Median	Range	(adj. p)[a]
Disease status							
EC	91 (45.0)	36654	6248 - 149743	<0.0001	4.16	0.83 – 11.74	0.0619
Benign	111 (55.0)	23121	1526 - 93115	(0.0006)	3.08	0.39 – 10.75	(0.3185)
Histology							
Type I	65 (74.7)	37715	6248 – 173402	0.1244	4.16	0.83 – 11.74	0.9594
Type II	22 (25.3)	34015	8183 – 72174	(0.5494)	4.29	0.83 – 12.48	(1.000)
EC differentiation							
Well differentiated G1	46 (61.3)	38921	6248 - 173402	0.7636	3.47	0.83 – 9.17	0.0051
Moderately differentiated G2	19 (25.3)	35638	12328 - 149743	(0.9998)	4.70	1.42 – 6.97	(0.0302)
Poorly differentiated G3	10 (13.3)	35581	8183 - 61204		5.69	0.83 – 36.7	
FIGO stage							
IA	58 (65.9)	33436	6248 - 109865	0.3607	3.52	0.83 – 6.87	0.0206
IB	12 (13.6)	37568	12384 - 118709	(0.9317)	4.72	2.02 – 13.66	(0.1174)
II	8 (0.09)	54979	8183 - 149743		6.26	1.77 – 12.48	
III	6 (0.07)	40824	34477 - 106658		4.35	2.93 – 8.38	
IV	4 (0.05)	55375	16834 - 59024		12.02	0.83 – 36.70	
Myometrial invasion							
No invasion	28 (32.6)	37885	6248 – 173402	0.6168	3.07	0.83 – 5.72	0.0080
< 50% myometrium	32 (37.2)	34727	6661 – 122861	(0.9968)	4.11	1.10 – 11.74	(0.0471)
> 50% myometrium	26 (30.2)	46184	8183 – 149743		4.70	0.83 – 18.37	
Lymphovascular invasion							
No	65 (74.7)	35089	6248 – 122861	0.2144	3.52	0.83 – 6.97	0.0004
Yes	22 (25.3)	47372	8183 – 149743	(0.7649)	5.36	0.83 – 18.37	(0.0024)
Metastasis							
No	78 (88.6)	35089	6248 - 122861	0.1854	4.16	0.83 – 11.74	0.2595
Yes	10 (11.4)	48380	16834 - 106658	(0.7078)	4.70	0.83 – 18.37	(0.8351)

[a]p-values were calculated using non-parametric Mann-Whitney tests or Kruskal-Wallis tests with post hoc test and Dunn's correction. Bonferroni-Šídák method was used for multiple comparison correction and adjusted p-values are listed in parenthesis.
Plasma levels of sTie-2, follistatin, neuroplin and G-CSF are included in Supplementary Table S1.

folds on each model; the threshold for deciding the prediction was calculated based on the ROC curve of the best model by maximising the difference between the true positive rate and the false positive rate on the training set.

The best performing model was then tested using the previously described testing set, again calculating the same basic metrics and validating the models still perform well on previously unseen data. To confirm that the model performance was significantly better than random guessing, Fisher's Exact test was used on the confusion matrix produced on the training and test datasets.

Two types of models were designed using the described approach: univariate models for each AF and BMI individually and multivariate models for the following combinations:

- Age + BMI + AFs, containing: age, BMI, neuropilin-1, sTie-2, IL-8, follistatin, leptin, G-CSF
- BMI + AFs, containing: BMI, neuropilin-1, sTie-2, IL-8, follistatin, leptin, G-CSF
- AFs only, containing: neuropilin-1, sTie-2, IL-8, follistatin, leptin, G-CSF
- BMI + AFs without leptin: BMI, neuropilin-1, sTie-2, IL-8, follistatin, G-CSF
- AFs only without leptin: neuropilin-1, sTie-2, IL-8, follistatin, G-CSF
- Selected features: age, IL-8, leptin and G-CSF

The "Selected features" model was designed based on the mljar automated feature selection capability, which works in two steps (30):

- A random input feature is created and a model trained on it; SHAP importance (31) is calculated for the feature.
- The model is trained on the rest of the features, and only those features that have a higher SHAP importance than

the introduced random feature are used in training. Those features included the aforementioned: age, IL-8, leptin and G-CSF.

Lastly, the feature importance was calculated using the permutation method (32). All models' output (predicted) variable was whether they belonged to the case or control group. The complete Jupyter Notebook (iPython) and Python scripts used for the model training, validation, plot drawing, and dataset schema are published on: https://github.com/klokedm/EndometrialCancerModelling.

3 Results

3.1 Characteristics of EC and control patients

The case group included 91 EC patients with a mean age of 62.1 ± 9.8 years (range: 32–86 years) and a mean body mass index (BMI) of 31.2 ± 7.7 kg/m2 (range: 18.8 – 58.5 kg/m2). 75 women (82,4%) were postmenopausal with an average duration of menopause of 12.6 ± 8.4 years. The detailed clinical characteristics are presented in Table 1.

Histology revealed 76 cases (83.5%) of endometrioid endometrial cancer (EEC), 9 cases (9.9%) of serous EC and one case (1.1%) of each of carcinosarcoma, clear cell EC and mixed type EC. The deep myometrial invasion was observed in 26 EC patients (30.2%), <50% invasion into the myometrium in 32 EC patients (37.2%), and no invasion into the myometrium in 28 EC patients (32.6%); this information was missing for five patients. LVI was observed in 22 patients (25.3%). According to the classification of the International Federation of Gynecology and Obstetrics (33), the following EC stages were observed: IA (n = 58, 65.9%), IB (n = 12, 13.6%), II (n = 8, 9.1%), III (n = 6, 6.8%), and IV (n = 4, 4.5%). Histopathological evaluation divided EC samples according to the degree of histological differentiation: G1, 47 cases; G2, 20 cases; and G3, 12 cases.

The control group included 111 patients with a mean age of 56.5 ± 10.5 years (range: 37–84 years) and a mean BMI of 27.0 ± 4.9 kg/m2 (range: 18.4–42.2 kg/m2). 62 women (55.9%) were postmenopausal, with an average duration of menopause of 12.7 ± 8.5 years. The detailed clinical characteristics are presented in Table 1.

When both groups were compared, there was a statistically significant difference in BMI and age distribution, as well as in menopausal status and in the presence of arterial hypertension (all $p < 0.001$). There were no differences between groups in hormonal therapy, smoking status or the presence of diabetes (Table 1).

3.2 Leptin is increased in EC patients and IL-8 in patients with LVI and MI

Univariate statistical analysis revealed a significant difference in the plasma concentration of leptin between EC and control patients (Figure 1; Table 2). The difference in IL-8 levels between EC and the control group was also substantial, although not statistically significant. IL-8 was significantly higher in patients with poorly differentiated G3 EC and in EC patients with present myometrial (MI) or lymphovascular invasion (LVI) compared to EC patients without invasion or control patients. Compared to control patients, leptin was significantly higher in EC patients regardless of MI or LVI or the presence of metastasis (Figure 2).

When EC patients were stratified according to the disease stages, we detected higher plasma levels of leptin, IL-8 and sTie-2 as EC progressed from stage IA through stage II (Figure 3, Table 2 and Supplementary Table 1). This trend was not seen in stages III and IV, which might be due to a low number of patients in these stages (6 and 4 patients, respectively). Leptin was also significantly higher in type 1 EC patients versus control patients, whereas IL-8 was higher in type 2 EC versus control patients, particularly in poorly differentiated endometrioid EC grade 3 (Figure 1; Table 2).

The prognostic potential of AFs was evaluated based on the presence of MI and LVI. There was a statistically significant difference in IL-8 plasma levels between patients with or without MI and LVI (Figure 2). Although IL-8 is also produced and secreted by adipocytes, and its levels increase with BMI, which is known to be associated with EC incidence and outcomes (34), we found no correlation between IL-8 and BMI with Pearson correlation coefficient of 0.0009 (Suplementary Table 8). Plasma levels of IL-8 were able to stratify EC patients according to the MI ($p = 0.0057$) and LVI ($p = 0.0005$), whereas BMI, which is not used in clinical practice to asses EC risk, was not successful in differentiation among groups: $p = 0.8669$ and 0.6156 for MI and LVI, respectively. The data is presented in Supplementary Figure 1.

3.3 Machine learning approaches identified several diagnostic models

The training dataset contained 161 patients (79.7%) and the testing dataset contained 41 patients (20.3%), and none of the 8 variables used in modelling were significantly different between the training and test datasets with p-values for: age (p=0.495), BMI (p=0.721), neuropilin-1 (p=0.277), sTie-2 (p=0.826), IL-8 (p=0.556), follistatin (p=0.785), leptin (p=0.848), G-CSF (p=0.256).

The AFs in the complete data set were weakly correlated (correlation coefficient < 0.3) with known risk factors (Age and

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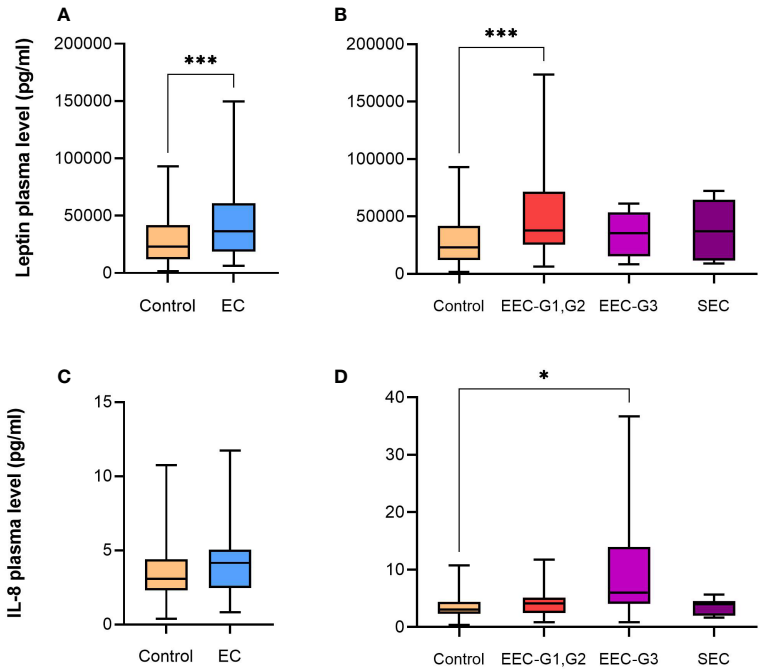

FIGURE 1
Box plots with median, minimum and maximum for plasma leptin and IL-8 levels (pg/mL). **(A, C)** Control patients and patients with endometrial cancer. **(B, D)** Control patients and patients with histological subtypes of endometrial cancer – Type I (EEC G1, G2) and Type II (EEC G3 and SEC). EC – endometrial cancer, EEC – endometrioid endometrial cancer, SEC – serous endometrial cancer, G – grade. Adjusted p values: *p < 0.05, ***p < 0.001.

BMI), with the exception of BMI and Leptin, which were strongly correlated (correlation coefficient > 0.7). All correlations are shown in Supplementary Table 8.

3.3.1. Model based on leptin performed significantly better than random guessing

The results obtained on the final ensemble using the training dataset (best metric for each metric in bold) are shown in Table 3. The table presents the Accuracy, Precision, F1 score and Receiver Operating Characteristic (ROC) Area Under Curve (AUC) metrics for all seven univariate models calculated on the training dataset using the metrics presented in Supplementary Table 2. The models with the highest accuracy were based on leptin and IL-8 on the training dataset, the model with the highest precision was based on follistatin, and the model with the highest F1 score and ROC AUC was based on leptin. The model based on leptin reached the best result on 3 out of 4 metrics: accuracy, F1 score and ROC AUC.

The results obtained on the final ensemble using the test dataset (best metric for each model in bold) are shown in Table 4. The table presents the Accuracy, Precision, F1 score and Area Under Curve (AUC) metrics for all seven univariate models calculated on the test dataset using the metrics presented in Supplementary Table 3. The model based on leptin reached the best result on all 4 metrics: accuracy, precision, F1 score and ROC AUC, and outperformed other AFs as well as BMI as an EC risk predictor.

Additionally, the Fisher Exact p-values calculated for confusion matrices on the test datasets on univariate models are shown in Supplementary Table 4. They show that all models, except the model based on neuropilin-1, performed significantly better than random guessing on the training dataset. However, only the model based on leptin performed significantly better than random guessing on both the training and the test datasets with a significance level of $p < 0.01$. The ROC curves for all seven final univariate models on the training and test datasets are shown in Figure 4.

3.3.2. The model, including the combination of all AFs, BMI and age, shows the best diagnostic characteristics

The results obtained on the final ensemble using the training dataset (best model for each metric in bold) are shown in Table 5. They show that the best results for all metrics were obtained for the model utilizing the subset of features determined using the method described in the Methodology section. However, all models achieved good results on all metrics, and discrepancies were minimal (Supplementary Table 5).

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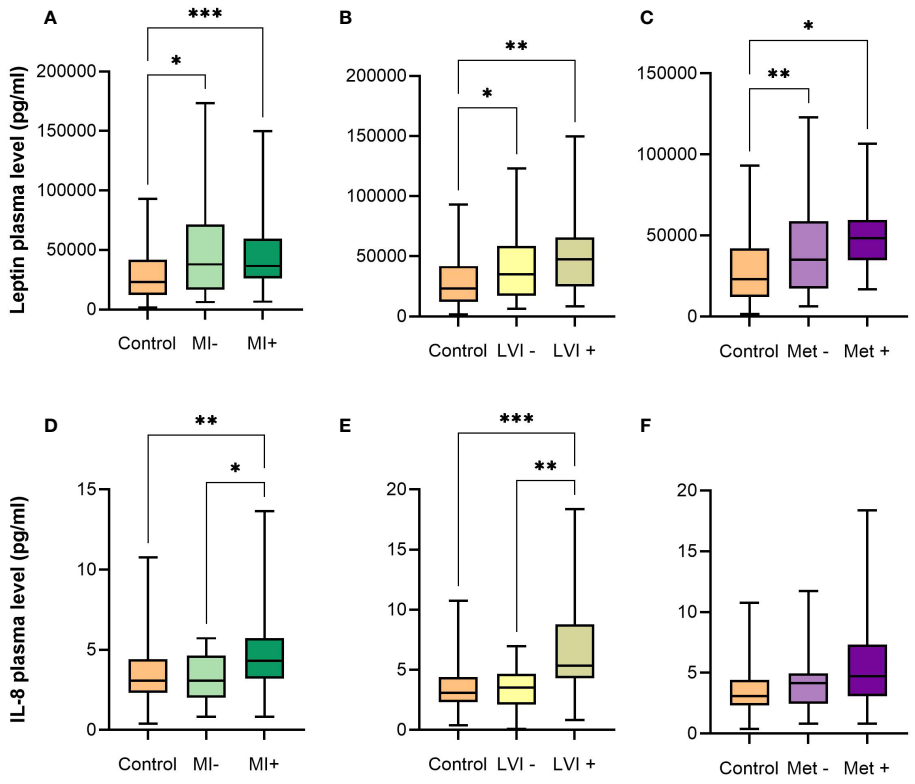

FIGURE 2
Box plots with median, minimum and maximum for plasma leptin and IL-8 levels (pg/mL). **(A, D)** Control patients and EC patients with absence or presence of myometrial invasion. **(B, E)** Control patients and EC patients with absence or presence of lymphovascular invasion. **(C, F)** Control patients and EC patients with absence or presence of metastasis. EC – endometrial cancer, MI – myometrial invasion, LVI – lymphovascular invasion, Met – metastasis. Adjusted p values: *p < 0.05, **p < 0.01, ***p < 0.001.

The results obtained on the final ensemble using the test dataset (best model for each model in bold) are presented in Table 6 (Supplementary Table 6) – the discrepancies are bigger on the test data in line with the expectations. However, metrics remain good, with the lowest accuracy being 63% and the highest accuracy being 80%, and lowest precision being 73% and the highest precision reaching 100%. The ROC AUC was in the range of 0.69 to 0.89 (Figure 5), indicating good model generalization to unseen data.

The "Selected Features" model was designed by automatically selecting the features with the estimated highest capability to discriminate between EC and control patients based on SHAP importance. Those features were: age, IL-8, leptin and G-CSF. The corresponding model reached a ROC AUC of 0.94

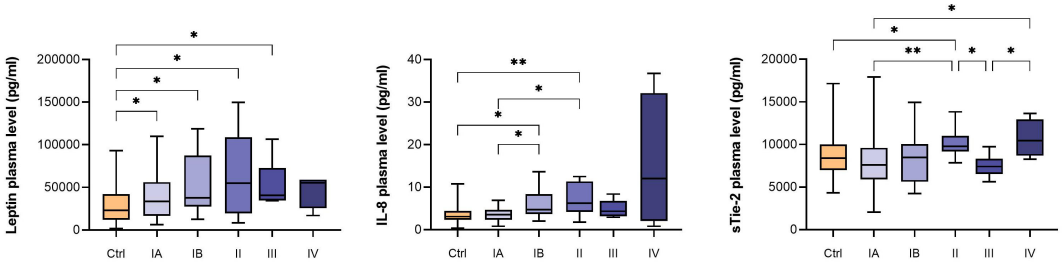

FIGURE 3
Box plots with median, minimum and maximum for plasma leptin, IL-8 and sTie-2 levels (pg/mL) for control patients and EC patients in different FIGO stages. P-values were calculated using Kruskal-Wallis tests with *post hoc* test without correction. *p < 0.05; **p < 0.01.

TABLE 3 Performance metrics of univariate models on the training dataset based on single angiogenic factors.

Model	Accuracy	Precision	F1 score	ROC AUC
BMI	0.66	0.65	0.59	0.68
Neuropilin-1	0.54	0.50	0.62	0.55
sTie-2	0.65	0.60	0.65	0.68
IL-8	**0.75**	0.74	0.71	0.83
Follistatin	0.73	**0.75**	0.67	0.77
Leptin	**0.75**	0.69	**0.74**	**0.80**
G-CSF	0.61	0.58	0.56	0.63

Best metric shown in bold.

on the training dataset and 0.81 on the test dataset. The model utilizing a combination of all AFs, BMI and age reached a ROC AUC of 0.89 on both the training and test dataset.

The Fisher Exact test analysis results on the test dataset for the multivariate models are presented in Supplementary Table 7. Similarly to the univariate models, all models performed significantly better than random chance on the training dataset, and all models where AFs were used together with additional clinical data also performed better than random guessing on the test dataset. Based on this performance, the models were able to generalize the patterns from the training data and could be useful for predicting the risk of EC on unseen data.

4 Discussion

Non-invasive diagnostic approaches invaluably assist clinicians in the detection, management and treatment of patients in daily clinical practice. Recently a proof-of-principle was provided in the area of cytological analysis for EC screening: O'Flynn et al. demontrated that endometrial cancer can be detected with high accuracy in urine and vaginal fluid (35). However, there are currently no validated diagnostic or prognostic biomarkers for EC that could accurately predict the presence and extent of disease, and thus pathohistological examination remains the gold standard for EC diagnosis.

In our previous discovery study, we investigated 37 AFs as potential biomarkers for EC. Preoperative plasma samples of 38 EC patients and 38 control patients were analyzed using Luminex xMAP^{TM} multiplexing technology. Six out of 37 AFs were present in significantly different concentrations between groups of patients. sTie-2 and G-CSF were lower in EC compared to control patients, and plasma level of leptin was higher in EC patients. Neuropilin-1 plasma level was higher in patients with type 2 EC (G3) compared to patients with lower grade cancer or controls. Follistatin level was higher in patients with LVI, and IL-8 plasma level was higher in patients with metastases (19). In this validation study, we further evaluated those six AFs on a larger group of patients. While our previous study was limited to post-menopausal women with endometrioid EC, in the present validation study, women with other EC types were also included, regardless of their menopausal status. In the previous study, where 60.5% of included EC patients were in IA stage EC, and no patients in stage II or IV were included, we detected lower values of sTie-2 in EC patients compared to the control group. In the current validation study, we observed a similar trend in the early stages, whereas the concentration of sTie-2 significantly increased as the disease progressed, particularly in FIGO stage IV. This is in accordance with results from Saito et al., who reported higher expression of Tie-2 in endometrial adenocarcinoma than in normal epithelial cells (36).

EC often involves patients burdened with other comorbidities, such as hypertension, diabetes or obesity (37). In our study, diabetes status was balanced between EC and control patients, and only leptin differed among patients when stratified according to hypertension status or obesity. The effect

TABLE 4 Performance metrics of univariate models on the test dataset based on single angiogenic factors.

Model	Accuracy	Precision	F1 score	ROC AUC
BMI	0.57	0.50	0.56	0.57
Neuropilin-1	0.35	0.33	0.40	0.39
sTie-2	0.41	0.31	0.31	0.50
IL-8	0.59	0.52	0.59	0.61
Follistatin	0.62	0.57	0.53	0.54
Leptin	**0.70**	**0.61**	**0.72**	**0.75**
G-CSF	0.59	0.52	0.62	0.55

Best metric shown in bold.

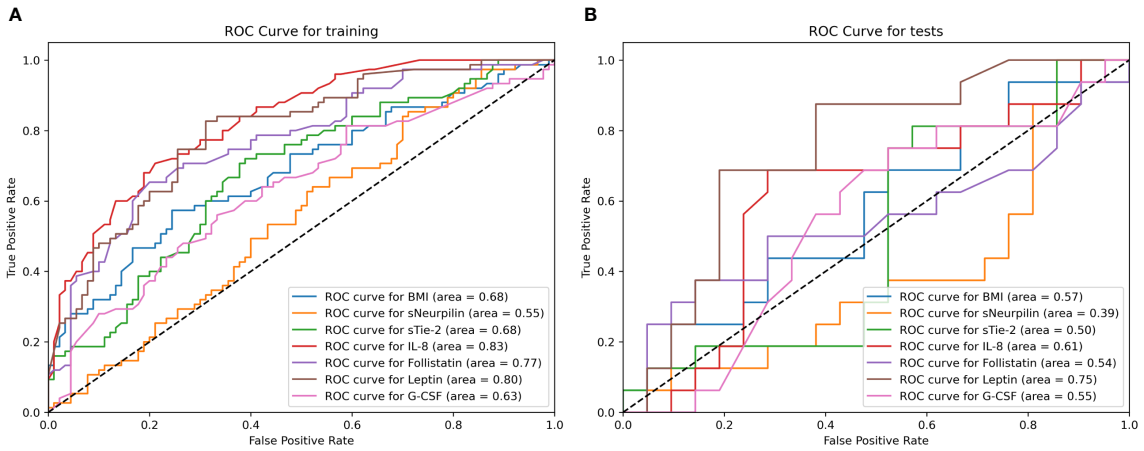

FIGURE 4
The Receiver Operating Characteristic (ROC) curve for all seven final univariate models on the training **(A)** and test **(B)** dataset calculated using the cross-folding method, as described in Section 2.4. **(A)** The curve shows how the true positive rates of individual models change according to the false positive rate, by varying the detection threshold in the model. The AUC metric for each curve is displayed in the parentheses. As can be seen, all models except neurpilin-1 perform better than random chance on the training dataset and the models are able to discern between case and control group with an AUC ≥ 0.63. **(B)** The curve shows how the true positive rates of individual models change according to the false positive rate by varying the detection threshold in the model. The AUC metric for each curve is displayed in the parentheses. As seen from the curve, only leptin and IL-8 based models deviate from the random line, and as shown in the performance metrics, only leptin reaches a statistically significant accuracy at the optimal ROC point. This indicates that the models (with the exception of the mentioned ones) overfit on the training data.

of BMI difference between groups was intensely further investigated through machine learning modelling.

Obesity is an established risk factor for EC and presents a larger risk for this malignancy than any other cancer type (38–41). Adipose tissue is an endocrine organ which synthesizes adipokines—biologically active substances participating in cell growth and differentiation, apoptosis, angiogenesis, and carcinogenesis. Leptin is among the most important adipokines during EC development, and numerous recent studies have already proposed it as a new candidate marker in determining the potential risk of EC (42–45). In accordance with the literature (46–50), our study showed higher leptin levels in EC patients, particularly in Type I EC, at higher stages and in patients with present metastasis or MI and LVI.

During carcinogenesis, leptin promotes tumor angiogenesis. Early studies showed that endothelial leptin receptor Ob-R generates a growth signal involving a tyrosine kinase-dependent intracellular pathway, promoting angiogenic processes (51, 52). Furthermore, leptin induces proliferation, migration, and invasion and suppresses apoptosis of cancer cells (42, 53). However, cancerogenic potential of leptin has been shown to differ between different tissues (54, 55). In EC, it has been shown in *in vitro* studies that leptin inhibits the apoptosis of endometrial carcinoma cells through activation of the nuclear factor κB-inducing kinase/IκB kinase pathway (49) and stimulates endometrial carcinoma cell proliferation *via* enhancing P450arom expression and estradiol synthesis (56).

Leptin has an important role in the regulation of energy balance and glucose metabolism and is considered to play an important part in the link between obesity and EC (57). There is still an ongoing debate in the literature on whether the effect of leptin on EC risk is related to higher BMI or whether it is an independent risk factor for EC. Wang et al. (58) performed a meta-analysis of 6 preceding studies and found that after

TABLE 5 Performance metrics of multivariate models based on multiple features on training data.

Model	Accuracy	Precision	F1 score	ROC AUC
Age+BMI+AFs	0.85	0.83	0.83	0.89
BMI + AFs	0.82	0.84	0.77	0.86
AFs	0.85	0.83	0.83	0.89
BMI + AFs without Leptin	0.84	0.79	0.83	0.92
AFs without Leptin	0.81	0.75	0.79	0.86
Selected Features	**0.86**	**0.85**	**0.84**	**0.94**

Best metric shown in bold.

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TABLE 6 Performance metrics of multivariate models based on multiple features on test data.

Model	Accuracy	Precision	F1 score	ROC AUC
Age+BMI+AFs	0.76	0.83	0.75	**0.89**
BMI + AFs	0.68	**1.00**	0.58	0.69
AFs	0.63	0.73	0.59	0.75
BMI + AFs without Leptin	**0.80**	0.89	**0.80**	0.83
AFs without Leptin	0.71	0.81	0.68	0.76
Selected Features	0.76	0.80	0.76	0.81

Best metric shown in bold.

adjusting for BMI, leptin was still associated with an increased risk of EC. On the other hand, another meta-analysis performed by Ellis et al. observed that BMI and diabetes appeared to affect the association between leptin levels and EC risk (43). Some other studies found a strong positive correlation between patients' BMI and serum leptin levels (46, 50), while a recent Mendelian randomization analysis showed a causal effect of BMI on EC but failed to find evidence for leptin to be causally implicated in EC risk (59).

By utilizing an automated machine learning approach and comparing univariate models applying BMI and leptin as predictor variables, our research indicates that leptin might be able to predict EC better than BMI. This would seem to be further indicated by the results of the automated feature prediction selection described in the methodology, which identified a combination of age, IL-8, leptin and G-CSF, but not BMI, as the most important features for multivariate model building and the performance of the corresponding model utilizing those features. This supports findings that leptin might be involved in EC development *via* pathways beyond obesity-related pathophysiology, including through angiogenesis (51, 52).

However, as always with machine learning models, this should be interpreted with care. Due to the relatively small data set, we observed overfitting on univariate models, and classification close to random on the test dataset could simply mean the model picked features that were not well generalizable. On the other hand, leptin-based multivariate models performed significantly better than random chance on the test dataset with an AUC very similar to the one reached on the training dataset. This indicates that the models were able to generalize and could be useful for predicting the risk of EC even on unseen data. Therefore, these models show good diagnostic characteristics, with AUC in the range from 0.69 to 0.89.

Another link between adipose tissue and EC is through chronic low-grade inflammation resulting from increased secretion of pro-inflammatory molecules such as cytokines and chemokines (60). IL-8 is a pro-inflammatory cytokine secreted by adipocytes, with well-defined functions in tumor-associated inflammation. It is chemotactic for lymphocytes and neutrophils

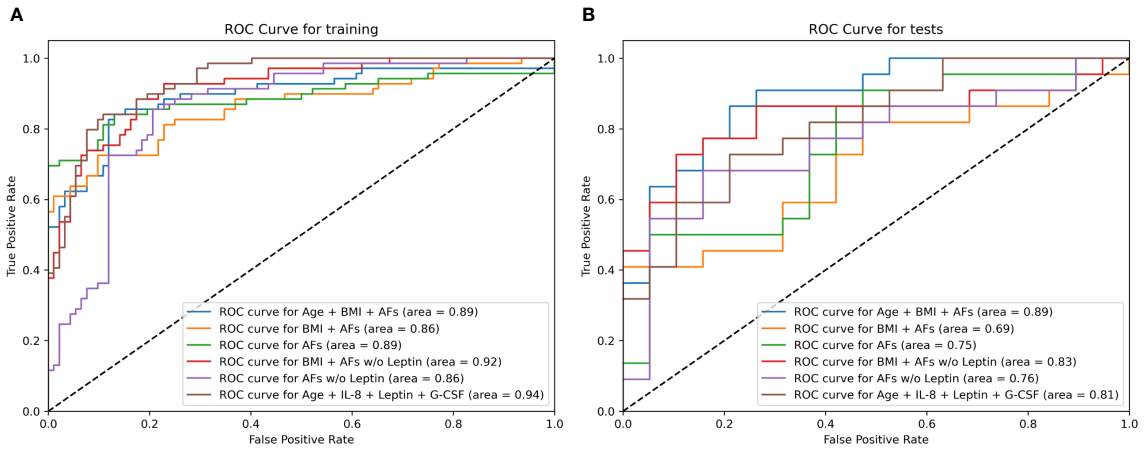

FIGURE 5
Receiver Operating Characteristic (ROC) curve for the six final multivariate models on the training **(A)** and test **(B)** dataset calculated using the cross-folding method, as described in Section 2.4. **(A)** The curve shows that all multivariate models were able to discern between case and control group on the training dataset with relatively good results (AUC > 0.85). **(B)** Most models utilizing multiple features were able to generalize to unseen data and maintained a relatively robust classification performance with ROC AUC > 0.80. The model utilizing a combination of all AFs, BMI and age reached an ROC AUC of 0.89 on both the training and test dataset.

and also has an important role in angiogenesis (61, 62). Its levels increase with BMI and waist circumference, which is also associated with EC incidence and outcomes (34, 63). Kotowicz et al. (64) demonstrated the clinical usefulness of IL-8 measurements as potential prognostic factors in type 1 EC, where elevated pretreatment IL-8 serum levels were independently associated with shorter disease-free and overall survival.

While in our study, leptin was significantly increased in type I EC, IL-8 was higher in type II EC. Its level also increased through higher grades, and it was particularly elevated in poorly differentiated EEC G3, however the difference between G1/G2 and G3 cases was not statistically significant. We assessed EEC G3 cancers together with type II tumors since several important reports have strongly demonstrated that high-grade endometrioid cancers have molecular characteristics, risk factors, clinical behavior, and prognosis overlapping with those of non-endometrioid cancers (65, 66). We demonstrated that higher levels of IL-8 are suspect for higher-grade tumors. However, there were only 10 G3 EC patients in our cohort. If this result could be validated on a larger set of patients, a simple blood test for IL-8 prior to the surgery might lead to a better stratification for the extent of surgery.

The angiogenic switch occurs early in the process of cancerogenesis. Correspondingly, we observed indicated trend for leptin, sTie-2 and IL-8 progression through early EC stages, which illuminates how growing tumor mass dictates a higher need for additional oxygen and nutrient supply and accelerates angiogenic activity. Nevertheless, due to low number of cases in each EC stage, this should be taken with care and studies on bigger cohort are needed to confirm this.

We also showed a statistically significant correlation between EC myometrial invasion and IL-8 levels. This was previously reported by Fujimoto et al., who suggested IL-8 might act as an angiogenic switch in myometrial invasion in stage I EC (67). According to the currently valid guidelines (10), LVI, among other histopathological characteristics, is the cornerstone of risk stratification. In our study, IL-8 levels significantly increased during LVI, which further demonstrates the role of IL-8 in the angiogenesis of EC. Since IL-8 is also associated with obesity, the prognostic value of IL-8 was compared to BMI based on the presence of MI and LVI. Plasma IL-8 level was a better prognostic biomarker than BMI in terms of EC patient stratification according to both MI and LVI. Levels of IL-8 were also higher in patients with present metastasis, which is in accordance with our previous results (19) and *in vitro* study on cell lines (68).

Due to the relatively low number of patients with LVI and MI in our dataset, the IL-8 link to MI and LVI could not be analysed using the machine learning approach; however, the data presented indicate that it would be a good candidate for a larger study with larger and more balanced datasets.

In our study, we also evaluated neuropilin-1, follistatin and G-CSF as potential biomarkers for EC. While they were individually able to differentiate between EC patients and control patients (with the exception of neuropilin-1), the univariate models utilizing those AFs did not generalize well to unseen (test) data (so-called overfitting). However, when used in conjunction with other data, the AFs significantly improved the classification capabilities of models, and the model utilizing a combination of all AFs, BMI and age reached a ROC AUC of 0.89 on both the training and test dataset, strongly indicating the usefulness of the combination of AFs.

The best multivariate model on the training set ("Selected Features") has also proven to be robust on both the training and test datasets, where it revealed good diagnostic characteristics with the ROC AUC of 0.94 and 0.81, respectively. It also greatly outperformed individual results of univariate models for included AFs, i.e. leptin, IL-8 and G-CSF.

Our study has also confirmed the importance of existing, well-known risk factors, namely age and BMI, and the values for both were significantly different between the EC and control patients (69, 70). To confirm AFs can valuably add to diagnostics of EC, we created and tested models using only the variables that are – at most – weakly correlated with known risk factors. Using those models, we have confirmed that even without knowing age, BMI or hypertension status, we can reach relatively robust results using only AFs (71% accuracy on the test dataset using AFs without leptin). Nevertheless, combining AFs with the existing risk factors yielded better results (80% accuracy on the test dataset), which confirms the value of already established risk factors in extended models.

Finally, the fact that models utilizing less data sometimes outperformed models utilizing more data might seem counterintuitive but would seem to hint at some combinations of factors containing more noise and thus causing overfitting. In our study, due to noise generation, models using a combination of both BMI and leptin performed weaker than models with either BMI or leptin alone, indicating some collinearity between them. Consequently, a larger study providing more data would be useful to fine-tune the developed diagnostic models and determine the minimal subset required to achieve good classification results.

Our study has the following limitations. First, relatively low number of patients in different EC subgroups – in relation to presence of LVI and MI, EC stage and grade, and EC subtypes. Second, the control group was chosen randomly among women with a prolapsed uterus or myoma who were admitted for surgical procedure, whereas ideal control group for endometrial cancer biomarker discovery would be symptomatic post-menopausal women. In our study patients in control group were relatively younger and with less additional comorbidities than patients with EC. Since these limitations may affect the generalisability of the study conclusions, the results of

this study should be validated on bigger cohort in a multicentric clinical study.

To conclude, AFs – especially leptin and IL-8, represent valuable biomarkers candidates with a potential for early diagnostics and risk stratification of EC. Leptin represents a candidate for a diagnostic biomarker of EC, while IL-8 might be valuable in EC patient stratification according to prognostic characteristics, e.g. LVI, MI and EC grade. Other AFs further increased the performance of multivariate models. As revealed through the machine learning approach, the plasma concentrations of AFs, in conjunction with other clinical data, show good diagnostic characteristics. They could, provided they are confirmed in a large-scale multicentre validation study, represent a valuable supplementary diagnostic tool for EC's early detection and prognostic characterization. This could guide the decision-making regarding the extent of surgery and the choice of adjuvant therapy for EC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the National Medical Ethics Committee of the Republic of Slovenia (No. 0120-515/2017/4 and 0120-541/2019/7. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LR: Conceptualisation, Data curation, Writing, Result Analysis, Discussion. MP: Methodology; Data analysis, Data curation. IR: Data curation, Writing, review & editing, Statistics, Modelling. MK: Data curation, Statistics, ML Modelling, Results, Writing – review & editing. BP: Methodology; Data curation. ŠS: Conceptualisation; Writing – review & editing; Resources; Supervision. TR: Conceptualisation; Writing – review & editing; Resources; Supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.972131/full#supplementary-material

References

1. Zhang S, Gong TT, Liu FH, Jiang YT, Sun H, Ma XX, et al. Global, regional, and national burden of endometrial cancer, 1990–2017: Results from the global burden of disease study, 2017. *Front Oncol* (2019) 9:1440. doi: 10.3389/fonc.2019.01440

2. Crosbie EJ, Kitson S, McAlpine JN, Mukhopadhyay A, Powell ME, Singh N. Endometrial cancer. *Lancet.* (2022) 399(10333): 1412–1428. doi: 10.1016/S0140-6736(22)00323-3

3. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol* (1983) 15(1):10–7. doi: 10.1016/0090-8258(83)90111-7

4. Wilczyński M, Danielska J, Wilczyński J. An update of the classical bokhman's dualistic model of endometrial cancer. *Przeglad Menopauzalny = Menopause Review* (2016) 15(2):63. doi: 10.5114/pm.2016.61186

5. Brinton LA, Felix AS, McMeekin DS, Creasman WT, Sherman ME, Mutch D, et al. Etiologic heterogeneity in endometrial cancer: evidence from a gynecologic oncology group trial. *Gynecol Oncol* (2013) 129(2):277–84. doi: 10.1016/j.ygyno.2013.02.023

6. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: More than two types. *Vol 15 Lancet Oncol Lancet Publishing Group;* (2014), 15: e268–78. doi: 10.1016/S1470-2045(13)70591-6

7. Cancer Genome Atlas Research Network, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. *Nature.* (2013) 497(7447):67–73. doi: 10.1038/nature12325

8. Knez J, Sobocan M, Belak U, Kavalar R, Zupin M, Büdefeld T, et al. Pre-treatment risk assessment of women with endometrial cancer: differences in outcomes of molecular and clinical classifications in the Slovenian patient cohort. *Radiol Oncol* (2021) 56(1):76–82. doi: 10.2478/raon-2021-0036

9. Nohuz E, De Simone L, Chêne G. Reliability of IOTA score and ADNEX model in the screening of ovarian malignancy in postmenopausal women. *J Gynecol Obstet Hum Reprod* (2019) 48(2):103–7. doi: 10.1016/j.jogoh.2018.04.012

10. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecologic Cancer* (2020) 31(1): ijgc-2020-002230. doi: 10.1136/ijgc-2020-002230

11. Rižner TL. Discovery of biomarkers for endometrial cancer: current status and prospects. *Expert Review of Molecular Diagnostics* (2016) 16(12):1315–36. doi: 10.1080/14737159.2016.1258302

12. Roškar L, Roškar I, Rižner TL, Smrkolj Š. Diagnostic and therapeutic values of angiogenic factors in endometrial cancer. *Biomolecules.* (2022) 12: 7 doi: 10.3390/biom12010007.

13. Risau W. Mechanisms of angiogenesis. *Nature* (1997) 386:671–4. doi: 10.1038/386671a0

14. Abulafia O, Sherer DM. Angiogenesis of the endometrium. *Obstet Gynecol* (1999) 94:148–53. doi: 10.1016/S0029-7844(99)00262-8

15. Weston G, Rogers PAW. Endometrial angiogenesis. *Best Pract Res Clin Obstet Gynaecol* (2000) 14:919–36. doi: 10.1053/beog.2000.0135

16. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature.* (2000) 407:249–57. doi: 10.1038/35025220

17. Zyła MM, Kostrzewa M, Litwińska E, Szpakowski A, Wilczyński JR, Stetkiewicz T. The role of angiogenic factors in endometrial cancer. *Przeglad Menopauzalny* (2014) 18:122–6. doi: 10.5114/pm.2014.42714

18. Yetkin-Arik B, Kastelein AW, Klaassen I, Jansen CHJR, Latul YP, Vittori M, et al. Angiogenesis in gynecological cancers and the options for anti-angiogenesis therapy. *Biochim Biophys Acta - Rev Cancer* (2021) 1875(1): 188446. doi: 10.1016/j.bbcan.2020.188446

19. Roškar L, Klančič T, Knific T, Rižner TL, Smrkolj Š. Tie-2, G-CSF, and leptin as promising diagnostic biomarkers for endometrial cancer: A pilot study. *J Clin Med* (2021) 10(4):765. doi: 10.3390/jcm10040765

20. Praiss AM, Huang Y, St. Clair CM, Tergas AI, Melamed A, Khoury-Collado F, et al. Using machine learning to create prognostic systems for endometrial cancer. *Gynecologic Oncol* (2020) 159(3):744–50. doi: 10.1016/j.ygyno.2020.09.047

21. Gargya P, Bálint BL. Histological grade of endometrioid endometrial cancer and relapse risk can be predicted with machine learning from gene expression data. *Cancers (Basel)* (2021) 13(17):4348. doi: 10.3390/cancers13174348

22. Motulsky HJ, Brown RE. Detecting outliers when fitting data with nonlinear regression – a new method based on robust nonlinear regression and the false discovery rate. *BMC Bioinf* (2006) 7(1):123. doi: 10.1186/1471-2105-7-123

23. Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. Scikit-learn: Machine learning in Python. *J Mach Learn Res* (2011) 12:2825–30. doi: 10.5555/1953048.2078195

24. Kokol P, Kokol M, Zagoranski S. Machine learning on small size samples: A synthetic knowledge synthesis. *Sci Progress* (2022) 105(1):003685042110297. doi: 10.1177/00368504211029777

25. Feurer M, Klein A, Eggensperger K, Springenberg J, Blum M, Hutter F. Efficient and robust automated machine learning. *Adv Neural Inf Process Syst 28* (2015) 2015:2962–70. doi: 10.5555/2969442.2969547

26. Feurer M, Eggensperger K, Falkner S, Lindauer M, Hutter F. Auto-sklearn 2.0: Hands-free AutoML *via* meta-learning. *J Machine Learn Res* (2020) 23(261): 1–61. doi: 10.48550/arXiv.2007.04074

27. Rahman M. M., Davis D. N. (2013). Machine learning-based missing value imputation method for clinical datasets. In IAENG transactions on engineering technologies. (Springer: Dordrecht), 245–57.

28. McKinney W.. (2010). Data structures for statistical computing in python. In *In Proceedings of the 9th Python in Science Conference*. 445 (1): 56–61.

29. The pandas development team. *pandas-dev/pandas: Pandas*. Zenodo (2020). doi: 10.5281/zenodo.3509134

30. Pińska A, Pioński P. *MLJAR: State-of-the-art automated machine learning framework for tabular data*. Poland: MLJAR Sp. z o.o (2021). Available at: https://github.com/mljar/mljar-supervised.

31. Lundberg SM, Lee SI. A unified approach to interpreting model predictions. *Adv Neural Inf Process Syst* (2017) 30, 4765–4774. doi: 10.5555/3295222.3295230

32. Fisher A, Rudin C, Dominici F. All models are wrong, but many are useful: Learning a variable's importance by studying an entire class of prediction models simultaneously. *J Mach Learn Res* (2019) 20(177):1–81. doi: 10.48550/arXiv.1801.01489

33. Amant F, Mirza MR, Koskas M, Creutzberg CL. Cancer of the corpus uteri. *Int J Gynecology Obstetrics.* (2018) 143(S2):37–50. doi: 10.1002/ijgo.12612

34. Kim CS, Park HS, Kawada T, Kim JH, Lim D, Hubbard NE, et al. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. *Int J Obes* (2006) 30(9):1347–55. doi: 10.1038/sj.ijo.0803259

35. O'Flynn H, Ryan NAJ, Narine N, Shelton D, Rana D, Crosbie EJ, et al. Diagnostic accuracy of cytology for the detection of endometrial cancer in urine and vaginal samples. *Nat Commun* (2021) 12:952. doi: 10.1038/s41467-021-21257-6

36. Saito M, Sato Y, Watanabe J, Kuramoto H, Kaba S, Fukuda T. Angiogenic factors in normal endometrium and endometrial adenocarcinoma. *Pathol Int* (2007) 57(3):140–7. doi: 10.1111/j.1440-1827.2006.02071.x

37. Shaw E, Farris M, McNeil J, Friedenreich C. Obesity and endometrial cancer. *Recent Results Cancer Res* (2016), 208:107–36. doi: 10.1007/978-3-319-42542-9_7

38. Reeves GK, Pirie K, Beral V, Green J, Spencer E, Bull D. Cancer incidence and mortality in relation to body mass index in the million women study: Cohort study. *Br Med J* (2007) 335(7630):1134–9. doi: 10.1136/bmj.39367.495995.AE

39. Onstad MA, Schmandt RE, Lu KH. Addressing the role of obesity in endometrial cancer risk, prevention, and treatment. *J Clin Oncol Am Soc Clin Oncology;* (2016) 34:4225–30. doi: 10.1200/JCO.2016.69.4638

40. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet.* (2008) 371(9612):569–78. doi: 10.1016/S0140-6736(08)60269-X

41. Daley-Brown D, Oprea-Ilies GM, Lee R, Pattillo R, Gonzalez-Perez RR. Molecular cues on obesity signals, tumor markers and endometrial cancer. *Horm Mol Biol Clin Investig* (2015) 21(1):89–106. doi: 10.1515/hmbci-2014-0049

42. Modzelewska P, Chludzińska S, Lewko J, Reszeć J. The influence of leptin on the process of carcinogenesis. *Wspolczesna Onkologia.* (2019) 23(2):63–8. doi: 10.5114/wo.2019.85877

43. Ellis PE, Barron GA, Bermano G. Adipocytokines and their relationship to endometrial cancer risk: A systematic review and meta-analysis. *Gynecologic Oncol* (2020) 158(2):507–16. doi: 10.1016/j.ygyno.2020.05.033

44. Ashizawa N, Yahata T, Quan J, Adachi S, Yoshihara K, Tanaka K. Serum leptin-adiponectin ratio and endometrial cancer risk in postmenopausal female subjects. *Gynecologic Oncol* (2010) 119(1):65–9. doi: 10.1016/j.ygyno.2010.07.007

45. Gong TT, Wu QJ, Wang YL, Ma XX. Circulating adiponectin, leptin and adiponectin-leptin ratio and endometrial cancer risk: Evidence from a meta-analysis of epidemiologic studies. *Int J Cancer* (2015) 137(8):1967–78. doi: 10.1002/ijc.29561

46. Boroń D, Nowakowski R, Grabarek BO, Zmarzły N, Opławski M. Expression pattern of leptin and its receptors in endometrioid endometrial cancer. *J Clin Med* (2021) 10(13):2787. doi: 10.3390/jcm10132787

47. Cymbaluk-Płoska A, Chudecka-Głaz A, Jagodzińska A, Pius-Sadowska E, Sompolska-Rzechuła A, Machaliński B, et al. Evaluation of biologically active substances promoting the development of or protecting against endometrial cancer. *OncoTargets Ther* (2018) 11:1363–72. doi: 10.2147/OTT.S155942

48. Zhang Y, Liu L, Li C, Ai H. Correlation analysis between the expressions of leptin and its receptor (ObR) and clinicopathology in endometrial cancer. *Cancer biomark* (2014) 14(5):353–9. doi: 10.3233/CBM-140415

49. Zhou X, Li H, Chai Y, Liu Z. Leptin inhibits the apoptosis of endometrial carcinoma cells through activation of the nuclear factor κB–inducing Kinase/IκB kinase pathway. *Int J Gynecologic Cancer* (2015) 25(5):770 LP–778. doi: 10.1097/IGC.0000000000000440

50. Madeddu C, Sanna E, Gramignano G, Tanca L, Cherchi MC, Mola B, et al. Correlation of leptin, proinflammatory cytokines and oxidative stress with tumor size and disease stage of endometrioid (Type I) endometrial cancer and review of the underlying mechanisms. *Cancers (Basel)* (2022) 14(2): 268. doi: 10.3390/cancers14020268

51. Bouloumié A, Drexler HC, Lafontan M, Busse R. Leptin, the product of ob gene, promotes angiogenesis. *Circ Res* (1998) 83(10):1059–66. doi: 10.1161/01.RES.83.10.1059

52. Sierra-Honigmann MR, Nath AK, Murakami C, García-Cardeña G, Papapetropoulos A, Sessa WC, et al. Biological action of leptin as an angiogenic factor. *Science.* (1998) 281(5383):1683–6. doi: 10.1126/science.281.5383.1683

53. Ray A, Cleary MP. The potential role of leptin in tumor invasion and metastasis. *Cytokine Growth Factor Rev* (2017) 38:80–97. doi: 10.1016/j.cytogfr.2017.11.002

54. Housa D, Housová J, Vernerová Z, Haluzík M. Adipocytokines and cancer. *Physiol Res* (2006) 55(3):233–44. doi: 10.33549/physiolres.930848

55. Somasundar P, Yu AK, Vona-Davis L, McFadden DW. Differential effects of leptin on cancer *in vitro*. *J Surg Res* (2003) 113(1):50–5. doi: 10.1016/S0022-4804(03)00166-5

56. Liu L, Wang L, Zheng J, Tang G. Leptin promotes human endometrial carcinoma cell proliferation by enhancing aromatase (P450arom) expression and estradiol formation. *Eur J Obstet Gynecol Reprod Biol* (2013) 170(1):198–201. doi: 10.1016/j.ejogrb.2013.04.004

57. Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, et al. Leptin in human physiology and pathophysiology. *Am J Physiol Endocrinol Metab* (2011) 301(4): E567–E584. doi: 10.1152/ajpendo.00315.2011

58. Wang PP, He XY, Wang R, Wang Z, Wang YG. High leptin level is an independent risk factor of endometrial cancer: A meta-analysis. *Cell Physiol Biochem* (2014) 34(5):1477–84. doi: 10.1159/000366352

59. Hazelwood E, Sanderson E, Tan VY, Ruth KS, Frayling TM, Dimou N, et al. Identifying molecular mediators of the relationship between body mass index and endometrial cancer risk: a mendelian randomization analysis. *BMC Med* (2022) 20(1):125. doi: 10.1186/s12916-022-02322-3

60. Friedenreich CM, Langley AR, Speidel TP, Lau DCW, Courneya KS, Csizmadi I, et al. Case-control study of inflammatory markers and the risk of endometrial cancer. *Eur J Cancer Prev* (2013) 22(4):374–9. doi: 10.1097/CEJ.0b013e32835b3813

61. Koch AE, Polverini PJ, Kunkel SL, Harlow LA, DiPietro LA, Elner VM, et al. Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Sci (1979)* (1992) 258(5089):1798–801. doi: 10.1126/science.1281554

62. Linkov F, Goughnour SL, Adambekov S, Lokshin A, Kelley JL, Sukumvanich P, et al. Inflammatory biomarker in adipose stem cells of women with endometrial cancer. *Biomarkers Med* (2018) 12(9):945–52. doi: 10.2217/bmm-2017-0355

63. Ciortea R, Mihu D, Mihu CM. Association between visceral fat, IL-8 and endometrial cancer. *Anticancer Res* (2014) 34(1):379–83.

64. Kotowicz B, Fuksiewicz M, Jonska-Gmyrek J, Berezowska A, Radziszewski J, Bidzinski M, et al. Clinical significance of pretreatment serum levels of VEGF and its receptors, IL- 8, and their prognostic value in type I and II endometrial cancer patients. *PLos One* (2017) 12(10):e0184576. doi: 10.1371/journal.pone.0184576

65. Voss MA, Ganesan R, Ludeman L, McCarthy K, Gornall R, Schaller G, et al. Should grade 3 endometrioid endometrial carcinoma be considered a type 2 cancer-a clinical and pathological evaluation. *Gynecologic Oncol* (2012) 124(1):15–20. doi: 10.1016/j.ygyno.2011.07.030

66. Setiawan VW, Yang HP, Pike MC, McCann SE, Yu H, Xiang YB, et al. Type I and II endometrial cancers: have they different risk factors? *J Clin Oncol* (2013) 31(20):2607–18. doi: 10.1200/JCO.2012.48.2596

67. Fujimoto J, Aoki I, Khatun S, Toyoki H, Tamaya T. Clinical implications of expression of interleukin-8 related to myometrial invasion with angiogenesis in uterine endometrial cancers. *Ann Oncol* (2002) 13(3):430–4. doi: 10.1093/annonc/mdf078

68. Berry KK, Varney ML, Dave BJ, Bucana CD, Fidler IJ, Singh RK. Expression of interleukin-8 in human metastatic endometrial carcinoma cells and its regulation by inflammatory cytokines. *Int J Gynecological Cancer* (2001) 11(1):54–60. doi: 10.1046/j.1525-1438.2001.011001054.x

69. Nevadunsky NS, Van Arsdale A, Strickler HD, Moadel A, Kaur G, Levitt J, et al. Obesity and age at diagnosis of endometrial cancer. *Obstet Gynecol* (2014) 124(2 Pt 1):300–6. doi: 10.1097/AOG.0000000000000381

70. Ghanbari Andarieh M, Agajani Delavar M, Moslemi D, Esmaeilzadeh S. Risk factors for endometrial cancer: Results from a hospital-based case-control study. *Asian Pac J Cancer Prev* (2016) 17(10):4791–6. doi: 10.22034/apjcp.2016.17.10.4791

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EDITED BY
Andrzej Semczuk,
Medical University of Lublin, Poland

REVIEWED BY
Shuangdi Li,
Shanghai First Maternity and Infant Hospital, China
Wu Ren,
Huazhong University of Science and Technology, China

*CORRESPONDENCE
Lijing Zhao
zhao_lj@jlu.edu.cn
Shuhua Zhao
zhaoshuhua-1966@163.com

†These authors have contributed equally to this work

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KNL1 is a prognostic and diagnostic biomarker related to immune infiltration in patients with uterine corpus endometrial carcinoma

Kang He[1†], Jingze Li[1†], Xuemiao Huang[1], Weixin Zhao[2], Kai Wang[1], Taiwei Wang[1], Junyu Chen[2], Zeyu Wang[1], Jiang Yi[3], Shuhua Zhao[2*] and Lijing Zhao[1*]

[1]Department of Rehabilitation, School of Nursing, Jilin University, Changchun, China, [2]The Department of Obstetrics and Gynecology, The Second Hospital of Jilin University, Changchun, Jilin, China, [3]Department of Rehabilitation, The Second Hospital of Jilin University, Changchun, Jilin, China

Background: The incidence and mortality of uterine corpus endometrial carcinoma (UCEC) are increasing yearly. There is currently no screening test for UCEC, and progress in its treatment is limited. It is important to identify new biomarkers for screening, diagnosing and predicting the outcomes of UCEC. A large number of previous studies have proven that KNL1 is crucial in the development of lung cancer, colorectal cancer and cervical cancer, but there is a lack of studies about the role of KNL1 in the development of UCEC.

Methods: The mRNA and protein expression data of KNL1 in The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO) and UALCAN databases and related clinical data were used to analyze the expression differences and clinical correlations of KNL1 in UCEC. A total of 108 clinical samples were collected, and the results of bioinformatics analysis were verified by immunohistochemistry. KNL1 and its related differentially expressed genes were used to draw a volcano map, construct a PPI protein interaction network, and perform gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), gene set enrichment analysis (GSEA) and immune infiltration analysis to predict the function of KNL1 during UCEC progression. The prognostic data of TCGA and 108 clinical patients were used to analyze the correlation of KNL1 expression with the survival of patients, and KM survival curves were drawn. The UCEC cell lines Ishikawa and Hec-1-A were used to verify the function of KNL1.

Results: KNL1 is significantly overexpressed in UCEC and is associated with a poor prognosis. KNL1 overexpression is closely related to cell mitosis, the cell cycle and

other functions and is correlated with the International Federation of Gynecology and Obstetrics (FIGO) stage, histological grade and other characteristics of UCEC patients. Knockdown of KNL1 expression in UCEC cell lines can inhibit their proliferation, invasion, metastasis and other phenotypes.

Conclusion: KNL1 is a prognostic and diagnostic biomarker associated with immune evasion in patients with UCEC.

KEYWORDS

KNL1, uterine corpus endometrial carcinoma, bioinformatics, prognosis, biomarker

Introduction

Uterine corpus endometrial carcinoma (UCEC) has the second highest incidence among types of gynecologic cancer (1, 2). In contrast to other malignant tumors, endometrial cancer's incidence and associated mortality have been increasing, and its age of onset has also demonstrated a pattern of becoming increasingly younger (3–5). Although the vast majority of patients with endometrial cancer are diagnosed at an early stage and have a good 5-year relative survival rate (1), patients with advanced or recurrent endometrial cancer have a poor response to therapy and a poor prognosis (6, 7). There is currently no screening test for UCEC, and its diagnosis is entirely based on symptoms; however, this approach has low specificity (8, 9). All of these elements highlight the lack of advances in the management of UCEC. The discovery and characterization of novel biomarkers for screening, diagnosing, and predicting the outcome of UCEC are crucial for patients with the disease.

Kinetochore Scaffold 1 (KNL1), also known as CASC5, D40, and AF15Q14, is primarily expressed in healthy testicles, various human cancer cell lines, and primary malignancies (10). It is a newly discovered member of the cancer testicular gene family and is located on chromosome 15 (11, 12). KNL1 can ensure high-fidelity chromosome segregation and is essential for maintaining mitosis (13–15). KNL1 has previously been identified as a possible lung adenocarcinoma driver gene (16). Experiments have shown that KNL1 can inhibit the apoptosis of colorectal cancer cells and promote their proliferation (17). Meanwhile, knockdown of KNL1 expression in cervical cancer HeLa cells inhibited their proliferation and induced apoptosis both *in vivo* and *in vitro* (18). All of the aforementioned findings imply that KNL1 may be crucial to the emergence, growth, and progression of various malignancies.

Nevertheless, there has not been enough research to conclusively show that KNL1 is involved in the emergence and progression of UCEC. In this study, The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) databases were used to analyze the expression of KNL1 and its correlation with clinical features, and the immunohistochemical results of 108 clinical specimens of UCEC were used to verify its expression. At the same time, a protein–protein interaction (PPI) network was constructed using KNL1 and its related differentially expressed genes, and gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), gene set enrichment analysis (GSEA) and immune infiltration analysis were performed to predict the function of KNL1 in promoting the occurrence and development of UCEC. Finally, the UCEC cell lines Ishikawa and Hec-1-A were used to verify the function of KNL1 and clarify the molecular mechanism by which KNL1 promotes the progression of UCEC.

Abbreviations: UCEC, Uterine corpus endometrial carcinoma; KNL1, Kinetochore scaffold 1; TCGA, The cancer genome atlas; GEO, Gene Expression Omnibus; PPI, Protein-protein interaction; GO, Gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; GSEA, Gene set enrichment analysis; FIGO, International Federation of Gynecology and Obstetrics; DEGs, Differentially expressed genes; OS, Overall survival; DSS, Disease-specific survival; PFI, Progress free interval; AOD, Average optical density; CCK-8, Cell counting kit-8; ANOVA, One-factor analysis of variance; IHC, Immunohistochemical.

Methods and materials

Data sources and preprocessing

RNA-seq data from the TCGA (https://portal.gdc.cancer.gov/) UCEC project and GTEx database describe the differential expression of KNL1 in unpaired and paired samples. The Toil process uniformized the data (19). The TCGA level 3 HTSeq-FPKM (Fragments Per Kilobase Per Million) format was translated to the TPM (transcripts per million reads) format and log2-transformed. All final TCGA-based analyses were conducted using TPM-formatted data. Using GEOquery [version 2.54.1] (20), the differential analysis data for KNL1 in dataset GSE17025 (21, 22) were extracted from the GEO database. These data were obtained by removing probes corresponding to multiple molecules, and when probes corresponding to the same molecule were encountered, only the probe with the highest signal value was retained. The data were then normalized once more using the normalize Between Arrays function of the limma package [version 3.42.2] (23). Using the CPTAC database in UALCAN (http://ualcan.path.uab.edu) (24, 25), differential expression of the KNL1 protein in UCEC and normal adjacent tissues was determined. R was used for all statistical analyses and visualizations (version 3.6.3).

Single-gene differential analysis and correlation analysis of KNL1

The DESeq2 package [version 1.26.0] and the STAT package [version 3.6.3] were used to conduct single-gene differential analysis

and single-gene correlation analysis of KNL1 in the UCEC project utilizing the TCGA database (26). The findings of the single-gene differential analysis were used to generate volcano plots with the ggplot2 software [version 3.3.3]. |$\log_2$ fold change (LogFC)|>1 and p.adj<0.05 were used as the thresholds for differentially expressed genes (DEGs). The STRING database was utilized to show the DEGs (27), the PPI network of DEGs was analyzed using the Cytoscape program, and the MCODE plugin was used to identify the HUB genes. The genes from the single-gene correlation analysis were then sorted by |Pearson value| in descending order, and the top 50 correlations were retrieved. The KNL1 single-gene coexpression heatmap was generated using the top 50 genes and the HUB gene by the ggplot2 [version 3.3.3] package.

Functional enrichment analysis

In the TCGA UCEC project, gene set enrichment analysis (GSEA) was utilized to investigate the putative signaling pathways based on differential expression analysis (KNL1 high-expression vs. KNL1 low-expression samples). The reference gene set was h.all.v7.2.symbols.gmt [Hallmarks]. An adjusted p value <0.05 was considered significantly enriched. After screening the DEGs based on the threshold (|LogFC |>1 and p.adj<0.05), Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to enhance the pathways associated with KNL1 in UCEC using the R packages "clusterProfiler" and "org.Hs.eg.db". p.adj<0.05 was considered significantly enriched.

Immunoinfiltration analysis of KNL1

GSVA [version 1.34.0] was used to examine the relative infiltration levels of 24 immune cells (28). For the immune infiltration algorithm, ssGSEA was employed, and Spearman correlation analysis was applied. The markers for twenty-four immune cells were derived from an article in Immunity (29). The samples were then separated into low and high KNL1 expression groups, the enrichment scores of various immune cell infiltrates in the various subgroups were computed, and the analysis was conducted using GSVA software [version 1.34.0]. Finally, the correlation between KNL1 and CD47, CD273, and TNFRSF4 was computed, and ggplot2 software [version 3.3.3] was used to depict it.

Analysis of the correlation between KNL1 mRNA expression and the prognosis of patients with UCEC

The survival data of UCEC patients were statistically analyzed using the survival package [version 3.2-10], and the results were visualized using the survminer package [version 0.4.9] to plot the overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) on Kaplan–Meier curves for the UCEC patients. Using the pROC package [version 1.17.0.1], ROC analysis was performed on the data to assess the accuracy of KNL1 for prognostication. All predictive data for the aforementioned survival study were from a Cell article (30). Finally, a dichotomous logistic regression model and clinical baseline datasheet were developed to predict the association between various clinicopathological characteristics and KNL1 expression.

Specimens

Jilin University's School of Nursing's Ethical Review Committee authorized the present study (Changchun, China). Paraffin–embedded specimens were collected at the Second Hospital of Jilin University (Changchun, China) from 108 patients with UCEC and 15 normal controls diagnosed between December 2012 and December 2019. Patients were informed about the UCEC-related study and agreed to participate. The criteria for inclusion were: i) initially diagnosed with UCEC and treated with standard surgery and/or radiotherapy and/or chemotherapy according to the FIGO stage and pathological type of the individual patient; ii) the diagnosis of UCEC was determined by an experienced gynecological pathologist; iii) the postoperative pathology results were interpreted by an experienced gynecological pathologist using FIGO staging criteria (Version 2009); and iv) complete follow-up data were available. The exclusion criteria were: i) a personal history of other malignant tumors; ii) preoperative radiation, chemotherapy, or hormonotherapy; and iii) a secondary uterine tumor. As stated in Supplementary Table 4, accessible clinical/pathological data were gathered from The Second Hospital of Jilin University's Medical Record Database. All 108 patients with UCEC were followed up, and their OS was determined.

Cell culture and stably transfected cell line development

The human UCEC cell lines Ishikawa and HEC-1-A were purchased from iCell Bioscience Inc., Shanghai. Ishikawa cells were cultured with Minimum Essential Medium (MEM, product code iCell-0012, iCell) supplemented with 10% fetal bovine serum (product code FS301-02; TransGen), 1% nonessential amino acids (NEAA, product code iCell-01000, iCell) and 1% penicillin-streptomycin (product code P1400, Solarbio). HEC-1-A cells were cultured with McCoy's 5A medium (product code iCell-0011, iCell) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The two cell lines were cultured at 37°C in a humidified atmosphere with 5% CO_2.

The lentiviral vector plasmid pLKO.1-Puro (product code FH1717; Hunan Fenghui Biotechnology Co., Ltd.) was utilized to construct the pLKO.1-Scramble and pLKO.1-shKNL1 plasmids. The interference sequences were 5'-GGUAAAAGUCCCAUAGAAATT-3' for shKNL1 and 5'-GTATAAGTCAACTGTTGAC-3' for shScramble. The lentiviruses used in this study were packaged using the 3 plasmid packaging system. After combining the lentiviral vector plasmids with the packaging plasmid PMD2.G (product code BR037, Fenghui), psPAX2 (product code BR036, Fenghui) and Lipofectamine™ 3000 transfection reagent (product code L3000150, Thermo Fisher), the complexed solution was introduced to HEK-

293T cells (product code iCell-h237, iCell). The medium was collected and filtered using a 0.22 μm filter after 48 and 72 hours. The medium was then kept at 4°C for up to one week before use.

To generate stably transfected cell lines, Ishikawa and HEC-1-A cells were seeded into 6-well plates (300,000 cells/well). Subsequently, 24 hours later, 1 ml of medium containing the above lentivirus was added to each well. After 48 hours, the medium was changed. Cells infected with viruses encoding the puromycin resistance gene were selected in 2 μg/mL puromycin. One week of puromycin selection was continued prior to cell collection and subsequent analysis.

Immunohistochemistry

Similar to an earlier study (31), immunohistochemical (IHC) staining was conducted. After 24 hours of fixation in 10% formalin at room temperature, the samples were embedded in paraffin and sectioned to a thickness of 3 μm. The sections were immersed in EDTA retrieval buffer (catalog number AR0023; Wuhan Boster Biological Technology, Ltd.) and cooked in a microwave. Then, 5% bovine serum albumin (product code AR1006; Boster Biological Technology, Inc.) was applied at room temperature for 20 minutes to prevent nonspecific binding. The histological sections were stained overnight at 4°C with rabbit anti-KNL1 antibody (product code DF13491; 1:100; Affinity), rabbit anti-CD56 antibody (product code GB112671; 1:750; Servicebio), rabbit anti-CD4 antibody (product code GB11064; 1:1000; Servicebio), and rabbit anti-B3GAT1 antibody (product code GB113461; 1:1000; Servicebio). The secondary antibody was goat antirabbit IgG coupled with horseradish peroxidase (product code GB23204; 1:200; Servicebio), and the staining technique was performed at 37°C for 30 minutes. Reactive products were observed using 3,3'diaminobenzidene (Boster Biological Technology, Inc.) as the chromogen, and the sections were counterstained for 2 minutes at room temperature with 0.1% hematoxylin (Boster Biological Technology, Inc.). Under a light microscope (AE2000, Motic) with an objective magnification of x200 or x400, images of the stained sections were recorded. The positive cell density was evaluated with Image-Pro Plus 6.0 (Media Cybernetics, Inc.), and the findings are reported as the average optical density (AOD) values. Two experienced pathologists from the Pathology Department of the Second Hospital of Jilin University graded the IHC staining independently under double-blind conditions.

Real time-PCR

Total RNA was isolated from fresh frozen tissue and stably transfected cells using an EasyPure RNA kit (product code ER101-01; TransGen), and first-strand cDNA was synthesized using a cDNA synthesis kit (product code AT311-02; TransGen) following the manufacturer's instructions. KNL1 transcription levels were determined by real-time PCR using the SYBR Green qPCR kit (product code AQ132; TransGen) according to the manufacturer's instructions, with GAPDH serving as the internal reference gene. The following primers were used: KNL1 gene, 5'-GATGG GGTGTCTTCAGAGGC-3' for forward and 5'-AGAGGACTC CTTGGGGGTTT-3' for reverse; GAPDH gene, 5'-GAAGGTG AAGGTCGGAGTC-3' for forward and 5'-GAAGATGGT GATGGGATTTC-3' for reverse. An ABI-Q3 was used to conduct PCR at 94°C for 30 seconds, followed by 45 cycles of amplification at 94°C for 5 seconds, 51°C for 15 seconds, and 72°C for 10 seconds (Thermo Fisher Scientific, Inc.). The expression levels of the mRNA were measured using the $2^{-\Delta\Delta Ct}$ method (31).

Cell counting kit-8 assay

Ishikawa, HEC-1-A, Ishikawa-shScramble, HEC-1-A-shScramble, Ishikawa-shKNL1 and HEC-1-A-shKNL1 cells were seeded into 96-well plates (3,000 cells/well). CCK-8 reagent (10 μl/well; product number BA00208, Bioss) was then added to each well 24, 48, and 96 hours later. After 1.5 hours of culture at 37°C, the absorbance of each well was measured at 450 nm with a microplate reader (E0226; Detie, Inc.

Invasion assay

For the invasion experiment, a Transwell chamber (Labselect, product code 14342) was used to determine the invasive potential of the UCEC cells listed above. The chamber was covered with Matrigel (BD Biosciences, product code 356234) per the manufacturer's instructions. A total of $3x10^4$ cells in 100 μL serum-free MEM or McCoy's 5A were placed in the upper chamber, while 600 μL 10% FBS media-based medium was placed in the lower chamber. After 30 hours of treatment at 37°C, the residual cells on the top surface were removed with a cotton swab, and the invasive cells were stained with 10% Giemsa. An optical microscope was used to record the images (AE2000, Motic).

Wound-healing assay

A wound-healing experiment was performed to assess the migratory capacity of the UCEC cells described above. Cells seeded in six-well plates ($3x10^5$ cells/well) were scratched with a 200 μl pipette tip to create a linear wound. The dislodged cells were washed and removed with PBS. Photographs were obtained using a digital camera and an optical microscope (Motic Corporation) to observe the movement of cells into the wounded region at 24 and 48 hours. All micrographs were obtained at the same magnification at the same time for each cell type.

Colony formation assay

HEC-1-A and Ishikawa cells were seeded onto 6-well plates at a density of 100 cells/well. Ten days later, the formation of typical colonies was observed. The cells were fixed with methanol and stained with 10% Giemsa (Biotopped, China). The number of visible colonies was counted to evaluate the colony formation ability of the cells. All experiments were conducted in three replicates.

Statistical analysis

The statistical analyses were conducted with the mean of three independent tests plus the standard deviation (SD). Statistical analyses were conducted utilizing SPSS 23.0 or R version 3.6.3; differences between groups were examined using one-factor analysis of variance (ANOVA) followed by Dunnett's *post hoc* test, Kruskal–Wallis test, or Student's t test. When $p<0.05$, differences were judged statistically significant. The Spearman correlation coefficient was calculated to determine the correlation between KNL1 and CD4, CD56 and B3GAT1.

Results

Differential expression of KNL1 in pancancer and UCEC

As shown in Supplementary Figure 1A, we found that in unpaired samples, the expression of KNL1 was higher in the following tumors than in normal tissues: adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), and uterine carcinosarcoma (UCS). Similarly, the expression of KNL1 was decreased in kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), and testicular germ cell tumors (TGCTs) compared to normal tissues.

As shown in Supplementary Figure 1B, in paired samples, the expression of KNL1 in BLCA, BRCA, COAD, ESCA, HNSC, LIHC, LUAD, LUSC, PRAD, STAD and UCEC was higher than that in adjacent tissues. The expression of KNL1 in KIRC and KIRP was lower than that in adjacent tissues. The number of tumor samples used in the pancancer analysis is presented in Supplementary Table 1. As shown in Figures 1A, B, in both unpaired and paired UCEC samples, the expression of KNL1 in tumors was higher than that in normal tissues. This point was validated by utilizing the mRNA and protein expression data of KNL1 in the GSE17025 and UALCAN databases, which were compatible with the results from the TCGA database, as shown in Figures 1C, D.

Evaluation of the expression of KNL1 in clinical samples of UCEC

The AOD of 108 UCEC clinical samples and 15 normal tissues was measured by immunohistochemical staining. As shown in Figure 2A, KNL1 expression was different in tissues with different degrees of differentiation. The expression of KNL1 protein in normal tissues is low, and the expression of KNL1 in tumor tissues gradually increases with a gradual decrease in tumor differentiation. The expression levels of KNL1 protein in 108 UCEC samples and 15 normal samples are shown in Figure 2B. The expression of KNL1 was significantly increased in tumor tissues. As shown in Figures 2C–F, KNL1 expression was different in patients with different FIGO stages, different tumor invasion statuses, different histologic grades, and different lymphatic metastases. A KNL1 expression box diagram of patients with other clinical features is shown in Supplementary Figure 2. Moreover, ROC curves of the protein expression data of KNL1 are shown in Figure 2G. The AUC=0.764, suggesting that KNL1 may be closely related to the occurrence and development of tumors.

Single-gene differential analysis and correlation analysis of KNL1

The results of single-gene differential analysis are shown in the volcano plot in Figure 3A. There were 850 genes that satisfied the threshold of $|LogFC|>1$ and $p.adj<0.05$, under which 243 genes were highly expressed and 607 genes were poorly expressed. These 850 genes were imported into the STRING database to construct differential protein interaction networks, and a total of 46 HUB genes were identified (MELK, E2F7, SMC2, ANLN, HMMR, OIP5, CDCA2, PBK, RAD51AP1, CENPI, CKAP2L, KIF14, FBXO5,

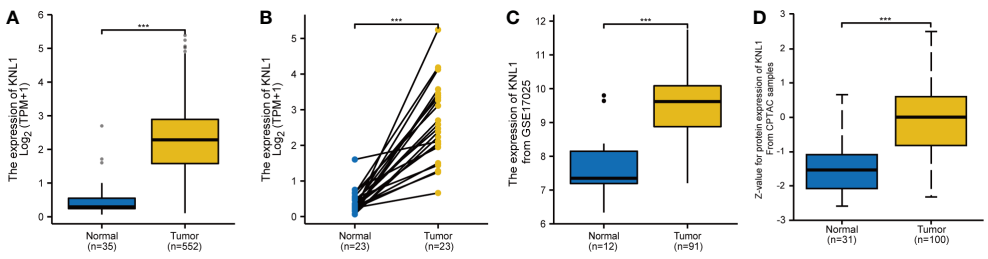

FIGURE 1
Differential expression analysis of KNL1 in patients with UCEC. **(A)** Differential analysis of KNL1 expression in unpaired UCEC samples. **(B)** Differential analysis of KNL1 expression in paired UCEC samples. **(C)** Differential analysis of KNL1 expression based on GSE17025 data. **(D)** Differential analysis of KNL1 protein expression based on CPTAC data. Significance identifier: ***, $p<0.001$.

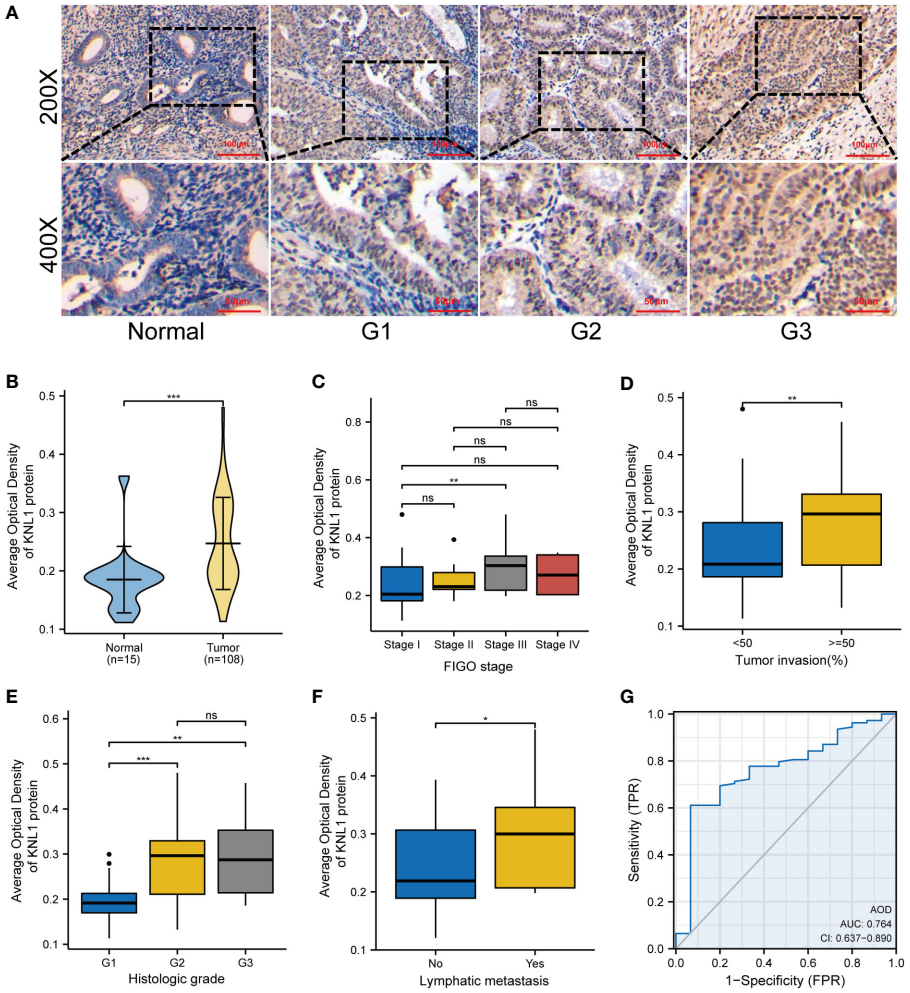

FIGURE 2
Expression and clinical correlation analysis of KNL1 in UCEC clinical samples. **(A)** Immunohistochemical results of KNL1 in normal endometrial tissues and UCEC tissues with different degrees of differentiation. **(B)** Group comparison of KNL1 immunohistochemical results in 108 UCEC clinical specimens and 15 normal endometrial cancer tissues. **(C–F)** Group comparison of KNL1 protein expression levels in samples with different clinical characteristics, **(C)** FIGO stage, **(D)** Tumor invasion, **(E)** Histologic grade, and **(F)** Lymphatic metastasis. **(G)** The diagnostic ROC curve of KNL1. Significance identifier: ns (no significance), p≥0.05; *, p< 0.05; **, p<0.01; ***, p<0.001.

FAM83D, CENPF, DLGAP5, CCNE2, FOXM1, TOP2A, NCAPG, SGOL2, DEPDC1, ASPM, KIF23, KIF15, BUB1, KIF11, MCM10, BUB1B, KIF18A, ERCC6L, NEK2, ECT2, NEIL3, ATAD2, NUSAP1, E2F8, DEPDC1 B, SMC4, MAD2L1, CENPE, KIF20B, CCNA2, CLSPN, ESCO2, and ARHGAP11A), as shown in Figure 3B. After that, we performed a correlation analysis and created a coexpression heatmap utilizing the 50 genes with the strongest connection with KNL1, as shown in Figure 3C. The heatmap of coexpression between the HUB genes and KNL1 is shown in Figure 3D.

Functional enrichment analysis of KNL1 in UCEC

KNL1 and its differentially expressed genes were used for GO and KEGG functional enrichment analyses. GO functional enrichment analysis showed that in terms of "biological process", pathways such as acute inflammatory response, humoral immune response, hormone metabolic process, chromosome organization involved in meiotic cell cycle, and meiotic cell cycle process were enriched. In terms of "molecular function", significant enrichment occurred in the pathways of G protein-coupled receptor binding, serine-type endopeptidase inhibitor activity, cytokine activity, hormone activity, and cysteine-type endopeptidase inhibitor activity involved in the apoptotic process. In terms of "cellular component", keratin filament, catenin complex, kinesin complex, mitotic spindle, condensed chromosome and other pathways were enriched, and the results are shown in Figures 4A, C and Supplementary Table 2.

The results of KEGG functional enrichment analysis showed that steroid hormone biosynthesis, metabolism of xenobiotics by cytochrome P450, drug metabolism - cytochrome P450, pentose and glucuronate interconversions, etc., were enriched, as shown in Figures 4B, D and Supplementary Table 2.

Finally, GSEA functional enrichment analysis was used to predict the function of KNL1 in the development of endometrial carcinoma, and it was found that KNL1 was closely associated with hallmark allograft rejection, hallmark complement, hallmark Kras signaling up, hallmark g2m checkpoint, hallmark mitotic spindle, hallmark mtorc1

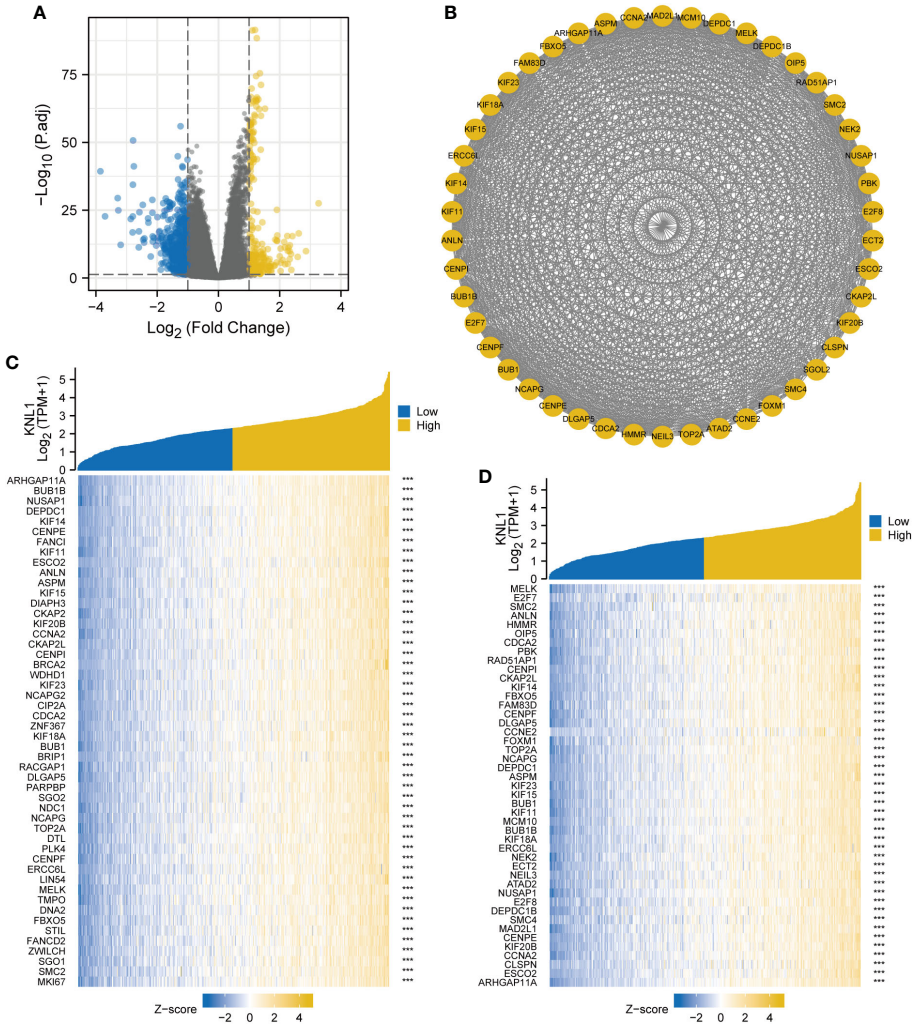

FIGURE 3
Single gene differential analysis and correlation analysis of KNL1. **(A)** Volcano map for single gene differential analysis of KNL1. **(B)** Protein interaction network diagram (PPI) of the HUB genes. **(C)** Heatmap of coexpression of the top 50 most correlated genes with KNL1 in single gene correlation analysis. **(D)** Heatmap of single gene coexpression of the HUB genes and KNL1.

signaling, hallmark e2f targets, hallmark myc targets v1 and other pathways, as shown in Figures 4E, F.

Immunoinfiltration analysis of KNL1 in UCEC

The relationship between the expression of KNL1 and the degree of infiltration of 24 immune cells was analyzed, and the results are shown in Figures 5A, C. The results showed that the expression of KNL1 had a significant positive correlation with the infiltration degree of Th2 cells, T helper cells, and Tcm cells, while the expression of KNL1 showed a significant negative correlation with the infiltration degree of pDCs, NK CD56bright cells, iDCs, and NK cells.

To validate the results of ssGSEA, we analyzed the correlation between the expression of KNL1 and the expression of various immune cell surface marker proteins and plotted a heatmap, shown in Figure 5B. The heatmap showed a strong correlation between KNL1 and CR2, CD1A, TPSAB1, B3GAT1, IL3RA, CD3D, and PTPRC, consistent with the previous analysis. Finally, as shown in Figures 5D–F, we analyzed the correlation between some common immunotherapeutic targets and KNL1 and found that the expression of KNL1 showed a significant positive correlation with the expression of CD47.

To verify the relationship between KNL1 expression and immune infiltration in patients with UCEC, immunohistochemical analysis of CD4, CD56 and B3GAT1 was performed using samples from 108 patients, shown in Figures 6A–D. The immunohistochemical results were used to analyze the correlation between KNL1 and CD4, CD56 and B3GAT1, and it was found that the expression of KNL1 was negatively correlated with the expression of CD56 and B4GAT1 and positively correlated with the expression of CD4, as shown in Figures 6E–G.

Effect of KNL1 expression on the prognosis of tumor patients

To determine the relationship between KNL1 expression and the prognosis of UCEC patients, we performed survival analysis using the

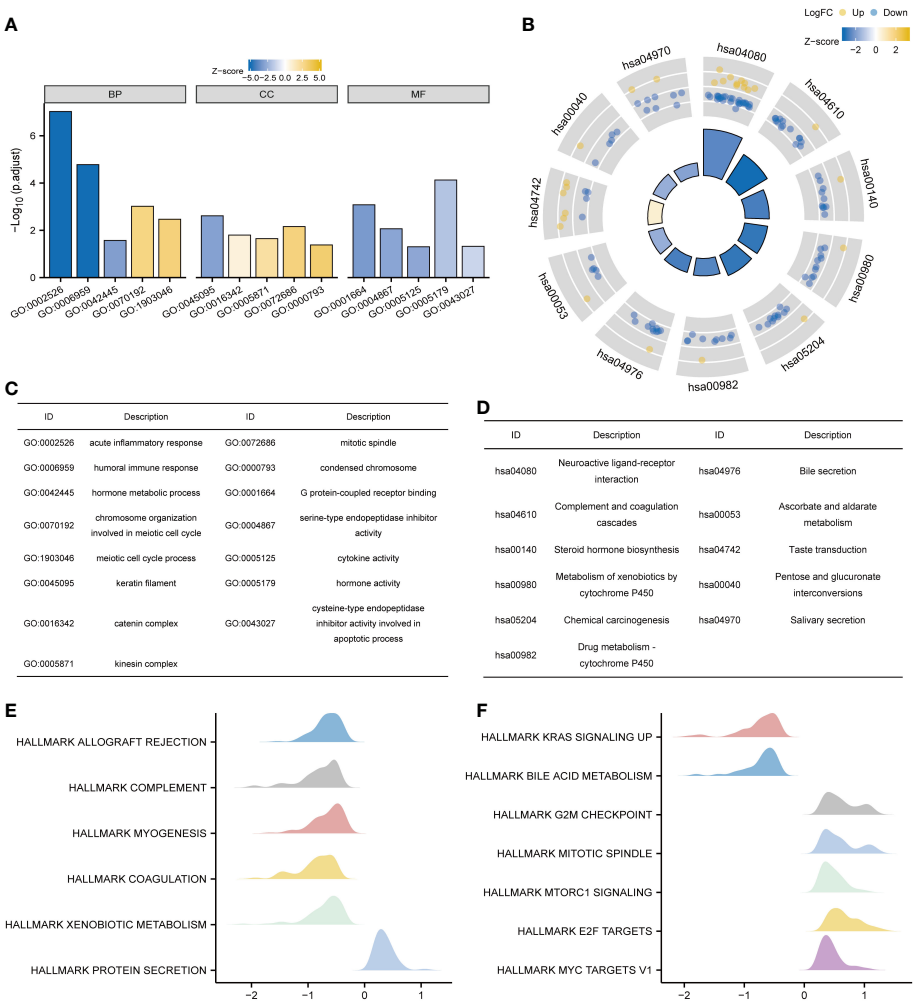

FIGURE 4
Functional enrichment analysis of KNL1 and related differentially expressed genes in UCEC. **(A)** Results of GO analysis. **(B)** Results of KEGG analysis. **(C, D)** GO and KEGG analysis category names corresponding to the GO and KEGG Identifier. **(E, F)** Results of KEGG analysis. When the abscissa was positive, KNL1 expression was positively correlated with this pathway, and when the abscissa was negative, the opposite was observed.

prognostic data of UCEC in TCGA, and the results are shown in Figures 7A–C. We found that high expression of KNL1 was correlated with worse overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI). As shown in Figure 7D, we also performed a ROC analysis to test the accuracy of KNL1 expression in predicting patient outcome and found that the AUC=0.952, suggesting that KNL1 expression is highly accurate in predicting the outcome of UCEC patients.

After that, we analyzed the relationship between KNL1 expression and various clinical characteristics of UCEC patients, for which the baseline data table is shown in Supplementary Table 3, and the results of the logistic analysis are shown in Table 1. The results in both Supplementary Table 3 and Table 1 suggest that the expression of KNL1 is closely related to the histologic grade of UCEC patients.

Finally, in addition to analyzing the correlation between KNL1 expression and the prognosis of UCEC patients, we also used the prognostic data of GBMLGG, LGG, BRCA, KIRP, KIRC, and PAAD in the TCGA database to analyze the association of KNL1 expression with the prognosis of these tumors, and the results are shown in Supplementary Figures 3A–F. The results showed that high expression of KNL1 led to worse OS of patients with these tumors, suggesting that KNL1 may be closely related to tumor progression.

We then analyzed the relationship between KNL1 expression and clinical features using the clinical information of 108 previously collected samples to verify the relationship between KNL1 and the prognosis of UCEC patients, and the results are shown in Figures 7E, F. The results in Figure E show that high expression of KNL1 was correlated with a poor prognosis in these 108 clinical patients, which further verifies the relationship between KNL1 expression and the OS of patients. Figure F shows the results of logistic regression analysis, indicating that there is a relationship between KNL1 expression and FIGO stage and histologic grade. The results in Supplementary Table 4 also show that the expression of KNL1 is significantly related to the degree of histologic grade, tumor invasion, FIGO stage and the expression of Ki67 protein, which is consistent with the previous analysis.

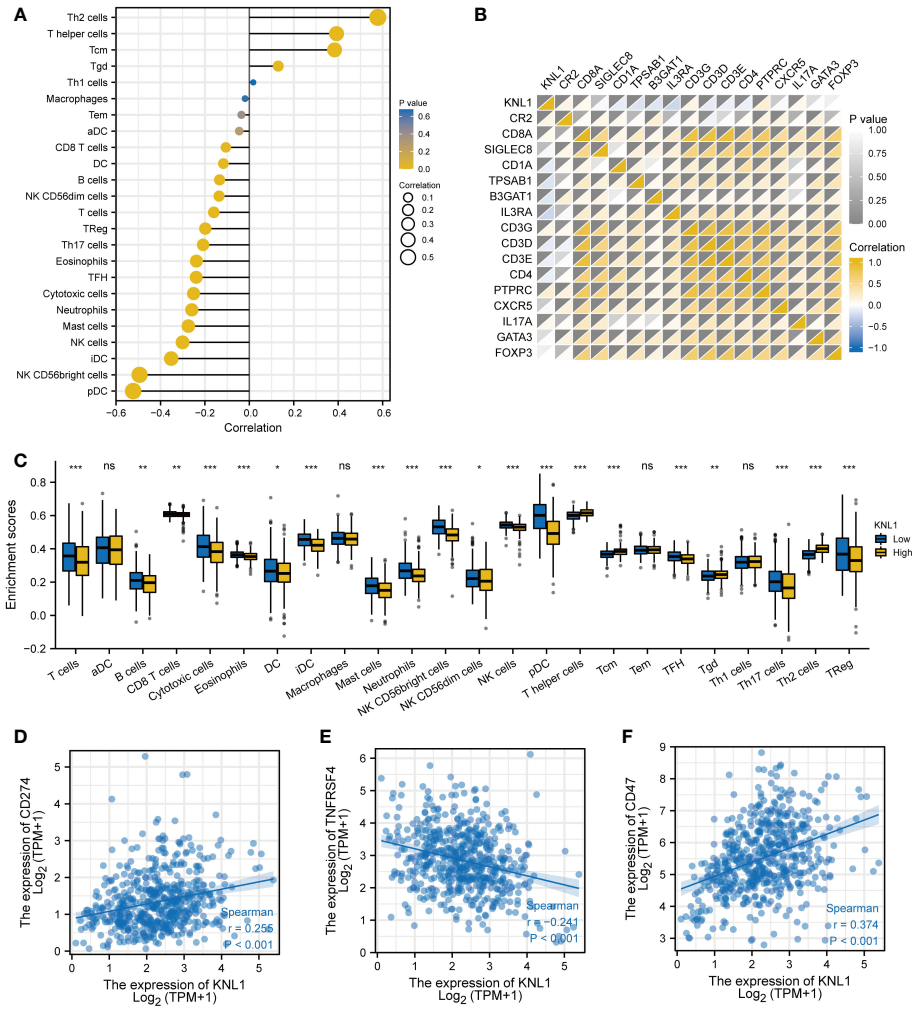

FIGURE 5
Immunoinfiltration analysis of KNL1. **(A)** Correlation analysis between KNL1 and 24 immune cell infiltration levels. **(B)** Heatmap of the correlation between KNL1 expression and various immune cell surface marker proteins: CR2 (B cells), CD8A (cytotoxic cells), SIGLEC8 (eosinophils), CD1A (iDCs), TPSAB1 (mast cells), B3GAT1 (NK cells), IL3RA (pDCs), CD3G (T cells), CD3D (T cells), CD3E (T cells), CD4 (T helper cells), PTPRC (Tcm), CXCR5 (Tfh), IL17A (Th17), GATA3 (Th2), and FOXP3 (Treg). **(C)** Infiltration levels of 24 kinds of immune cells in samples with different KNL1 expression levels. **(D–F)** Correlation analysis between KNL1 and the expression levels of CD274, TNFRSF4 and CD47. Significance identifier: ns (no significance), p≥0.05; *, p< 0.05; **, p<0.01; ***, p<0.001.

Effect of KNL1 knockdown on the proliferation, invasion and metastasis of endometrial cancer cells

After knocking down the expression of KNL1 in HEC-1-A and Ishikawa cell lines, the expression of KNL1 was confirmed to be significantly reduced, as shown in Figure 8A. We then performed CCK-8 assays and found that cell proliferation was significantly reduced after knockdown, as shown in Figures 8B, C. We also performed a wound-healing assay and found that the metastatic ability of cells with KNL1 knockdown was significantly weaker than that of the control group over time, as shown in Figures 8H–J. We also performed Transwell experiments and the invasive ability of cells with KNL1 knockdown was also significantly weakened, as shown in Figures 8F, G. Finally, we performed a colony formation assay and found that knockdown of KNL1 expression in cells was followed by a decrease in their colony formation ability Figures 8D, E).

Discussion

To date, there is a lack of good biomarkers for screening and diagnosing UCEC (32). Finding and identifying new biomarkers for early screening and diagnosis is particularly important for patients at high risk of UCEC (33, 34). In addition, due to the limitations of clinical staging, the final pathological diagnosis and staging are based on surgical specimens (35, 36). Therefore, it is also necessary to study the prognostic biological indicators of UCEC, which will help to classify UCEC patients into low-risk and high-risk groups before surgery to improve individualized treatment (37).

In this study, using RNA-seq data from the TCGA and GEO databases, it was found that KNL1 was highly expressed in UCEC, suggesting that KNL1 was related to the occurrence and development of UCEC. Using 108 cases of endometrial carcinoma and 15 cases of normal endometrium, we found that the expression of KNL1 protein in tumors was higher than that in normal tissues. Its expression level

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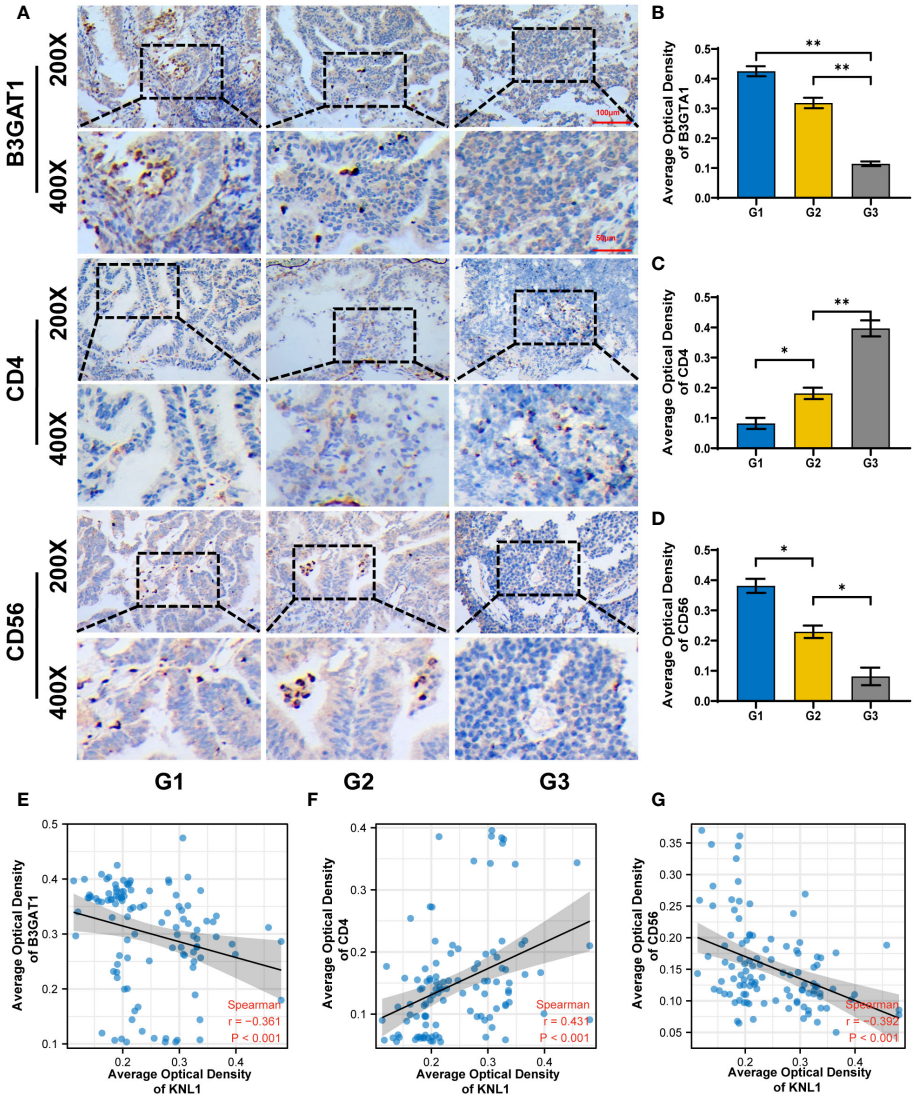

FIGURE 6
Correlation between KNL1 expression and the expression of CD4, CD56 and B3TAG1 in patients with UCEC. **(A)** Immunohistochemical images of CD4, CD56 and B4GAT1 in UCEC patients with different histologic grades. **(B–D)** Histogram of the immunohistochemical results for CD4, CD56, and B4GAT1. **(E–G)** Scatter plot of the correlation between the expression levels of CD4, CD56, and B3GAT1 and KNL1. Significance identifier:p≥0.05; *, p< 0.05; **, p<0.01.

was related to FIGO stage, tumor invasion, histologic grade and lymphatic metastasis. These results suggest that KNL1 may be a useful diagnostic molecular marker for UCEC and could predict the outcome of patients with UCEC. In addition, the ROC diagnostic curve drawn with the data obtained from the clinical samples showed that the AUC=0.764, further indicating that KNL1 could be useful in UCEC diagnosis.

To clarify the role of KNL1 in the occurrence and development of UCEC, 46 HUB genes closely related to the function of KNL1 and the most relevant 50 genes were identified by single gene differential analysis and single gene correlation analysis, including the KIF protein family, SMC protein family, BUB protein family, MELK and CENPF. Previous studies have found that CENPF, MELK, PBK, TOP2A and NEK2 are upregulated in breast cancer and this is associated with a poor prognosis. CENPF, MELK and PBK are related to CD4+ T cells, and TOP2A is related to CD8+ T cells (38, 39). In addition, MELK regulates cell cycle progression (40), leading to a worse prognosis in patients with adrenal cortical carcinoma and Wilms tumor (41, 42), and it could be a novel target for cancer therapy (43). The expression of E2F family proteins and BUB family proteins is also significantly related to the cell cycle and can promote the proliferation of tumor cells (44–47). E2F family proteins have also been shown to be potential targets for molecular diagnosis and targeted therapy of clear cell carcinoma and liver cancer (48). The expression of the SMC family is closely associated with B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils, and DCs (49), which can be potential therapeutic targets for HCC, and it has been demonstrated that inhibitors targeting SMC2, SMC3, and SMC4 can be a practical therapeutic strategy for HCC (50, 51). All of the above results suggest that KNL1 may participate in cell mitosis and the cell cycle and thus play an important role in the occurrence and development of tumors.

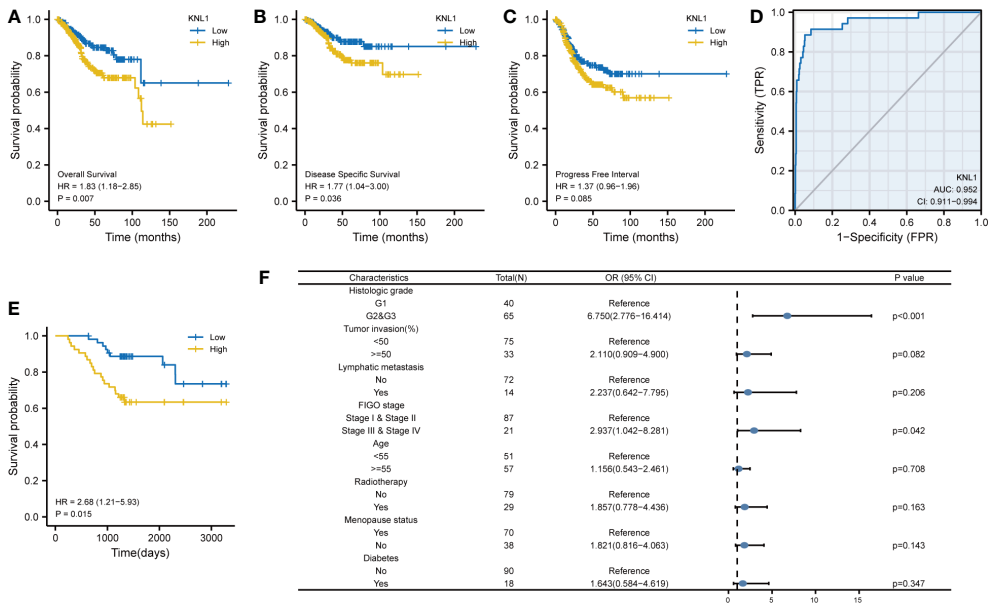

FIGURE 7
Correlation of KNL1 expression with the outcomes of UCEC patients. **(A–C)** KM survival curves stratified by KNL1 expression for overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI). **(D)** Prognostic ROC curve; the area under the ROC curve was between 0.5 and 1. The closer the AUC is to 1, the better the diagnostic effect is. The AUC has a low accuracy when it is between 0.5 and 0.7, a moderate accuracy when it is between 0.7 and 0.9, and a high accuracy when it is above 0.9. **(E)** KM OS curves stratified by KNL1 expression of 108 clinical samples of UCEC. **(F)** Results of binary logistic regression analysis of the correlation between the KNL1 expression level and the clinical characteristics of the 108 patients. The data were incomplete, as some records were lost.

To further understand the molecular mechanism of KNL1 in tumorigenesis and development, functional enrichment analysis of GO, KEGG and GSEA was performed using KNL1 and its related differentially expressed genes. GO analysis showed that KNL1 was involved in the humoral immune response, keratin filament, mitotic spindle and other biological processes. There is increasing evidence that the humoral immune response is associated with tumorigenesis (52). As a cytoskeletal protein of epithelial cells, keratin is involved in regulating apoptosis, growth and migration of tumor cells. An elevated level of keratin in the serum or tumor tissue of tumor patients has been used for the clinical diagnosis of tumors, and the expression level of keratin is negatively correlated with the survival of tumor patients and can be used as a prognostic marker (53–57).

The correct arrangement of mitotic spindles during cell division is essential for cell fate determination, tissue organization, and development. Changes in the dynamics and control of the microtubules that compromise the mitotic spindle leads to chromosomal instability, which in turn leads to the production of tumor cells (58, 59).

KEGG analysis also showed that KNL1 function was related to the biosynthesis of steroid hormones, the metabolism of cytochrome P450 and other pathways. Estrogen, as a steroid hormone, can bind to estrogen receptors and affect the progression of endometrial cancer (60). Previous studies have reported that high expression of cytochrome P450 can induce the development of tumors and inactivate anticancer drugs (61).

Consistent with the results of the GO analysis, the results of GSEA also showed that KNL1 was significantly enriched in many pathways related to mitosis. KNL1 is also closely related to the functions of the KRAS, mTORC1 and MYC genes. Previous studies have found that the KRAS gene acts as a switch in the body, regulating signaling pathways such as tumor cell growth and angiogenesis. Mutations in the KRAS gene cause continuous stimulation of cell growth, leading to tumorigenesis (62). mTORC1 can regulate cell proliferation, metabolism and survival by integrating growth factor signals and cell energy status. mTORC1 dysfunction plays a key role in tumor cell proliferation and metastasis (63). As a transcription factor with extensive functions, MYC is mainly activated by amplification,

TABLE 1 The results of the logistic regression model obtained from the RNA-seq data in the TCGA database.

Characteristics	Total (N)[a]	Odds Ratio(OR)	P value
Clinical stage (Stage IV & Stage II & Stage III vs. Stage I)	552	1.361 (0.965-1.924)	0.080
Age (>60 vs. <=60)	549	1.038 (0.734-1.466)	0.834
BMI (>30 vs. <=30)	519	0.950 (0.669-1.348)	0.773
Histological type (Mixed & Serous vs. Endometrioid)	552	1.121 (0.765-1.644)	0.559
Histologic grade (G2 & G3 vs. G1)	541	3.395 (2.114-5.605)	<0.001
Tumor invasion(%) (>=50 vs. <50)	474	0.976 (0.679-1.403)	0.898
Menopause status (Post vs. Pre & Peri)	506	0.759 (0.423-1.349)	0.349
Diabetes (Yes vs. No)	451	1.206 (0.796-1.829)	0.377

[a]Data incomplete as some record data were lost.

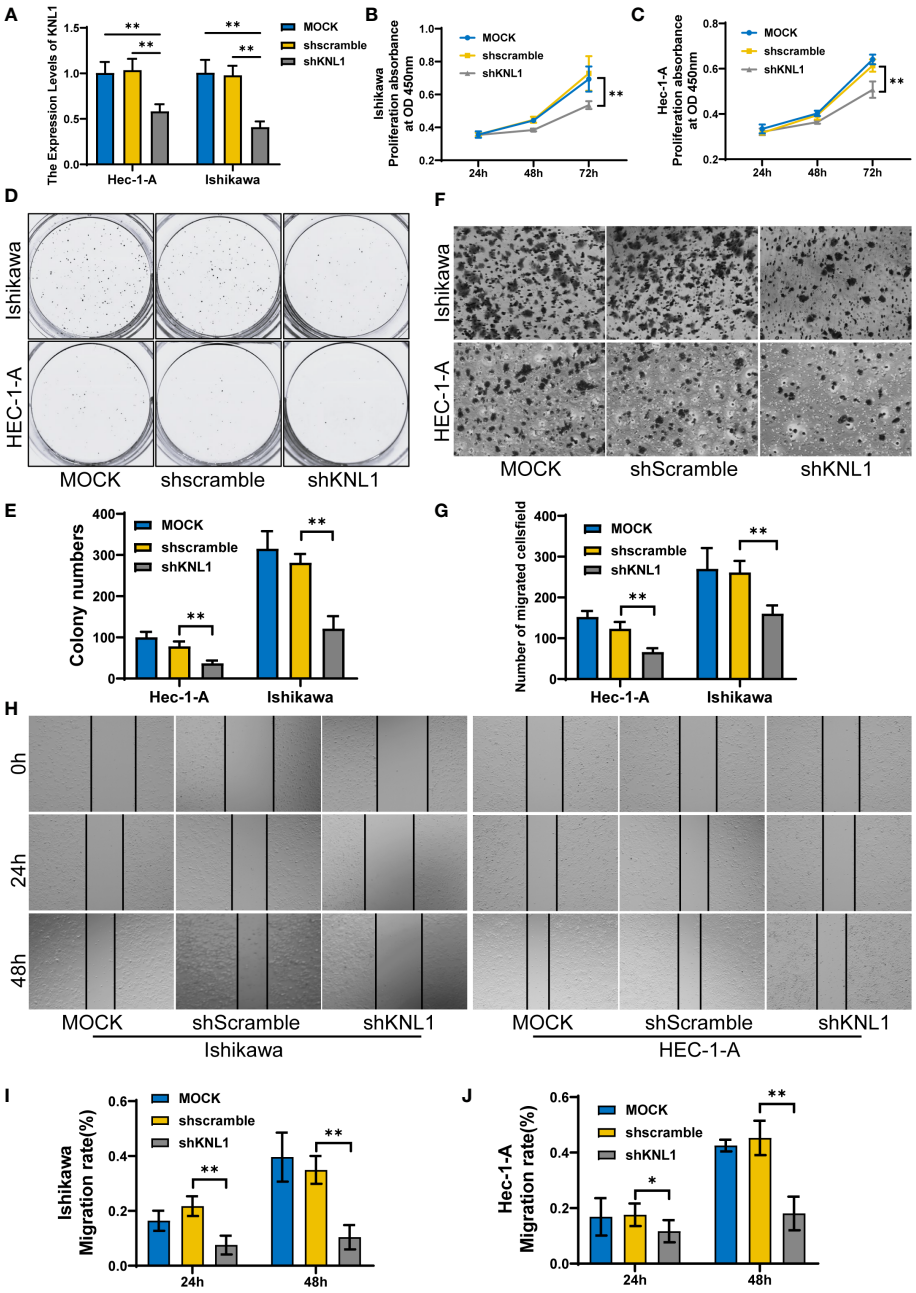

FIGURE 8
Effect of knockdown of KNL1 expression in UCEC cell lines on tumor cell proliferation, invasion and other phenotypes. **(A)** Ishikawa and Hec-1-A cells were transfected with shKNL1, and the level of KNL1 was evaluated by qRT–PCR. **(B, C)** The proliferation of Ishikawa and Hec-1-A cells was examined by CCK-8 **(D, E)** and colony-formation assays. **(F, G)** The migration of Ishikawa and Hec-1-A cells was examined by Transwell assays. **(H–J)** The metastatic capacity of Ishikawa and Hec-1-A cells was examined by wound-healing assays. Significance identifier: p≥0.05; *, p< 0.05; **, p<0.01.

chromosomal translocation and rearrangement, regulates cell differentiation and proliferation through various mechanisms, and participates in the occurrence, development and evolution of tumors (64).

Given the correlation between KNL1-related genes and T cells, this study further explored the relationship between KNL1 and immune cell infiltration in tumors. KNL1 was positively correlated with the infiltration of Th2 cells, T helper cells and Tcm cells and negatively correlated with the infiltration of pDCs, iDCs and NK cells. This result was confirmed by immunohistochemical analysis of 108 endometrial carcinoma samples. Studies have shown that pDCs can promote the antitumor immune response (65), iDCs can promote the activation of T cells, and NK cells play a key role in immune regulation through interactions with DCs (66). During tumor progression, the transition from Th1/Th2 balance to Th2 dominance is crucial. Th2 cells are not conducive to cellular immune antitumor effects. Restoring the Th1/Th2 balance is of great significance in tumor therapy (67). The results of this study indicate that upregulation of KNL1 expression may be adverse to the antitumor immune response of the body, and it is significantly positively correlated with the immunotherapy target CD47, suggesting that KNL1 may be a potential immunotherapy target for tumor immunotherapy. Additional proteomics and larger sample size

studies are needed for further verification of this possibility in the future.

Taking into consideration the upregulation of KNL1 expression in tumor tissues and its inhibition of antitumor immunity, we speculated that KNL1 might be correlated with the prognosis of patients with endometrial cancer. Using KNL1 expression network data and clinical data, KM survival analysis showed that high KNL1 expression predicted a poor prognosis. The ROC curve analysis showed that KNL1 had a high accuracy in predicting the outcomes of patients.

When analyzing the correlation between KNL1 expression and the clinical characteristics of patients, this study found that the expression of KNL1 was only correlated with the histologic grade of patients by using RNA-seq data analysis from online databases. However, immunohistochemical analysis of 108 clinical samples showed that KNL1 protein expression was correlated with FIGO stage, tumor invasion, histologic grades and lymphatic metastases of patients. The inconsistency between these results may be because the former was obtained from an analysis of RNA-seq data at the transcription level, while the latter was obtained from immunohistochemical analysis results at the protein level. The mRNA abundance does not necessarily have a linear relationship with the protein expression level of its translated products. There are many levels of regulation of protein content, and the transcription level is only one level. In addition, mRNA degradation, protein degradation, protein modification, protein folding and other factors may cause the mRNA abundance and protein expression levels to be inconsistent. These factors can all lead to differences in the final results (68). Meanwhile, the protein expression level in this study was quantified using the results of immunohistochemical analysis, and the sample size used in the analysis was only 108 cases, which may lead to bias in the analysis results. More proteomics and larger sample size studies are needed in the future to verify the relationship between the protein expression level of KNL1 and the clinical characteristics of patients.

Finally, to verify the function of KNL1, this study used the endometrial cancer cell lines HEC-1-A and Ishikawa to downregulate the expression of KNL1 by stable transfection of shRNA. Knockdown of KNL1 expression weakened cell viability and decreased the metastatic and invasive abilities of the tumor cells. This result further verified that KNL1 is closely related to the occurrence and development of tumors and is involved in the invasion and metastasis of tumor cells. Therefore, KNL1 can be used as a potential molecular target for tumor therapy.

In conclusion, the upregulation of KNL1 expression can promote the occurrence, metastasis and invasion of UCEC and inhibit the antitumor immune response. Therefore, KNL1 can be used as an independent risk factor for UCEC and is a potential molecular marker for diagnosing, treating and predicting the outcome of UCEC, which can help doctors make more reasonable treatment plans for patients.

At the same time, this study has certain limitations. First, there is a large difference between the numbers of tumor samples and normal samples, and further research is needed to narrow this difference in sample sizes in the future. In addition, the application of a single biomarker is unlikely to be sufficiently accurate for prognostication and diagnosis, and a combination of several different biomarkers needs to be further evaluated in the future. This can lead to the identification of algorithms with better diagnostic characteristics. This study verified the effect of KNL1 on UCEC cells, but the results related to pathway enrichment still need to be further verified by *in vitro* and *in vivo* experiments. This study is a retrospective study, and more prospective studies are needed in the future to reduce the bias inherently caused by retrospective studies.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: The TCGA database (https://portal.gdc.cancer.gov/), The UALCAN database (http://ualcan.path.uab.edu), and GSE17025 of The GEO database (https://www.ncbi.nlm.nih.gov/geo/).

Ethics statement

The present study was approved by the Ethics Committee of the School of Nursing, Jilin University (Changchun, China). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SZ and LZ conceived and designed the study. KH, JL acquired the data performed the statistical analysis. KW and XH performed the experiments and analysis the data. WZ and TW drafted the manuscript. JC, ZW and JY contributed to revising the manuscript for intellectual content and language editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1090779/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Differential expression analysis results of KNL1 in pancancer patients. **(A)** Results of differential analysis of KNL1 expression in 33 tumors based on TCGA database data. **(B)** Pancancer analysis of paired samples based on data from the TCGA database.

SUPPLEMENTARY FIGURE 2
Expression of KNL1 in UCEC patients with different histological types. **(A)** Box diagram of KNL1 expression obtained from RNA-seq data in the TCGA database. **(B)** Box plot of KNL1 expression using immunohistochemical analysis of 108 clinical samples.

SUPPLEMENTARY FIGURE 3
KM overall survival curves stratified by KNL1 expression in different tumors. **(A)** KM survival curve of GBMLGG, **(B)** KM survival curve of LGG, **(C)** KM survival curve of BRCA, **(D)** KM survival curve of KIRP, **(E)** KM survival curve of KIRC, and (F) KM survival curve of PAAD.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654

2. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. *Lancet (London England)* (2005) 366(9484):491–505. doi: 10.1016/S0140-6736(05)67063-8

3. Wong YF, Cheung TH, Lo KWK, Yim SF, Siu NSS, Chan SCS, et al. Identification of molecular markers and signaling pathway in endometrial cancer in Hong Kong Chinese women by genome-wide gene expression profiling. *Oncogene* (2007) 26(13):1971–82. doi: 10.1038/sj.onc.1209986

4. Zhang Y, Liu Z, Yu X, Zhang X, Lü S, Chen X, et al. The association between metabolic abnormality and endometrial cancer: a large case-control study in China. *Gynecol Oncol* (2010) 117(1):41–6. doi: 10.1016/j.ygyno.2009.12.029

5. Llauradó M, Ruiz A, Majem B, Ertekin T, Colás E, Pedrola N, et al. Molecular bases of endometrial cancer: new roles for new actors in the diagnosis and the therapy of the disease. *Mol Cell Endocrinol* (2012) 358(2):244–55. doi: 10.1016/j.mce.2011.10.003

6. Neri M, Peiretti M, Melis GB, Piras B, Vallerino V, Paoletti AM, et al. Systemic therapy for the treatment of endometrial cancer. *Expert Opin Pharmacother* (2019) 20(16):2019–32. doi: 10.1080/14656566.2019.1654996

7. Brooks RA, Fleming GF, Lastra RR, Lee NK, Moroney JW, Son CH, et al. Current recommendations and recent progress in endometrial cancer. *CA: Cancer J For Clin* (2019) 69(4):258–79. doi: 10.3322/caac.21561

8. Dueholm M, Hjorth IMD, Dahl K, Hansen ES, Ørtoft G. Ultrasound scoring of endometrial pattern for fast-track identification or exclusion of endometrial cancer in women with postmenopausal bleeding. *J Minim Invasive Gynecol* (2019) 26(3):516–25. doi: 10.1016/j.jmig.2018.06.010

9. Clarke MA, Long BJ, Del Mar Morillo A, Arbyn M, Bakkum-Gamez JN, Wentzensen N. Association of endometrial cancer risk with postmenopausal bleeding in women: A systematic review and meta-analysis. *JAMA Internal Med* (2018) 178(9):1210–22. doi: 10.1001/jamainternmed.2018.2820

10. Takimoto M, Wei G, Dosaka-Akita H, Mao P, Kondo S, Sakuragi N, et al. Frequent expression of new cancer/testis gene D40/AF15q14 in lung cancers of smokers. *Br J Cancer* (2002) 86(11):1757–62. doi: 10.1038/sj.bjc.6600328

11. Wei G, Takimoto M, Yoshida I, Mao PZ, Koya RC, Miura T, et al. Chromosomal assignment of a novel human gene D40. *Nucleic Acids Symp Ser* (1999) 1999(42):71–2. doi: 10.1093/nass/42.1.71

12. Hayette S, Tigaud I, Vanier A, Martel S, Corbo L, Charrin C, et al. AF15q14, a novel partner gene fused to the MLL gene in an acute myeloid leukaemia with a t (11,15)(q23;q14). *Oncogene* (2000) 19(38):4446–50. doi: 10.1038/sj.onc.1203789

13. Vleugel M, Tromer E, Omerzu M, Groenewold V, Nijenhuis W, Snel B, et al. Arrayed BUB recruitment modules in the kinetochore scaffold KNL1 promote accurate chromosome segregation. *J Cell Biol* (2013) 203(6):943–55. doi: 10.1083/jcb.201307016

14. Bajaj R, Bollen M, Peti W, Page R. KNL1 binding to PP1 and microtubules is mutually exclusive. *Structure* (2018) 26(10). doi: 10.1016/j.str.2018.06.013

15. Liu D, Vleugel M, Backer CB, Hori T, Fukagawa T, Cheeseman IM, et al. Regulated targeting of protein phosphatase 1 to the outer kinetochore by KNL1 opposes aurora b kinase. *J Cell Biol* (2010) 188(6):809–20. doi: 10.1083/jcb.201001006

16. Cui Y, Zhang C, Ma S, Guo W, Cao W, Guan F. CASC5 is a potential tumour driving gene in lung adenocarcinoma. *Cell Biochem Funct* (2020) 38(6):733–42. doi: 10.1002/cbf.3540

17. Bai T, Zhao Y, Liu Y, Cai B, Dong N, Li B. Effect of KNL1 on the proliferation and apoptosis of colorectal cancer cells. *Technol Cancer Res Treat* (2019) 18:1533033819858668. doi: 10.1177/1533033819858668

18. Urata YN, Takeshita F, Tanaka H, Ochiya T, Takimoto M. Targeted knockdown of the kinetochore protein D40/Knl-1 inhibits human cancer in a p53 status-independent manner. *Sci Rep* (2015) 5:13676. doi: 10.1038/srep13676

19. Vivian J, Rao AA, Nothaft FA, Ketchum C, Armstrong J, Novak A, et al. Toil enables reproducible, open source, big biomedical data analyses. *Nat Biotechnol* (2017) 35(4):314–6. doi: 10.1038/nbt.3772

20. Davis S, Meltzer PS. GEOquery: a bridge between the gene expression omnibus (GEO) and BioConductor. *Bioinformatics* (2007) 23(14):1846–7. doi: 10.1093/bioinformatics/btm254

21. Day RS, McDade KK, Chandran UR, Lisovich A, Conrads TP, Hood BL, et al. Identifier mapping performance for integrating transcriptomics and proteomics experimental results. *BMC Bioinf* (2011) 12:213. doi: 10.1186/1471-2105-12-213

22. Day RS, McDade KK. A decision theory paradigm for evaluating identifier mapping and filtering methods using data integration. *BMC Bioinf* (2013) 14:223. doi: 10.1186/1471-2105-14-223

23. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* (2015) 43(7):e47. doi: 10.1093/nar/gkv007

24. Zhang Y, Chen F, Chandrashekar DS, Varambally S, Creighton CJ. Proteogenomic characterization of 2002 human cancers reveals pan-cancer molecular subtypes and associated pathways. *Nat Commun* (2022) 13(1):2669.

25. Chen F, Chandrashekar DS, Varambally S, Creighton CJ. Pan-cancer molecular subtypes revealed by mass-spectrometry-based proteomic characterization of more than 500 human cancers. *Nat Commun* (2019) 10(1):5679. doi: 10.1038/s41467-019-13528-0

26. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* (2014) 15(12):550. doi: 10.1186/s13059-014-0550-8

27. Jensen LJ, Kuhn M, Stark M, Chaffron S, Creevey C, Muller J, et al. STRING 8–a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res* (2009) 37(Database issue):D412–D6. doi: 10.1093/nar/gkn760

28. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinf* (2013) 14:7. doi: 10.1186/1471-2105-14-7

29. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* (2013) 39(4):782–95. doi: 10.1016/j.immuni.2013.10.003

30. Liu J, Lichtenberg T, Hoadley KA, Poisson LM, Lazar AJ, Cherniack AD, et al. An integrated TCGA pan-cancer clinical data resource to drive high-quality survival outcome analytics. *Cell* (2018) 173(2).

31. Chen J, Xu D, Wang T, Yang Z, Yang Y, He K, et al. HMGB1 promotes the development of castration–resistant prostate cancer by regulating androgen receptor activation. *Oncol Rep* (2022) 48(5). doi: 10.3892/or.2022.8412

32. Bignotti E, Ragnoli M, Zanotti L, Calza S, Falchetti M, Lonardi S, et al. Diagnostic and prognostic impact of serum HE4 detection in endometrial carcinoma patients. *Br J Cancer* (2011) 104(9):1418–25. doi: 10.1038/bjc.2011.109

33. Antonsen SL, Høgdall E, Christensen IJ, Lydolph M, Tabor A, Loft Jakobsen A, et al. HE4 and CA125 levels in the preoperative assessment of endometrial cancer patients: a prospective multicenter study (ENDOMET). *Acta Obstet Gynecol Scand* (2013) 92(11):1313–22. doi: 10.1111/aogs.12235

34. Rižner TL. Discovery of biomarkers for endometrial cancer: current status and prospects. *Expert Rev Mol Diagn* (2016) 16(12):1315–36.

35. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al. ESMO-ESGO-ESTRO consensus conference on endometrial cancer: diagnosis, treatment and follow-up. *Ann Oncol* (2016) 27(1):16–41. doi: 10.1093/annonc/mdv484

36. Sundar S, Balega J, Crosbie E, Drake A, Edmondson R, Fotopoulou C, et al. BGCS uterine cancer guidelines: Recommendations for practice. *Eur J Obstet Gynecol Reprod Biol* (2017) 213:71–97. doi: 10.1016/j.ejogrb.2017.04.015

37. Townsend MH, Ence ZE, Felsted AM, Parker AC, Piccolo SR, Robison RA, et al. Potential new biomarkers for endometrial cancer. *Cancer Cell Int* (2019) 19:19. doi: 10.1186/s12935-019-0731-3

38. Paizula X, Mutailipu D, Xu W, Wang H, Yi L. Identification of biomarkers related to tumorigenesis and prognosis in breast cancer. *Gland Surg* (2022) 11(9):1472–88. doi: 10.21037/gs-22-449

39. Zhang J, Lin H, Hou L, Xiao H, Gong X, Guo X, et al. Exploration of the breast ductal carcinoma in situ signature and its prognostic implications. *Cancer Med* (2022). doi: 10.1002/cam4.5071

40. Hardeman AA, Han YJ, Grushko TA, Mueller J, Gomez MJ, Zheng Y, et al. Subtype-specific expression of MELK is partly due to copy number alterations in breast cancer. *PloS One* (2022) 17(6):e0268693. doi: 10.1371/journal.pone.0268693

41. Laha D, Grant RRC, Mishra P, Boufraqech M, Shen M, Zhang Y-Q, et al. Preclinical assessment of synergistic efficacy of MELK and CDK inhibitors in adrenocortical cancer. *J Exp Clin Cancer Res* (2022) 41(1):282. doi: 10.1186/s13046-022-02464-5

42. Du J, Xiao J, Zhuo Y, Nie M, Yang J, Luo X, et al. LncRNA OSTM1-AS1 acts as an oncogenic factor in wilms' tumor by regulating the miR-514a-3p/MELK axis. *Anticancer Drugs* (2022) 33(8):720–30. doi: 10.1097/CAD.0000000000001320

43. Makki Almansour N. Computational exploration of maternal embryonic leucine zipper kinase (MELK) as a cancer drug target. *Saudi J Biol Sci* (2022) 29(7):103335. doi: 10.1016/j.sjbs.2022.103335

44. Hao F, Wang N, Zhang Y, Xu W, Chen Y, Fei X, et al. E2F7 enhances hepatocellular carcinoma growth by preserving the SP1/SOX4/Anillin axis *via* repressing miRNA-383-5p transcription. *Mol Carcinog* (2022) 61(11):975–88. doi: 10.1002/mc.23454

45. Liu Z-G, Su J, Liu H, Yang X-J, Yang X, Wei Y, et al. Comprehensive bioinformatics analysis of the E2F family in human clear cell renal cell carcinoma. *Oncol Lett* (2022) 24(4):351. doi: 10.3892/ol.2022.13471

46. Tan Y, Zhang M, Chen X, Deng Y. Identifying a three-gene signature and associated drugs for hepatitis b virus-related hepatocellular carcinoma using comprehensive bioinformatics analysis. *Tohoku J Exp Med* (2022) 258(2):149–57. doi: 10.1620/tjem.2022.J069

47. Vleugel M, Omerzu M, Groenewold V, Hadders MA, Lens SMA, Kops GJPL. Sequential multisite phospho-regulation of KNL1-BUB3 interfaces at mitotic kinetochores. *Mol Cell* (2015) 57(5):824–35. doi: 10.1016/j.molcel.2014.12.036

48. Zeng K, Song G, Chen B, Gao X, Liu C, Miao J, et al. Comprehensive analysis to identify the RP11-478C19.2/E2F7 axis as a novel biomarker for treatment decisions in clear cell renal cell carcinoma. *Transl Oncol* (2022) 25:101525. doi: 10.1016/j.tranon.2022.101525

49. Nie H, Wang Y, Yang X, Liao Z, He X, Zhou J, et al. Clinical significance and integrative analysis of the SMC family in hepatocellular carcinoma. *Front In Med* (2021) 8:727965. doi: 10.3389/fmed.2021.727965

50. Yan W, Wang D-D, Zhang H-D, Huang J, Hou J-C, Yang S-J, et al. Expression profile and prognostic values of SMC family members in HCC. *Medicine* (2022) 101(42): e31336. doi: 10.1097/MD.0000000000031336

51. Houlard M, Cutts EE, Shamim MS, Godwin J, Weisz D, Presser Aiden A, et al. MCPH1 inhibits condensin II during interphase by regulating its SMC2-kleisin interface. *Elife* (2021) 10.

52. Monroy-Iglesias MJ, Crescioli S, Beckmann K, Le N, Karagiannis SN, Van Hemelrijck M, et al. Antibodies as biomarkers for cancer risk: a systematic review. *Clin Exp Immunol* (2022) 209(1):46–63. doi: 10.1093/cei/uxac030

53. Homberg M, Magin TM. Beyond expectations: novel insights into epidermal keratin function and regulation. *Int Rev Cell Mol Biol* (2014) 311:265–306. doi: 10.1016/B978-0-12-800179-0.00007-6

54. Karsch S, Büchau F, Magin TM, Janshoff A. An intact keratin network is crucial for mechanical integrity and barrier function in keratinocyte cell sheets. *Cell Mol Life Sci* (2020) 77(21):4397–411. doi: 10.1007/s00018-019-03424-7

55. Zhang N, Zhang R, Zou K, Yu W, Guo W, Gao Y, et al. Keratin 23 promotes telomerase reverse transcriptase expression and human colorectal cancer growth. *Cell Death Dis* (2017) 8(7):e2961. doi: 10.1038/cddis.2017.339

56. Bozza WP, Zhang Y, Zhang B. Cytokeratin 8/18 protects breast cancer cell lines from TRAIL-induced apoptosis. *Oncotarget* (2018) 9(33):23264–73. doi: 10.18632/oncotarget.25297

57. Bilandzic M, Rainczuk A, Green E, Fairweather N, Jobling TW, Plebanski M, et al. Keratin-14 (KRT14) positive leader cells mediate mesothelial clearance and invasion by ovarian cancer cells. *Cancers* (2019) 11(9). doi: 10.3390/cancers11091228

58. Marquis C, Fonseca CL, Queen KA, Wood L, Vandal SE, Malaby HLH, et al. Chromosomally unstable tumor cells specifically require KIF18A for proliferation. *Nat Commun* (2021) 12(1):1213. doi: 10.1038/s41467-021-21447-2

59. Noatynska A, Gotta M, Meraldi P. Mitotic spindle (DIS)orientation and DISease: cause or consequence? *J Cell Biol* (2012) 199(7):1025–35. doi: 10.1083/jcb.201209015

60. Nyholm HC, Nielsen AL, Lyndrup J, Norup P, Thorpe SM. Biochemical and immunohistochemical estrogen and progesterone receptors in adenomatous hyperplasia and endometrial carcinoma: correlations with stage and other clinicopathologic features. *Am J Obstetrics Gynecol.* (1992) 167(5):1334–42. doi: 10.1016/S0002-9378(11)91712-8

61. Sausville LN, Williams SM, Pozzi A. Cytochrome P450 epoxygenases and cancer: A genetic and a molecular perspective. *Pharmacol Ther* (2019) 196:183–94. doi: 10.1016/j.pharmthera.2018.11.009

62. Reck M, Carbone DP, Garassino M, Barlesi F. Targeting KRAS in non-small-cell lung cancer: recent progress and new approaches. *Ann Oncol* (2021) 32(9):1101–10. doi: 10.1016/j.annonc.2021.06.001

63. Chen J, Ou Y, Luo R, Wang J, Wang D, Guan J, et al. SAR1B senses leucine levels to regulate mTORC1 signalling. *Nature* (2021) 596(7871):281–4. doi: 10.1038/s41586-021-03768-w

64. Dang CV. MYC on the path to cancer. *Cell* (2012) 149(1):22–35. doi: 10.1016/j.cell.2012.03.003

65. Zhang Q, He Y, Luo N, Patel SJ, Han Y, Gao R, et al. Landscape and dynamics of single immune cells in hepatocellular carcinoma. *Cell* (2019) 179(4). doi: 10.1016/j.cell.2019.10.003

66. Yofe I, Dahan R, Amit I. Single-cell genomic approaches for developing the next generation of immunotherapies. *Nat Med* (2020) 26(2):171–7. doi: 10.1038/s41591-019-0736-4

67. Sharma A, Rajappa M, Saxena A, Sharma M. Cytokine profile in Indian women with cervical intraepithelial neoplasia and cancer cervix. *Int J gynecol. Cancer* (2007) 17 (4):879–85. doi: 10.1111/j.1525-1438.2007.00883.x

68. Liu Y, Beyer A, Aebersold R. On the dependency of cellular protein levels on mRNA abundance. *Cell* (2016) 165(3):535–50. doi: 10.1016/j.cell.2016.03.014

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EDITED BY
Janina Tokarz,
Helmholtz Association of German Research Centres (HZ), Germany

REVIEWED BY
Subathra Adithan,
Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), India
Zhenyu Shu,
Zhejiang Provincial People's Hospital, China
Kundan Thind,
Alberta Health Services, Canada
Kevin Albuquerque,
University of Texas Southwestern Medical Center, United States

*CORRESPONDENCE
Chengwei Wang
2214757292@qq.com

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Multiparametric magnetic resonance imaging-based radiomics nomogram for predicting tumor grade in endometrial cancer

Xiaoning Yue[1], Xiaoyu He[1], Shuaijie He[1], Jingjing Wu[1], Wei Fan[1], Haijun Zhang[2] and Chengwei Wang[1]*

[1]Department of CT&MRI, The First Affiliated Hospital of Medical College, Shihezi University, Shihezi, China, [2]Department of Pathology, The First Affiliated Hospital of Medical College, Shihezi University, Shihezi, China

Background: Tumor grade is associated with the treatment and prognosis of endometrial cancer (EC). The accurate preoperative prediction of the tumor grade is essential for EC risk stratification. Herein, we aimed to assess the performance of a multiparametric magnetic resonance imaging (MRI)-based radiomics nomogram for predicting high-grade EC.

Methods: One hundred and forty-three patients with EC who had undergone preoperative pelvic MRI were retrospectively enrolled and divided into a training set (n =100) and a validation set (n =43). Radiomic features were extracted based on T2-weighted, diffusion-weighted, and dynamic contrast-enhanced T1-weighted images. The minimum absolute contraction selection operator (LASSO) was implemented to obtain optimal radiomics features and build the rad-score. Multivariate logistic regression analysis was used to determine the clinical MRI features and build a clinical model. We developed a radiomics nomogram by combining important clinical MRI features and rad-score. A receiver operating characteristic (ROC) curve was used to evaluate the performance of the three models. The clinical net benefit of the nomogram was assessed using decision curve analysis (DCA), net reclassification index (NRI), and integrated discrimination index (IDI).

Results: In total, 35/143 patients had high-grade EC and 108 had low-grade EC. The areas under the ROC curves of the clinical model, rad-score, and radiomics nomogram were 0.837 (95% confidence interval [CI]: 0.754–0.920), 0.875 (95% CI: 0.797–0.952), and 0.923 (95% CI: 0.869–0.977) for the training set; 0.857 (95% CI: 0.741–0.973), 0.785 (95% CI: 0.592–0.979), and 0.914 (95% CI: 0.827–0.996) for the validation set, respectively. The radiomics nomogram showed a good net benefit according to the DCA. NRIs were 0.637 (0.214–1.061) and 0.657 (0.079–

1.394), and IDIs were 0.115 (0.077–0.306) and 0.053 (0.027–0.357) in the training set and validation set, respectively.

Conclusion: The radiomics nomogram based on multiparametric MRI can predict the tumor grade of EC before surgery and yield a higher performance than that of dilation and curettage.

KEYWORDS

endometrial cancer, histological grade, magnetic resonance imaging, radiomics, nomogram

Introduction

The incidence of endometrial carcinoma (EC) has risen steadily in recent years and the standard operation for EC consists of hysterectomy and bilateral salpingo-oophorectomy (1, 2). The 2020 the European Society of Gynaecological Oncology the European Society for Radiotherapy & Oncology and the European Society of Pathology (ESGO-ESTRO-ESP) guidelines recommend pelvic and abdominal para-aortic lymph node dissection for patients with high-intermediate-risk/high-risk EC (high-grade EC and myometrial invasion ≥ 50%), but not low-risk EC (low-grade EC, myometrial invasion< 50%, and lymphatic vascular space invasion [LVSI] negative) (3). The prognosis of EC is related to tumor grade, deep myometrial invasion (DMI), LVSI, and lymph node metastasis (LNM). Tumor grade is an important predictor of disease outcome and LNM as well as an important cornerstone for determining the extent of surgical treatment (4, 5).

Almost all patients with EC undergo preoperative dilation and curettage (D&C) or hysteroscopic biopsy. A recent review showed moderate agreement between D&C and the final surgical pathology (6). The underestimation of the pathological grade will lead to inadequate treatment and risk of LNM in the future, whereas overestimation of the pathological grade will lead to excessive surgical treatment and cause unnecessary complications in patients (7, 8). One study showed that the inconsistent diagnosis of preoperative pathological grading is an important reason for the high mortality rate (9). Consequently, it is necessary to develop an accurate and noninvasive preoperative method to predict the tumor grade of EC.

In addition to diagnostic curettage, magnetic resonance imaging (MRI) has the greatest potential to predict tumor grade. Most studies have predicted the pathological grade of EC using conventional MRI features or apparent diffusion coefficient (ADC) values (10, 11). However, owing to the subjective influence of measurement level and experience, some quantitative indicators are difficult to represent the heterogeneity of the whole tumor. Their value in evaluating tumor grade remains controversial. Radiomics is a non-invasive method for quantitatively assessing tumor heterogeneity by digitally analyzing a large number of image features extracted from medical images with high throughput. In addition, radiomics can link image features with phenotypes by establishing descriptive and predictive models, which may provide useful information for differential tumor diagnosis and evaluation of tumor response to treatment (12–14). In EC, previous studies have demonstrated that radiomics performs well in assessing the depth of myometrial invasion (MI), LVSI, LNM, and prognosis (9, 15–17). Therefore, we believe that radiomics is a promising tool for predicting preoperative tumor grade.

This study aimed to develop a radiomics nomogram based on multiparametric MRI to predict high-grade EC and compare the net clinical benefit of the radiomics nomogram with that of preoperative D&C.

Materials and methods

Patients

This study was approved by the Ethics Committee of our institution and the requirement for patient informed consent was waived. Between January 2017 and March 2022, 182 patients with a histopathological diagnosis of EC underwent preoperative pelvic MRI. The inclusion criteria were as follows: (1) patients with EC confirmed by postoperative histopathology. (2) MRI was performed within 2 weeks before the operation in our hospital, and (3) no adjuvant therapy was performed before MRI examination. The exclusion criteria were as follows: (1) tumor was less than two layers on MRI or the maximum diameter of the tumor was less than 10 mm (n = 23), (2) image quality pitfalls (n = 2), (3) no DCE-MRI (n = 7), (4) incomplete histopathology report (n = 3), and (5) combined with other pelvic malignancies (n = 4). Finally, a total of 143 patients (average age 55.52 ± 10.46 years) were enrolled and randomly divided into the training set (100 patients, 27 of whom had high-grade EC) and the validation set (43 patients, eight of whom had high-grade EC) at a ratio of 7:3 by stepwise sampling. A flow chart of the inclusion and exclusion criteria for the patients is shown in Figure 1.

MRI protocols

Axial TI-weighted imaging (T1WI), sagittal and coronal T2-weighted imaging (T2WI) without fat suppression, axial fat suppression T2WI, axial diffusion-weighted imaging (DWI [b = 0 and 800 s/mm^2]), and three planes (axial, sagittal, and coronal) of

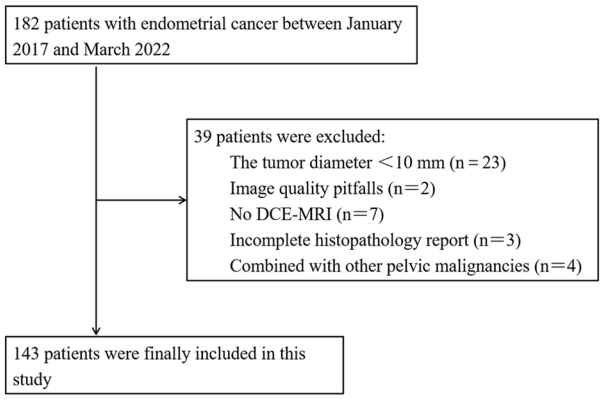

FIGURE 1
Flowchart of included and excluded patients with endometrial cancer.

dynamic contrast-enhanced T1-weighted images (DCE-T1WI) of the pelvis were performed using a 3.0 T magnetic resonance machine (GE Discovery MR 750 W, Milwaukee, WI) and one 1.5 T MR machine (Philips, Maltiva, the Netherlands). All the images were acquired using an eight-channel phased array surface coil. The patients fasted for 4–6h before MRI scans to reduce artifacts caused by bowel peristalsis. There were eight dynamic phases in DCE-T1WI. The first was a mask film. Before the second dynamic phase scanning, a contrast agent (gadolinium chelate, GE Healthcare) was injected into the cubital vein of the patient with a dosage of 0.2 ml/kg and an injection rate of 2–3 ml/s. Each dynamic phase was scanned for 18–20 s. The details of the MRI scanning protocols are listed in Supplementary Table S1A.

Classification of tumor grade

Two pathologists divided endometrioid adenocarcinomas into well differentiated (grade 1), moderately differentiated (grade 2), and poorly differentiated (grade 3) according to the proportion of non-squamous solid components in the tumor tissue (18). For the difference in 5-year survival and prognosis, we considered grade 1/grade 2 endometrioid adenocarcinoma as low-grade EC, grade 3 adenocarcinoma, and non-endometrioid adenocarcinoma (e.g., clear, serous cell carcinomas, etc.) as high-grade EC, which has a less favorable prognosis (1).

Clinical and conventional MRI features

Clinical data, including patient age, CA125 (within 2 weeks before surgery), HE4 (within 2 weeks before surgery), and tumor grade by preoperative D&C, were obtained through the hospital information management system. Pathological reports should include tumor differentiation, depth of MI, CSI, and FIGO stage.

Two radiologists (A and B with 5 and 12 years of experience, respectively) reviewed the MRI images of each patient, blinded to the pathological and clinical data. The evaluation items included maximum tumor diameter (mean value of the tumor on axial T2WI, DWI, and DCE-T1WI), depth of MI, CSI, and LNM. Disagreements were re-evaluated by another senior physician.

Image segmentation and radiomics feature extraction

The region of interest (ROI) was manually delineated in each layer of the tumor on axial T2WI, DWI, and DCE-T1WI images (the seventh dynamic scanning period) by radiologist A and automatically converted into three-dimensional images to obtain the volume of interest (VOI) using the 3D-Slicer software (v.4.11.0, https://www.slicer.org). Subsequently, radiologist B randomly selected 40 patients to draw the ROI in the same manner. All ROIs were drawn considering cystic, necrotic, and bleeding areas within the tumor, but avoiding the normal muscularis adjacent to the tumor tissue and hematoma outside the tumor. A flowchart of the radiomics feature extraction is shown in Figure 2.

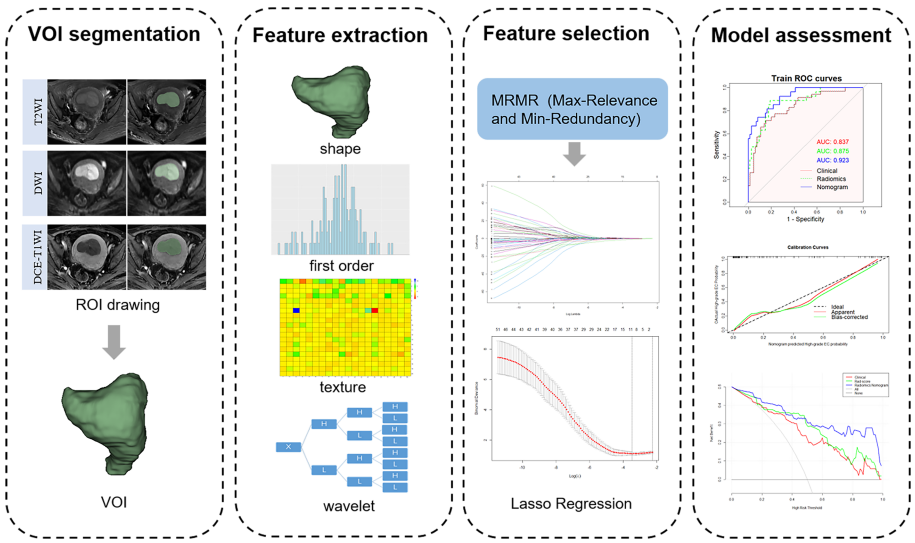

FIGURE 2
Workflow of radiomics analysis and model building.

Before extracting radiomics features, MRI images must be preprocessed to compensate for the difference in signal intensity caused by different field strengths and scanning protocols. Image preprocessing included resampling the image to a voxel size of 1 mm^3 and discretizing the voxel intensity value with a fixed bin width of 25 mm to standardize the gray intensity of each image and reduce image noise.

In total, 851 radiomics features extracted from each VOI of T2WI, DWI, and DCE-T1WI images included shape-based, first-order, and texture features (including GLCM, GLDM, GLSZM, GLRLM, and NGTDM). The intraclass correlation coefficient (ICC) was used to evaluate the reproducibility of radiomics features. To explore more information inside the tumor to highlight the differences between tumor grades, the first-order features and texture features were transformed by wavelet transform, and eight wavelet decomposition features of different frequency bands were obtained. Detailed information on all the features is provided in Supplementary Table S2A. All radiomics features were preprocessed using *Z*-score standardization to eliminate the influence of different gray values.

Features selection and radiomics score construction

The radiomics features with ICC ≥ 0.75 into R software (v4.2.0, https://www.R-project.org). First, 80 radiomics features with the greatest correlation with tumor grade were selected based on the maximum relevance and minimum redundancy (mRMR) algorithm. These features were further reduced in dimension and screened using least absolute shrinkage and selection operator (LASSO) regression. The regularization parameter λ was adjusted by 10-fold cross-validation to select robust features and construct a radiomics score (rad-score) by linear combinations weighted by the corresponding coefficients of the selected features.

Development of clinical model and radiomics nomogram

Univariate and multivariate logistic regression analyses were used for clinical and conventional MRI features associated with tumor grade. Features with statistically significant differences were considered independent risk factors and were used to establish the clinical model. Next, a radiomics nomogram was established by combining the above independent risk factors with the rad-score using logistic regression. A calibration curve was drawn, and the *p*-value of the Hosmer-Lemeshow test was used to evaluate the fitting effect of the model.

Clinical usefulness

The clinical feasibility of the radiomics nomogram, rad-score and clinical model was evaluated by decision curve analysis (DCA). The net benefits of both under different probability thresholds were analyzed by comparing the clinical decision curves of the radiomics nomogram and preoperative D&C. The net reclassification index (NRI) and integrated discrimination index (IDI) were calculated to analyze the advantages of the radiomics nomogram in predicting high-grade EC compared with those of D&C. Finally, the clinical impact curve (CIC) was used to analyze the loss-benefit ratio of the nomogram and preoperative D&C compared with the actual postoperative pathological results of each patient under different probability thresholds.

Statistical analysis

The normality of all parameters was checked using the Shapiro–Wilk test. Quantitative data were analyzed using the *t*-test or Mann-Whitney *U* test, and qualitative data were analyzed using the chi-square test. Stepwise logistic regression was performed to establish models for predicting high-grade EC from the statistically significant variables. The predictive performance indicators obtained in the training and validation sets included receiver operating characteristic (ROC) curves and correlation areas under the curve (AUCs). The prediction efficiency of the models was compared using the Delong' test. $P< 0.05$ indicates statistical significance. Statistical analysis of all data was conducted using the R software (v4.2.0, https://www.R-project.org). The "Irr" package was used for ICC analysis. The "mRMRe" package and "glmnet" package were used for screening and dimensionality reduction of image features. The "rms" package was required to obtain nomogram and calibration curve. The analysis of DCA required the installation of "rmda" package. Finally, NRI and IDI were calculated using "predidicABEL" package.

Results

Clinical features and model construction

The clinical and pathological features of the 143 patients were balanced between the training and validation sets, and the difference between the two sets was not statistically significant (Table 1). The pathological grade was high-grade EC in of 35/143 patients (24.5%) and low-grade EC in 108/143 patients (75.5%). Univariate *t*-test analysis showed that age, HE4, DMI (MR_DMI), CSI (MR_CSI), and LNM (MR_LNM) on MRI reports were significantly different between high-grade and low-grade ECs, but no statistically significant association between maximum tumor diameter and CA125 and tumor grade was found (Supplementary Table S3A). Univariate and multivariate logistic regression analyses indicated that age, MR_DMI, MR_CSI, and MR_LNM were independent risk factors for high-grade EC.

Discordance between the preoperative D&C and final pathological results was observed in 34/143 patients (23.8%). The tumor grade in 24/143 patients (16.8%) was underestimated, of which 10/24 patients (41.7%) with preoperative grade 1/2 were found to be grade 3, and 14/24 patients (58.3%) with preoperative grade 1 were

TABLE 1 Patient characteristics.

Characteristics	Training set (n100)	Validation set (n43)	*P* value
Age (y)	55.5±10.5	55.5±10.4	0.979
CA125	45.8±57.4	31.5±31.7	0.128
HE4	107.6±94.8	109.1±90.4	0.933
Tumor size	50.4±20.0	53.5±35.9	0.516
MR_DMI			0.692
Absent	64 (64.0%)	29 (67.4%)	
Present	36 (36.0%)	14 (32.6%)	
MR_CSI			0.435
Absent	78 (78.0%)	36 (83.7%)	
Present	22 (22.0%)	7 (16.3%)	
MR_LNM			0.219
Absent	87 (87.0%)	38 (88.4%)	
Present	13 (13.0%)	5 (11.6%)	
FIGO stage			0.277
IA	48 (48.0%)	27 (62.8%)	
IB	12 (12.0%)	6 (14.0%)	
II	10 (10.0%)	5 (11.6%)	
IIIA	7 (7.0%)	1 (2.3%)	
IIIB	2 (2.0%)	1 (2.3%)	
IIIC	18 (18.0%)	2 (4.7%)	
IVB	3 (3.0%)	1 (2.3%)	
Histopathology DMI			0.202
Absent	66 (66.0%)	33 (76.7%)	
Present	34 (34.0%)	10 (23.3%)	
Histopathology CSI			0.033
Absent	72 (72.0%)	38 (88.4%)	
Present	28 (28.0%)	5 (11.6%)	
Histopathology LNM			0.468
Absent	80 (80.0%)	38 (88.4%)	
Present	20 (20.0%)	5 (11.6%)	

FIGO, Federation of International of Gynecologists and Obstetricians; HE4, human epididymis protein 4; MR_DMI, MRI-reported deep myometrium invasion; MR_CSI, MRI-reported cervical stromal invasion; MR_LNM, MR-reported lymph node metastasis

found to be grade 2. In contrast, the pathological grade was overestimated in 10/143 patients (7.0%), including 3/10 grade 1/grade 2 patients (30.0%) who were preoperatively diagnosed with grade 3 or non-endometrioid cancer and 7/10 grade 1 patients (70.0%) who were preoperatively diagnosed with grade 2.

Two radiologists at our institution retrospectively analyzed the MR images of the patients with EC. The sensitivity for the diagnosis of DMI, CSI, and LNM was 86.4, 66.67, and 40.0%, respectively, and the specificity for the diagnosis of DMI, CSI, and LNM was 89.9, 93.64, and 95.4%, respectively.

Radiomics features selection and radiomics score development

After ICC analysis, 2,225 features (739 T2WI features, 736 DWI features, and 750 DCE-T1WI features) were retained. The mRMR algorithm was used to screen out the 80 features most related to high-grade EC, and then LASSO regression was used to avoid radiomics feature overfitting, taking λ as the minimum value (Supplementary Figure S1A). Finally, 11 features with nonzero coefficients were retained to construct the rad-score (Figure 3). The formula for

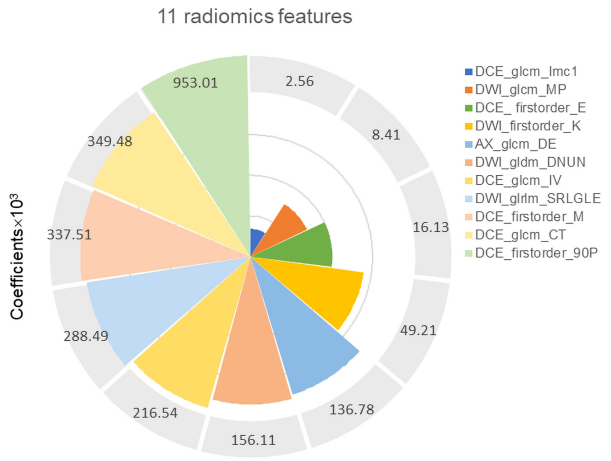

FIGURE 3
Eleven robust radiomics features and corresponding coefficients for rad-score construction.

calculating the rad-score is as follows:

$$Rad-score = -1.1563 - 0.2885 * M1 + 0.0161 * M2 - 0.9530 * M3 + 0.0026 * M4 - 0.2165 * M5 + 0.3375 * M6 + 0.1561 * M7 + 0.1368 * M8 + 0.0492 * M9 + 0.3495 * M10 - 0.0084 * M11.$$

M1 = DCE_wavelet.LLH_firstorder_Entropy;
M2 = DWI_wavelet.LHH_glrlm_ShortRunLowGrayLevelEmphasis;
M3 = DCE_wavelet.LLH_firstorder_90Percentile;
M4 = DCE_wavelet.HLH_glcm_IMC1;
M5 = DCE_wavelet.LHL_glcm_InverseVariance;
M6 = DCE_original_firstorder_Maximum;
M7 = DWI_wavelet.HHH_gldm_DependenceNonUniformityNormalized;
M8 = AX_wavelet.HHH_glcm_DifferenceEntropy;
M9 = DWI_wavelet.HHH_firstorder_Kurtosis;
M10 = DCE_wavelet.LLH_glcm_ClusterTendency;
M11 = DWI_wavelet.HH_Hglcm_MaximumProbability.

Prediction performance and validation of radiomics nomogram

The radiomics nomogram was established using logistic regression by combining the above four clinical and MRI factors (age, MR_DMI, MR_CSI, and MR_LNM) with the rad-score (Table 2), which was visualized by the nomogram in Figure 4. The AUCs of the clinical model, rad-score, and radiomics nomogram were 0.837 (95% confidence interval [CI]: 0.754–0.920), 0.875 (95% CI: 0.797–0.952) and 0.923 (95% CI: 0.869–0.977) in the training set, and 0.857 (95% CI: 0.741–0.973), 0.786 (95% CI: 0.592–0.979), and 0.914 (95% CI: 0.827–0.998) in the validation set. The prediction performance of the three models is shown in Table 3, with the ROC curves shown in Figures 5A, B.

The radiomics nomogram yielded the best prediction performance for both sets. The calibration curves are shown in Figures 5C, D, indicating that the nomogram prediction results were in good agreement with the pathological grade of EC in the training and validation sets (p = 0.551 and 0.998, respectively). Delong's test demonstrated that the difference between the nomogram and clinical model was statistically significant in the training and validation sets (p = 0.019 and 0.031, respectively). However, the difference between the rad-score and clinical model was not statistically significant (all $p > 0.05$).

Clinical practicability

The DCA of the three models showed that the developed radiomics nomogram had a higher net benefit than the rad-score and clinical model at most threshold probabilities in the training (Figure 6A) and validation sets (Supplementary Figure S2A), and a higher net benefit than the actual D&C at threshold probabilities of 0–0.46 and greater than 0.67. The CIC showed the loss-benefit ratio obtained by the radiomics nomogram and D&C at different probability thresholds (Figures 6B, C). The reclassification measures of discrimination indicated that, compared with those of D&C, the NRIs of the radiomics nomogram were 0.637 (95% CI: 0.214–1.061, p = 0.003) and 0.657 (95% CI: 0.079–1.394, p = 0.05), and IDIs of radiomics nomogram were 0.115 (95% CI: 0.077–

TABLE 2 Univariable and multivariable Logistic regression analyses results for high-grade EC.

Characteristics	Univariable analysis		Multivariable analysis	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age	1.084 (1.037, 1.138)	0.001	1.090 (1.012, 1.186)	0.028
HE4	1.049 (1.029, 1.117)	0.014	1.008 (1.001, 1.018)	0.060
MI_MR	6.573 (2.559, 17.874)	0.001	5.268 (1.323, 24.498)	0.023
MR_ CSI	6.500 (2.303, 19.426)	0.001	6.547 (1.287, 39.904)	0.028
MR_LNM	8.625 (2.511, 34.918)	0.001	7.847 (2.106, 35.293)	0.012
Radiomics score	11.031 (4.280, 35.601)	0.001	9.237 (2.723, 43.079)	0.001

OR, odds ratio; CI, confidence interval; EC, endometrial cancer.

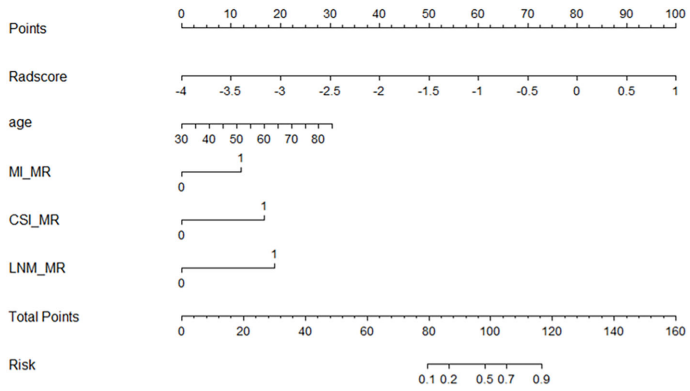

FIGURE 4
Nomogram for predicting the tumor grade of endometrial cancer, established based on multiparameter magnetic resonance imaging and patient age.

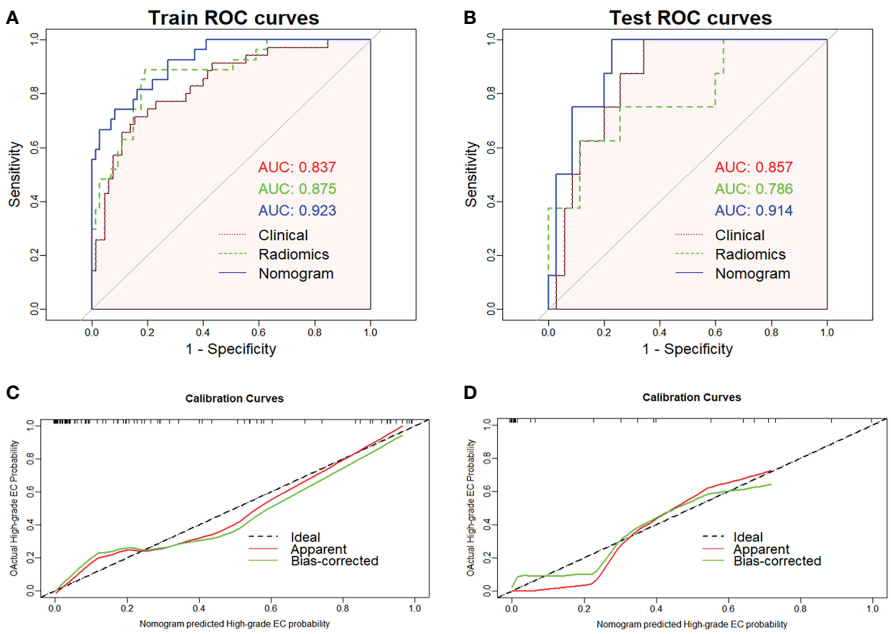

FIGURE 5
Receiver operating characteristic curves of the three models predicting high-grade endometrial cancer in the training **(A)** and validation sets **(B)**. The graphs **(C)** and **(D)** show that the calibration curve of nomogram has good calibration ability in both the training and validation sets, respectively.

0.306, p = 0.241) and 0.053 (95% CI: 0.027–0.357, p = 0.788) in the training set and validation set, respectively.

Discussion

In this study, we developed a radiomics nomogram based on MRI radiomics features for noninvasive preoperative prediction of tumor grade in EC. The radiomics nomogram can improve the accuracy of distinguishing high-grade EC before surgery, and DCA showed that the nomogram has clinical practicability in assessing preoperative risk stratification of EC. Because the required parameters are easy to obtain, the nomogram is expected to be a powerful tool for gynecologists to develop individualized treatments.

Predictive value of clinical model for high-grade EC

Two radiologists retrospectively analyzed the MRI images of each patient, and the sensitivity and specificity of the diagnosis of DMI, CSI, and LNM were consistent with those of previous studies (19–21). Many studies (5, 22–24) have confirmed that patient age, DMI, CSI, and LNM are important prognostic factors in high-risk patients with EC. Our study indicated that advanced age, MRI-reported DMI, CSI,

TABLE 3 Predictive performance of the clinical model, radiomics score, and radiomics nomogram for high-grade endometrial cancer.

Cohort	Models	AUC (95%CI)	ACC	SEN	SPE	NPV	PPV
Training set	Clinical model	0.837 (0.754, 0.920)	0.801	0.714	0.846	0.846	0.714
	Radiomics score	0.875 (0.797, 0.952)	0.830	0.889	0.808	0.952	0.632
	Radiomics nomogram	0.923 (0.869, 0.977)	0.877	0.741	0.918	0.905	0.769
Validation set	Clinical model	0.857 (0.741, 0.973)	0.721	1.000	0.657	1.000	0.400
	Radiomics score	0.786 (0.592, 0.979)	0.837	0.625	0.886	0.912	0.556
	Radiomics nomogram	0.914 (0.827, 0.998)	0.839	1.000	0.771	1.000	0.500

AUC, area under curve; ACC, accuracy; SEN, sensitivity; SPE, specificity; PPV, positive predictive value, NPV, negative predictive value.

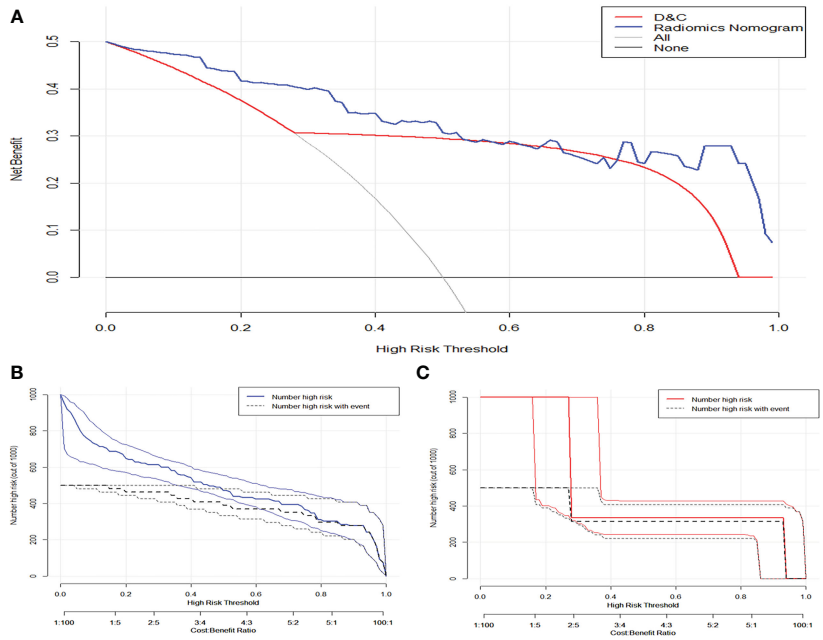

FIGURE 6
(A) The clinical decision curve demonstrated that nomogram has higher net benefits than preoperative curettage at a threshold probability of 0–0.47 and > 0.67. The solid blue and orange lines in figures **(B)** and **(C)** represent the clinical impact curves of the nomogram and the actual preoperative DC, respectively. The black dashed line represents the postoperative pathological results of patients with endometrial cancer, and the closer the solid line is to the black dashed line, the better the prediction effect.

and LNM were independent risk factors for high-grade EC. We found that the serum HE4 level of high-grade EC was significantly higher than that of low-grade EC. Although serum HE4 level was not an independent predictor of high-grade EC in this study, serum HE4 was connected with the prognostic factors of tumor grade, FIGO stage, and LNM in EC (25). For gynecologists, preoperative serum HE4 levels are of great clinical value for assessing EC risk stratification. In addition, CA125 was not significantly different between low-grade and high-grade EC, which is inconsistent with the findings of Zheng et al. (26). Serum CA125 is closely related to extrauterine invasion and LNM (20, 27). Therefore, we speculate that this may be caused by different pathological features, such as the FIGO stages. In addition, the mean maximum tumor diameter in the three sequences was not related to tumor grade. Although the clinical model combined conventional MRI features with patient age, ROC and DCA analyses revealed that it had limited usefulness in predicting the pathological grade of EC.

Predictive value of rad-score for high-grade EC

Radiomics can extract massive features from MRI images, which can effectively solve the problem of tumor heterogeneity that is difficult to quantitatively evaluate (28). In this study, we screened 11 radiomics features that were strongly correlated with tumor grade to construct the rad-score. Among them, the DCE sequence extracted more radiomics features (7/11) than the other two sequences, suggesting that DCE-MRI could provide more tumor information using a contrast agent. The higher the grade of the tumor, the greater the angiogenesis and vascular permeability, which makes the necrotic cystic changes of the tissue more clearly displayed (29). In addition, among all types of radiomics features, high-dimensional abstract wavelet features accounted for the largest proportion, which indicates that wavelet signs can capture clinical information that is not easily perceived visually and can better reflect tumor

heterogeneity. Therefore, radiomics can play a significant role in predicting prognostic factors of EC in the future.

Radiomics nomogram further improved the accuracy of prediction

The radiomics-based nomogram included patient age, MR_DMI, MR_CSI, MR_LNM, and rad-score. Compared with that of the radiomics score and clinical model, the nomogram had improved accuracy, better predictive performance, and higher net benefit. Bereby-Kahane et al. (30) suggested that texture features based on two-dimensional MRI were of limited value in predicting high-grade endometrial adenocarcinoma, with a sensitivity of 52% and a specificity of 75%. A recent study (26) developed a radiomics nomogram based on radiomics features, CA125, and body mass index, with a sensitivity of 88.8% and specificity of 81.5% for predicting high-grade EC. The prediction performance was higher than that of the previous study, but the specificity was lower than that of our study. Unfortunately, only shape features, first-order features, and partial texture features were covered in their study. In our study, the radiomics nomogram not only included conventional MRI features assessed by two radiologists but also feature extraction from multiple sequences (T2WI, DWI, and DCE-MRI), which can provide a practical clinical tool for preoperative risk stratification of EC.

The nomogram had great potential compared with D&C in predicting tumor grade

Although almost all patients underwent D&C or endometrial biopsy before surgery, the accuracy of preoperative pathological grading evaluation was uneven due to limited tumor tissue samples, tumor heterogeneity, and operator experience. A previous meta-analysis showed a 67% (95% CI: 0.60–0.75) agreement rate between preoperative endometrial sampling and final histopathology, with 21% of tumor grades underestimated and 25% of tumor grades overestimated (31). A recent review (6) obtained similar results and concluded that preoperative EC sampling is not always the best predictor of the final pathological grade of EC. In this study, we found that the concordance rate between D&C and final pathological diagnosis was approximately 76.2%, 16.8% of the tumor grade was upgraded and approximately 41.7% of the patients with these upgrades were upgraded from low-grade to high-grade, which was not different from the results of previous studies. However, inadequate grading may lead gynecologists to incorrectly assess the risk of LNM and select suboptimal treatment plans (6). In theory, radiomics can noninvasively obtain information about tumors and predict tumor heterogeneity and aggressiveness. Therefore, we compared the radiomics nomogram with the curettage results, and DCA reported that the radiomics nomogram can get higher net benefit. In addition, the NRI showed that the discrimination ability of the radiomics nomogram was significantly improved compared with that of D&C in the training and validation sets. Considering that the NRI measures the improvement of a certain threshold and cannot evaluate the overall improvement of the model, we recalculated the IDI. The IDI indicated that about five to 11 patients would benefit from the prediction of radiomics nomogram. In general, we believe that the radiomics nomogram has advantages over preoperative D&C in differentiating low-grade EC from high-grade EC.

With the rapid development of radiomics technology, a more precise and accurate quantitative assessment of lesions and radiomics has the advantages of being noninvasive and reproducible. We believe that radiomics will become a safer and more reliable clinical tool for predicting tumor grade and evaluating EC prognosis in the future.

Our study had some limitations. First, this retrospective study only included patients who met the inclusion and exclusion criteria, which might have resulted in selection bias. Second, all enrolled patients underwent diagnostic curettage before the MRI scan, which may cause the tumor volume seen on MRI to be smaller than the actual size, and the evaluation of tumor grade by the maximum diameter of the tumor in this study will be disturbed. Third, different field strengths and machine types may cause image heterogeneity. Therefore, we resampled and normalized the images and standardized the extracted features to reduce differences. Finally, this was a single-center small sample study, it cannot be denied that there may be an imbalance in the distribution of pathological features in the validation set. Therefore, a larger sample size and external validation are needed to verify the robustness and reproducibility of the radiomics nomogram.

In conclusion, we developed a radiomics nomogram based on MRI radiomics and clinical data that has good diagnostic performance for identifying high- and low-grade EC. The nomogram had a good net clinical benefit compared with that of D&C and provided an effective noninvasive tool for gynecologists to assess EC risk stratification before surgery.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by The First Affiliated Hospital of Shihezi University School of Medicine. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

XY: Conceptualization, Data curation, Methodology, Writing–original draft. XH: Data curation, Software, Methodology, Formal analysis. SH: Data curation, Investigation. JW: Data curation, Investigation. Formal analysis. WF: Software, Supervision. HZ: Data curation. CW: Conceptualization, Supervision, Writing-review &editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1081134/full#supplementary-material

SUPPLEMENTARY FIGURE 1
(A) Radiomics features were selected by least absolute shrinkage and selection operator (LASSO) logistic regression model in the training set. **(B)** The penalty parameter log (λ) was selected using 10-fold cross-validation through the minimum criterion, with the dashed line on the left representing the minimum log (λ) and the dashed line on the right representing log (λ) one standard error from the minimum.

SUPPLEMENTARY FIGURE 2
The clinical decision curves of three models for predicting high-grade endometrial cancer in the training **(A)** and validation sets **(B)**.

References

1. Koskas M, Amant F, Mirza M, Creutzberg C. Cancer of the corpus uteri: 2021 update. *Int J Gynecol Obstet: Off Organ Int Fed Gynaecology Obstetrics* (2021); 155 (Suppl. S1): 45–60. doi: 10.1002/ijgo.13866

2. Fitzmaurice C, Akinyemiju TF, Al Lami FH, Alam T, Alizadeh-Navaei R, Allen C, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016: A systematic analysis for the global burden of disease study. *JAMA Oncol* (2018) 4 (11):1553–68. doi: 10.1001/jamaoncol.2018.2706

3. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer Off J Int Gynecological Cancer Soc* (2021) 31(1):12–39. doi: 10.1136/ijgc-2020-002230

4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA: Cancer J Clin* (2019) 69 (1):7–34. doi: 10.3322/caac.21551

5. Bogani G, Dowdy S, Cliby W, Ghezzi F, Rossetti D, Frigerio L, et al. Management of endometrial cancer: issues and controversies. *Eur J Gynaecological Oncol* (2016) 37(1):6–12.

6. Lukanović D, Matjašič M, Kobal B. Accuracy of preoperative sampling diagnosis for predicting final pathology in patients with endometrial carcinoma: A review. *Trans Cancer Res* (2020) 9(12):7785–96. doi: 10.21037/tcr-20-2228

7. Koh W, Abu-Rustum N, Bean S, Bradley K, Campos S, Cho K, et al. Uterine neoplasms, version 1.2018, NCCN clinical practice guidelines in oncology. *J Natl Compr Cancer Netw* (2018) 16(2):170–99. doi: 10.6004/jnccn.2018.0006

8. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al. ESMO-ESGO-ESTRO consensus conference on endometrial cancer: diagnosis, treatment and follow-up. *Ann Oncol Off J Eur Soc Med Oncol* (2016) 27(1):16–41. doi: 10.1093/annonc/mdv484

9. Bhardwaj V, Sharma A, Parambath S, Gul I, Zhang X, Lobie P, et al. Machine learning for endometrial cancer prediction and prognostication. *Front Oncol* (2022) 12:852746. doi: 10.3389/fonc.2022.852746

10. Reyes-Pérez JA, Villaseñor-Navarro Y, Jiménez de Los Santos ME, Pacheco-Bravo I, Calle-Loja M, Sollozo-Dupont I. The apparent diffusion coefficient (ADC) on 3-T MRI differentiates myometrial invasion depth and histological grade in patients with endometrial cancer. *Acta Radiologica (Stockholm Sweden 1987)* (2020) 61(9):1277–86. doi: 10.1177/0284185119898658

11. Kakkar C, Gupta K, Jain K, Narang V, Singh A, Saggar K, et al. Diagnostic accuracy of calculated tumor volumes and apparent diffusion coefficient values in predicting endometrial cancer grade. *Int J Appl Basic Med Res* (2022) 12(1):37–42. doi: 10.4103/ijabmr.ijabmr_553_21

12. Kumar V, Gu Y, Basu S, Berglund A, Eschrich SA, Schabath MB, et al. Radiomics: the process and the challenges. *Magnetic resonance Imaging* (2012) 30(9):1234–48. doi: 10.1016/j.mri.2012.06.010

13. Kickingereder P, Burth S, Wick A, Götz M, Eidel O, Schlemmer HP, et al. Radiomic profiling of glioblastoma: Identifying an imaging predictor of patient survival with improved performance over established clinical and radiologic risk models. *Radiology* (2016) 280(3):880–9. doi: 10.1148/radiol.2016160845

14. Lee SH, Park H, Ko ES. Radiomics in breast imaging from techniques to clinical applications: A review. *Korean J Radiol* (2020) 21(7):779–92. doi: 10.3348/kjr.2019.0855

15. Rodríguez-Ortega A, Alegre A, Lago V, Carot-Sierra JM, Ten-Esteve A, Montoliu G, et al. Machine learning-based integration of prognostic magnetic resonance imaging biomarkers for myometrial invasion stratification in endometrial cancer. *J Magnetic Resonance Imag JMRI* (2021) 54(3):987–95. doi: 10.1002/jmri.27625

16. Long L, Sun J, Jiang L, Hu Y, Li L, Tan Y, et al. MRI-Based traditional radiomics and computer-vision nomogram for predicting lymphovascular space invasion in endometrial carcinoma. *Diagn Interventional Imag* (2021) 102(7-8):455–62. doi: 10.1016/j.diii.2021.02.008

17. Liu XF, Yan BC, Li Y, Ma FH, Qiang JW. Radiomics nomogram in assisting lymphadenectomy decisions by predicting lymph node metastasis in early-stage endometrial cancer. *Front Oncol* (2022) 12:894918. doi: 10.3389/fonc.2022.894918

18. Amant F, Mirza MR, Koskas M, Creutzberg CL. Cancer of the corpus uteri. *Int J Gynaecol Obstet* (2018) 143 Suppl 2:37–50. doi: 10.1002/ijgo.12612

19. Jose T, Singh A, Vardhan S. Pre-surgical staging in endometrial cancer: An opportunity for risk stratification and triage? *Med J Armed Forces India* (2021) 77 (2):205–13. doi: 10.1016/j.mjafi.2020.09.009

20. Zamani N, Modares Gilani M, Zamani F, Zamani MH. Utility of pelvic MRI and tumor markers HE4 and CA125 to predict depth of myometrial invasion and cervical involvement in endometrial cancer. *J Family Reprod Health* (2015) 9(4):177–83.

21. Teng F, Zhang YF, Wang YM, Yu J, Lang X, Tian WY, et al. Contrast-enhanced MRI in preoperative assessment of myometrial and cervical invasion, and lymph node metastasis: Diagnostic value and error analysis in endometrial carcinoma. *Acta Obstet Gynecol Scand* (2015) 94(3):266–73. doi: 10.1111/aogs.12570

22. Wang Y, Bi Q, Deng Y, Yang Z, Song Y, Wu Y, et al. Development and validation of an MRI-based radiomics nomogram for assessing deep myometrial invasion in early stage endometrial adenocarcinoma. *Acad Radiol* (2022), S1076-6332(22)00320-8. doi: 10.1016/j.acra.2022.05.017

23. Yan BC, Li Y, Ma FH, Feng F, Sun MH, Lin GW, et al. Preoperative assessment for high-risk endometrial cancer by developing an MRI- and clinical-based radiomics nomogram: A multicenter study. *J Magnetic Resonance Imag JMRI* (2020) 52(6):1872–82. doi: 10.1002/jmri.27289

24. Kim SI, Kim JW. Endometrial cancer. *N Engl J Med* (2021) 384(6):586. doi: 10.1056/NEJMc2035378

25. Degez M, Caillon H, Chauviré-Drouard A, Leroy M, Lair D, Winer N, et al. Endometrial cancer: A systematic review of HE4, REM and REM-b. *Clinica Chimica Acta; Int J Clin Chem* (2021) 515:27–36. doi: 10.1016/j.cca.2020.12.029

26. Zheng T, Yang L, Du J, Dong Y, Wu S, Shi Q, et al. Combination analysis of a radiomics-based predictive model with clinical indicators for the preoperative assessment of histological grade in endometrial carcinoma. *Front Oncol* (2021) 11:582495. doi: 10.3389/fonc.2021.582495

27. Panyavaranant P, Manchana T. Preoperative markers for the prediction of high-risk features in endometrial cancer. *World J Clin Oncol* (2020) 11(6):378–88. doi: 10.5306/wjco.v11.i6.378

28. Sala E, Mema E, Himoto Y, Veeraraghavan H, Brenton JD, Snyder A, et al. Unravelling tumour heterogeneity using next-generation imaging: radiomics, radiogenomics, and habitat imaging. *Clin Radiol* (2017) 72(1):3–10. doi: 10.1016/j.crad.2016.09.013

29. Aerts HJ, Velazquez ER, Leijenaar RT, Parmar C, Grossmann P, Carvalho S, et al. Decoding tumour phenotype by noninvasive imaging using a quantitative radiomics approach. *Nat Commun* (2014) 5:4006. doi: 10.1038/ncomms5006

30. Bereby-Kahane M, Dautry R, Matzner-Lober E, Cornelis F, Sebbag-Sfez D, Place V, et al. Prediction of tumor grade and lymphovascular space invasion in endometrial adenocarcinoma with MR imaging-based radiomic analysis. *Diagn Interventional Imag* (2020) 101(6):401–11. doi: 10.1016/j.diii.2020.01.003

31. Visser N, Reijnen C, Massuger L, Nagtegaal I, Bulten J, Pijnenborg J. Accuracy of endometrial sampling in endometrial carcinoma: A systematic review and meta-analysis. *Obstetrics Gynecol* (2017) 130(4):803–13. doi: 10.1097/aog.0000000000002261

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EDITED BY
Andrzej Semczuk,
Medical University of Lublin, Poland

REVIEWED BY
Alexey Koval,
University of Geneva, Switzerland
Rakesh Kumar,
Shri Mata Vaishno Devi University, India

*CORRESPONDENCE
Jure Knez
jure.knez@ukc-mb.si

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The association of Wnt-signalling and EMT markers with clinical characteristics in women with endometrial cancer

Živa Ledinek[1], Monika Sobočan[2,3], Damjan Sisinger[1], Marko Hojnik[1], Tomaž Büdefeld[4], Uroš Potočnik[4,5,6] and Jure Knez[2,3]*

[1]Department of Pathology, University Medical Centre Maribor, Maribor, Slovenia, [2]Divison for Gynaecology and Perinatology, University Medical Centre Maribor, Maribor, Slovenia, [3]Department of Gynaecology and Obstetrics, Faculty of Medicine University of Maribor, Maribor, Slovenia, [4]Center for Human Molecular Genetics and Pharmacogenomics, Faculty of Medicine, University of Maribor, Maribor, Slovenia, [5]Laboratory for Biochemistry, Molecular Biology and Genomics, University of Maribor, Maribor, Slovenia, [6]Department for Science and Research, University Medical Centre Maribor, Maribor, Slovenia

Endometrial cancer is the most common gynecologic malignancy in the developed world. Risk stratification and treatment approaches are changing due to better understanding of tumor biology. Upregulated Wnt signaling plays an important role in cancer initiation and progression with promising potential for development of specific Wnt inhibitor therapy. One of the ways in which Wnt signaling contributes to progression of cancer, is by activating epithelial-to-mesenchymal transition (EMT) in tumor cells, causing the expression of mesenchymal markers, and enabling tumor cells to dissociate and migrate. This study analyzed the expression of Wnt signaling and EMT markers in endometrial cancer. Wnt signaling and EMT markers were significantly correlated with hormone receptors status in EC, but not with other clinico-pathological characteristics. Expression of Wnt antagonist, Dkk1 was significantly different between the ESGO-ESTRO-ESP patient risk assessment categories using integrated molecular risk assessment.

KEYWORDS

Wnt pathway, EMT - epithelial to mesenchymal transformation, endometrial cancer (EC), β-catenin (B-catenin), DKK1, E-cadherin, N-cadherin

1 Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in the developed world. With average overall 5-year survival rate of 76% and over 90% in early-stage disease, the number of estimated deaths in 2020 still exceeded 97,000 (1–3). Prognosis of patients with EC depends on pathomorphological as well as molecular characteristics of tumors, the latter becoming an integral part of the latest World Health

Organization (WHO) Classification of Female Genital Tumors (2, 4, 5). Currently, four molecular subtypes of EC have been proposed based on genetic characteristics of tumors: (i) POLE (DNA Polymerase Epsilon) ultra-mutated tumors, (ii) mismatch repair-deficient (MMRd) tumors, (iii) p53-mutant tumors (p53abn), and (iv) tumors of no specific molecular profile (NSMP) (5). Molecular classification of EC has offered new insight in the process of carcinogenesis and progression of EC and it has provided new potential targets for treatment and different prognostic subgroups of patients (6–8).

One of important mechanisms that has been linked to tumorigenesis as well as progression of EC is dysfunction of Wnt/β-catenin signaling pathway (9–11). The canonical Wnt/β-catenin signaling pathway is activated by binding of Wnt ligands to heterodimers of Frizzled (FZD) receptors and lipoprotein receptor-related protein (LRP) co-receptors on the surface of the cell. This leads to inactivation of β-catenin destruction complex in the cytoplasm, enabling β-catenin to be transferred to the nucleus, where it forms a complex with the lymphoid enhancer factor (LEF) and T-cell factor (TCF), leading to transcription of cell cycle regulator genes (11, 12). Mutations of catenin beta-1 (*CTNNB1*) gene, occurring in approximately 20-25% of ECs, present an alternative way of activating Wnt/β-catenin signaling pathway through inefficient destruction of β-catenin. Clinically relevant mutations in exon 3 of *CTNNB1* gene prevent phosphorylation and ubiquitination of the protein hence having the same result as binding of Wnt ligands (11, 13–15). Mutations of *CTNNB1* are characteristic for NSMP molecular subtype of EC. Wnt signaling is tightly regulated by Wnt inhibitors, among them the group of Dickkopf (DKK) proteins. Among four members of DKK family proteins, DKK1 – competitive inhibitor against Wnt3a is a prototypical Wnt antagonist and the most extensively studied DKK protein (16). Dysregulation of DKK1 has recently emerged as a potential biomarker of cancer progression and prognosis for several types of malignancies. Its overexpression in endometrial cancer suggests a negative feedback loop between DKK1 expression and Wnt signaling activation (17, 18). Wnt signaling is also regulated by steroid sex hormones. Estrogens and progesterone maintain a dynamic balance between the proliferation and differentiation of endometrium, that is essential for the prevention of abnormal endometrial growth that rises the risk of developing EC (19). Expression of β-catenin was found to be positively correlated with the expression of ER receptors in EC, suggesting a synergy between estrogens and Wnt signaling (10). On the other hand, intact progesterone signaling has a potential to inhibit Wnt signaling by induction of Wnt inhibitors, such as forkhead box 1 protein (FOXO1) (20, 21). Apart from rather well-established role of estrogens and progesterone in the carcinogenesis of EC, androgen receptor (AR) expression also contributes to the progression and prognosis of the disease and may be connected to the Wnt signaling. AR expression in EC is more commonly present in primary tumors and is often lost in metastatic disease (22–24). Tangen et al. discovered a correlation between the loss of AR expression and more aggressive nature of EC and worse prognosis in EC patients (25). Although a correlation between the Wnt signaling and expression of AR has been studied in some cancers (26, 27), there are no studies in EC yet.

Another important mechanism of carcinogenesis and progression of EC is epithelial-to-mesenchymal transition (EMT). EMT leads to the loss of intracellular junctions and of apical-basal polarity in tumor cells as well as the reorganization of the cytoskeleton, increased motility of individual cells, and degradation of the extracellular matrix proteins (6). One of the hallmarks of EMT is the loss of epithelial surface markers, most notably E-cadherin and subsequently the expression of mesenchymal markers, such as N-cadherin and vimentin (28–30). The phenomenon of balanced downregulation of E-cadherin expression and N-cadherin overexpression is described as "cadherin switch" and is regarded as a marker of EMT (6, 30). Wnt signaling is needed for both, the initiation and the maintenance of the mesenchymal phenotype of tumor cells which is mainly achieved by activating the transcription of target genes, contributing to EMT process (31, 32) and is also connected to loss of E-cadherin expression, enabling translocation of β-catenin to participate in Wnt cascade (33). A connection between the Wnt signaling and EMT suggests that Wnt inhibitors, such as DKK proteins could prevent the EMT, making them potential therapeutical targets (18, 20, 34).

This research aims to evaluate the correlation of clinico-pathological and traditional molecular markers of EC with novel biomarkers implicated in more/less aggressive subtypes of EC. Through analyzing the interconnection of Wnt signaling markers and EMT markers the aim of this research is to elucidate the potential role of these novel candidates in prognosis of EC.

2 Material and methods

2.1 Patient selection and characteristics

This was a prospective cohort study, including all consecutive patients between January 2020 and March 2022 at the University Medical Centre Maribor. All recruited patients underwent surgical treatment of EC after a multidisciplinary tumor board evaluation. Patients' demographic data such as age at the time of diagnosis, BMI and clinical data were recorded. Exclusion criteria were treatment for benign or pre-cancerous conditions or if there was no available tissue for additional IHC staining.

2.2 Molecular analysis

Molecular analysis was performed as previously described using the integrated molecular characterization approach (35). Mutational status of *CTNNB1* gene was determined by Sanger sequencing of exon 3 following the previously described methodology (35). Identification of clinically relevant (15) missense mutations of the following amino acids: D32, G34, S33, S37, T41, D207 and V516, the last one being a splice site variant.

2.3 Histopathological characteristics and immunohistochemistry

Standardized pathohistological assessment and additional immunohistochemical staining was performed on post-operative specimens with confirmed endometrial cancer. In 4% of cases (n=3) post-operative specimen showed only benign tissue. In these cases, additional immunohistochemical staining was performed on pre-operative biopsy samples. All samples were assessed at the Department of Pathology of the University Medical Centre Maribor. During routine clinical evaluation, samples are assessed for estrogen (ER), progesterone (PR) expression and morphological characteristics contributing to clinical decision-making as previously described in (35).

In addition to standardized pathology report, samples were selected for ancillary immunohistochemical staining. Paraffine embedded tissue blocks were selected and 4 µm thick slices of tumor tissue were transferred in sections to SuperFrost slides (Thermo Fisher Scientific). Immunostaining was done by standard method in an automatic stainer (BenchMark ULTRA, Ventana Medical Systems, Inc.). Immunostaining was performed for androgen receptors (AR) (rabbit monoclonal antibody, clone SP107, F. Hoffmann-La Roche Ltd., RTU), MMRd (MLH1, MSH2, PMS2, MSH6), p53, β-catenin (mouse monoclonal antibody; clone 17C2; Dako Cytomation Glostrup; at 1:10 dilution), E-cadherin (mouse monoclonal antibody; clone NCH-38; Dako Cytomation Glostrup; at 1:40 dilution), N-cadherin (mouse monoclonal antibody; clone 5D5; GeneTex; at 1:1000 dilution) and DKK1 (rabbit monoclonal antibody; clone SC03-86; Invitrogen – ThermoFisher; at 1:100 dilution). We used marker specific positive and negative controls on every slide. Standard tissue block no. 1 (liver, tonsil, and pancreatic tissue) was used as control for E-cadherin and β-catenin staining. To test and optimize the protocols for N-cadherin and Dkk1, tissue controls were chosen according to the manufacturer's recommendation – hepatocellular carcinoma, colorectal carcinoma, and placental tissue. Reactions in control tissue are shown in Supplementary Figure 1. N-cadherin had overall stronger immuno-positive reactions, compared to Dkk1. A research protocol for N-cadherin and Dkk1 staining was established for EC evaluation. Both markers are not used in routine clinical practice. Since E-cadherin and beta-catenin are validated and frequently used molecular markers in clinical practice, standard tissue blocks were used as controls. Overall reactions in control tissue were similar on every slide with almost no difference in staining intensity. To test and optimize the protocols for N-cadherin and Dkk1, tissue controls were chosen according to the manufacturer's recommendations. There were internal negative controls in the chosen tissue samples. Reaction of markers with tissue controls is shown in Supplementary Figure 1.

The sample evaluation was done independently by two pathology experts (DS and MH), who were blinded to each other's grades. If there was discrepancy between their grading, higher than 20%, samples were re-evaluated, and the experts settled a final score. Expression of all immunohistochemical markers (β-catenin, Dkk1, E-cadherin and N-cadherin) was evaluated by counting the number of immuno-positive tumor cells and expressed by percentage. Tumor cells were deemed positive if there was a clear membranous (β-catenin) (36, 37), membranous and/or cytoplasmic (E-cadherin, N-cadherin) (29, 38) and cytoplasmic (Dkk1) (10, 39, 40) reaction. At the same time, intensity of reaction was scored on the scale from 0 to 3 (0 = no reaction, 1 = mild, 2 = moderate, 3 = strong). Examples of scoring system is shown in Figure 1. Using the described parameters, we calculated the standard H score, as previously reported (39, 41). H score was calculated by multiplying the percentage of positive cells with staining intensity. Additionally, we recorded the β-catenin nuclear staining, that could be used as a surrogate for Wnt signaling, in tumor cells and categorized it as either focal or diffuse, based on the extent of nuclear reaction in tumor cells (42, 43).

Whole Slide Images (WSI) were taken using Aperio ScanScope CS under the same conditions. WSI were then exported as.jpeg format using Aperio Slide Manager software and were not edited, only cropped to the same size.

2.4 Statistical analysis

Descriptive analysis was used for numeric variables, using median (Me) and range. Absolute and relative frequencies were reported for categorical variables. Expression levels of molecular markers were all expressed as median value or either % of expression or median H-score. To assess the correlation between two numeric variables, namely the % of hormone receptor expression and H-score of other molecular marker expression, Spearman correlation coefficient was calculated, and scatter plot diagrams (Supplementary Material) were used to present the results. Correlations between numeric and categorical variables were evaluated using non-parametric tests, either Mann-Whitney U test or Kruskal-Wallis H-test. Results were presented by reporting U value when Mann-Whitney U test was used and H value when Kruskal-Wallis H test was used, along with the level of significance (p value). Statistical significance was set at $p<0.05$. Statistical analysis was performed using SPSS for Windows, Version 25.0.0 (IBM Corp., Armonk, NY, USA).

3 Results

3.1 Patient characteristics

Sixty-five women were included in this study. Their clinico-pathological characteristics are depicted in Table 1.

3.2 Correlation between expression of molecular markers

Expression of selected molecular markers (β-catenin, E-cadherin, N-cadherin and Dkk1) and hormone receptors (ER, PR and AR) in tumour tissue was evaluated and is presented in Figure 2 and Supplementary Table 1. Expression of ER was found to be positively correlated with expression of PR ($r(64) = 0.844$, $p<0.05$), AR ($r(55) = 0.597$, $p<0.05$), β-catenin ($r(64) = 0.065$, $p<0.05$), N-

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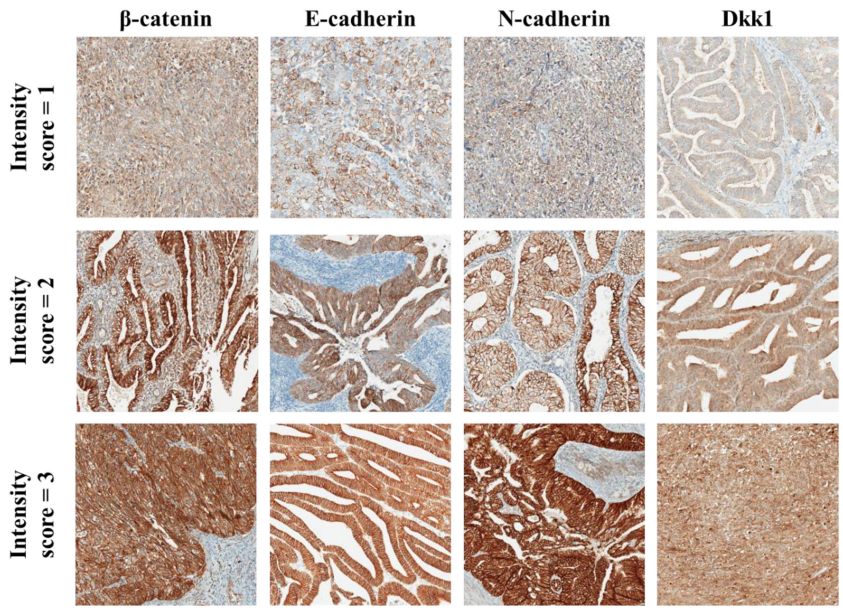

FIGURE 1
Examples of IHC staining for β-catenin, E-cadherin, N-cadherin, and Dkk1 in EC tumour cells. Staining interpretation was done by assessing staining intensity as weak (intensity score = 1), moderate (intensity score = 2) and strong (intensity score = 3). Intensity score was multiplied by the % of tumour cells with positive reaction and the result was recorded as H score (range from 0 to 300). All micrographs are taken at 100x magnification.

TABLE 1 Patient characteristics.

Median age at time of diagnosis (n=65)			69 years (41-87)	
Median Body Mass Index (BMI) (n=65)			31 17-43)	
			n (%)	CI 95%
Menopausal status	Pre-menopausal		6 (9%)	[4-10]
	Post-menopausal		59 (91%)	[82 - 96]
EC subtype	Type I		55 (85%)	[74 - 92]
	Type II		10 (15%)	[8 - 26]
EC grade	Low grade (G1-2)		50 (88%)	[77 - 94]
	High grade (G3)		7 (12%)	[6 - 23]
LVSI	absent		46 (71%)	[59 - 81]
	focal		2 (3%)	[1 - 10]
	diffuse		17 (26%)	[17 - 38]
Myometrial invasion	≤ 50%		32 (49%)	[37 - 61]
	> 50%		33 (51%)	[39 - 63]
FIGO stage	Stage I	IA	32 (49%)	[37 - 61]
		IB	16 (25%)	[15 - 36]
	Stage II		1 (2%)	[0 - 7]
	Stage III		13 (20%)	[12 -30]
	Stage IV		3 (5%)	[1 - 12]
Integrated molecular subgroup	POLEmut		4 (6%)	[2 - 14]

(Continued)

TABLE 1 Continued

	MMRd	23 (35%)	[25 - 47]
	NSMP among them CTNNB1mut	32 (49%) 4 (6%)	[37 - 61]
	p53abn	6 (9%)	[4 - 18]
ESGO-ESTRO-ESP patient risk assessment	low risk	29 (45%)	[33 - 57]
	intermediate risk	8 (12%)	[6 - 22]
	high-intermediate risk	4 (6%)	[2 - 14]
	high risk	21 (32%)	[21 - 44]
	advanced carcinoma	3 (5%)	[1 - 12]

EC, Endometrial cancer; LVSI, Lympho-vascular infiltration; FIGO stage, The International Federation of Gynecology and Obstetrics; ESGO-ESTRO-ESP, European Society for Gynaecologic Oncology - European Society Radiation Oncology – European Society for Pathology.

cadherin (r(64) = 0.280, p<0.05) and Dkk1 (r(64) = 0.263, p<0.05). Expression of PR was positively correlated with expression of ER, AR (r(55) = 0.554, p<0.05) and β-catenin (r(65) = 0.287, p<0.05). Expression of AR was positively correlated with expression of ER, PR, β-catenin (r(55) = 0,308, p<0.05) and N-cadherin (r(55) = 0.332, p<0.05). Expression of β-catenin was positively correlated with expression of ER, PR, AR, E-cadherin (r(65) = 0.345, p<0.05), N-cadherin (r(65) = 0.649, p<0.05) and Dkk1 (r(65) = 0.392, p<0.05). Expression of E-cadherin was positively correlated with expression of β-catenin and N-cadherin (r(65) = 0.452, p<0.05). Expression of N-cadherin was positively correlated with expression of ER, AR, β-catenin, E-cadherin and Dkk1 (r(65) = 0.365, p<0.05). Expression of Dkk1 was positively correlated with expression of ER, β-catenin and N-cadherin.

3.3 Expression correlation of β-catenin with EMT markers and hormone receptors in EC

Nuclear expression of β-catenin was found in 45 (69.2%; 95% CI [57.4%, 79.4%]) ECs. Pattern of expression was mostly focal with smaller groups of tumour cells showing positive nuclear reaction. Diffuse nuclear positivity was shown in 2 cases (3,1%).

We compared the membranous expression of β-catenin, cadherins and Wnt antagonist Dkk1 in tumour tissue as well as expression of hormone receptors (ER, PR and AR) against nuclear expression of β-catenin. Results are shown in Table 2. Expression of N-cadherin was higher for tumours with nuclear expression of β-catenin (Me [H-score] = 270) than for tumours without nuclear β-catenin expression (Me [H-score] = 253), U = 296.5, p < 0.05. Expression of Dkk1 was also found to be higher for tumours with nuclear expression of β-catenin (Me [H-score] = 115) than for tumours without nuclear β-catenin expression (Me [H-score] = 102), U = 270.5, p < 0.05. Expression of membranous β-catenin was found to be higher in tumours with nuclear β-catenin expression (Me [H-score] = 250), compared to tumours without nuclear β-catenin expression (Me [H-score] = 235), U = 254.5, p < 0.05. There was no statistically significant correlation between nuclear expression of β-catenin and the expression of E-cadherin. Expression of ER receptors was higher in tumours with nuclear expression of β-catenin (Me [%] = 100) compared to tumours without nuclear expression of β-catenin (Me [%] = 70), U = 183.0,

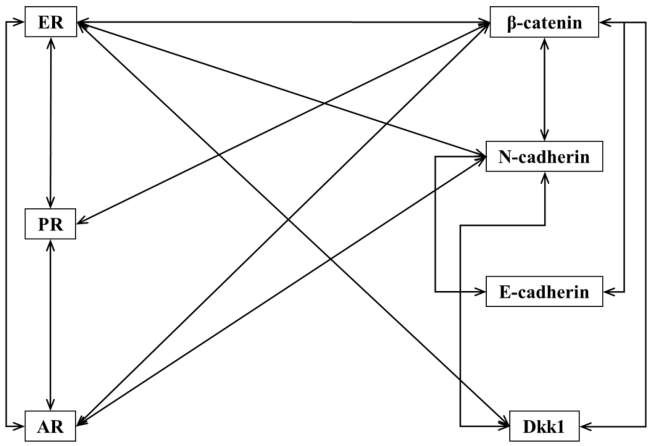

FIGURE 2
Correlations between expression of different molecular markers. Lines connect IHC markers with positive and statistically significant correlation in their expression in tumours cells.

TABLE 2 Correlations between expression of hormone receptors and molecular markers in tumours with and without nuclear expression of β-catenin.

Molecular marker	Nuclear expression of β-catenin		
	PRESENT (Median value)	ABSENT (Median value)	Mann-Whitney U-test
ER	100%	70%	U = 183.0; p < 0.05
PR	100%	45%	U = 180.0; p < 0.05
AR	30%	10%	U = 163.0; p < 0.05
β-catenin (membranous)	H score: 250	H score: 235	U = 254.5; p < 0.05
E-cadherin	H score: 223	H score: 223	U = 430.5; p = 0.782
N-cadherin	H score: 270	H score: 253	U = 296.5; p < 0.05
Dkk1	H score: 115	H score: 102	U = 270.5; p < 0.05

p< 0.05. The same was true for the expression of PR in tumours with nuclear expression of β-catenin (Me [%] = 100) versus without (Me [%] = 45), U = 180.0, p < 0.05 and for expression of AR in tumours with nuclear expression of β-catenin (Me [%] = 30) versus without (Me [%] = 10), U = 163.0, p < 0.05.

3.4 Correlation between expression of molecular markers and clinical characteristics

Expression of molecular markers (β-catenin, E-cadherin, N-cadherin and Dkk1) and hormone receptors (ER, PR and AR) was compared to clinical data and histopathological characteristics of the tumours, listed in Table 1. All results are shown in Supplementary Tables 2, 3.

Comparison between the ESGO-ESTRO-ESP patient risk assessment categories and expression of molecular markers showed significant correlations with the expression of Dkk1, H = 10.196, p < 0.05. The expression of Dkk1 was lower in low-risk (H-score = 105) and high-intermediate risk groups (H-score = 65), compared to intermediate-risk (H-score = 112) ang high-risk (H-score = 111) groups, but was highest in advanced carcinoma (H-score = 130). There was no significant change between expression of hormone receptors or other markers and patient risk groups.

There was no significant difference between expression of any of the molecular markers or hormone receptors and presence of LVSI, stage of the disease, myometrial invasion, FIGO stage or integrated molecular subgroups as shown in Supplementary Table 2. Higher expression of ER and PR was detected in Type I compared to Type II tumour and in low grade compared to high grade tumours.

4 Discussion

This prospective study shows that Wnt signalling is significantly involved in driving behaviour of endometrial cancer. Wnt signalling and expression of EMT markers in EC were significantly correlated with hormone receptors status in EC, but not with other clinico-pathological characteristics. Expression of Wnt antagonist, Dkk1 was significantly different among the ESGO-ESTRO-ESP patient risk assessment categories, being the highest in advanced carcinoma, lowest in high-intermediate risk group and approximately the same in low-risk, intermediate-risk, and high-risk groups.

The historical discrimination between type I and type II EC shows the influence of hormone status on pathogenesis and progression of EC. Recent studies have elucidated the impact of ER and PR expression on clinicopathological characteristics of EC, such as tumour invasiveness and FIGO stage. Loss of hormone receptor expression has been linked to worse prognosis and lower overall survival of patients with EC (44–47). Results of our study showed significant difference between the ER and PR expression, tumour type, and tumour grade, but not other clinicopathological characteristics. Most likely explanation why our results do not concur with previous studies is that our study compared a combined H-score with other tumour characteristics, whereas most of other studies used two-tier grading of hormone receptor status (positive or negative) and only set a specific cut-off value. Our approach has also been suggested to be more appropriate in clinical practise (48).

Important mechanism of EC carcinogenesis is Wnt signalling pathway. Its result is translocation of β-catenin into the cell nucleus, triggering target gene expression of cell cycle regulators. Nuclear β-catenin expression, determined by IHC, has been widely studied as a potential surrogate for Wnt signalling and *CTNNB1* gene mutations. Such mutations of exon 3 in *CTNNB1* gene occur in up to 20% of tumours, more often in low grade, early ECs (15). In our study 6% of women with EC had *CTNNB1* mutations, which is lower than expected (49, 50). *CTNNB1* mutational status in EC was not associated with any clinicopathological characteristics of the tumours, or with expression of hormone nor other molecular markers. However, it is possible to assess presence of Wnt signalling by IHC determination of β-catenin, regardless of mutational status of *CTNNB1* gene (36, 43).

Comparing Wnt signalling to expression of other markers in this study revealed positive correlation with the expression of all hormone receptors, membranous expression of β-catenin and

expression of N-cadherin as well as Wnt antagonist, Dkk1. Wnt signalling in normal endometrium is regulated also by the expression of Wnt antagonists, such as Dkk1. Our results showed higher expression of Dkk1 in tumours with nuclear β-catenin expression, suggesting negative feedback loop between Wnt signalling and Wnt antagonists, as has been proposed by previous studies (18, 51). So far Dkk1 expression was found to be higher in benign endometrial tissue, compared to EC and was also found to be higher in low grade EC compared to high grade EC (10, 40), supporting the theory of downregulation of Wnt antagonists' expression in EC (17, 52). Our results were in concordance with research done so far (10, 40), we showed lower expression of Dkk1 in high grade EC compared to low grade EC, but the difference was not statistically significant. We are among the first to compare expression of Dkk1 to molecular characteristics of EC, as well as integrated risk groups, based on new ESGO-ESTRO-ESP guidelines. Studies that have compared IHC expression of Dkk1 among different FIGO stages or histological grades of EC so far are scarce (10, 40, 53) and do not consider the potential influence of molecular classification. We did not find any significant correlation between molecular groups themselves, but we found the expression of Dkk1 to be significantly different between ESGO-ESTRO-ESP risk groups, being upregulated in advanced carcinoma. However, our results did not show linear increase in Dkk1 expression across the integrated risk groups, which could be a consequence of a small sample size. ESGO-ESTRO-ESP risk groups are based on a combination of pathohistological characteristics (histological type, tumour grade, LVSI), FIGO grade and molecular classification of EC (4). Since Dkk1 could be one of potential therapeutic targets (52, 54) further studies are needed to determine, whether there is a difference between expression of Dkk1 in tumour tissue and serum of the patient and how any of those would influence the potential use of therapeutics for EC.

Alterations in cellular adhesion molecules, are important mechanism of tumour progression and metastasis. Lower expression of membranous E-cadherin or complete loss of E-cadherin expression has been associated with higher FIGO grade, deep myometrial invasion, risk of tumour recurrence, and metastatic disease (36, 55, 56). Our study showed similar patterns of lower E-cadherin expression in tumours of Type II compared to Type I endometrial cancer, presence of LVSI and deeper myometrial invasion, but not in tumours of higher grade or higher FIGO stage. However, none of our results were statistically significant. Other authors have reported correlation between low expression of E-cadherin and higher expression of other cadherins, most importantly N-cadherin, marker of mesothelial differentiation and thus indicator of EMT (6, 38, 57). Our study, on the contrary, showed a positive correlation between expression of both cadherins, a phenomenon that has not yet been recognized. The phenomenon, called "cadherin switch" has been implicated, often described in other types of cancer, i.e. breast cancer or ovarian cancer (58, 59), but also in EC (29). We compared the expression of cadherins with nonparametric test, comparing the mean H-score value, like other studies (39, 41), since cut-off for defining positive or negative expression of cadherins has not been validated in any of the previous studies. In comparison to most other studies, we studied the average overall expression of cadherins in tumour tissue. We did not compare or distinguish between only membranous or cytoplasmic staining to take into an account a possible different intracellular location of the marker, also we did not compare the IHC reaction in centre of the tumour-to-tumour front, where differences have been most observed (6, 38). Our N-cadherin staining has been very strong overall, having a very high mean H-score, regardless of tumour type, stage, or grade.

There are different limitations of this study which are connected to the explorative nature of the methodology as well as the cohort itself. As previously discussed in cancers, where IHC receptor expression is important in therapeutic decision-making, cut-off values for predictive outcomes need to be validated in larger cohorts. While there has been advancement in our understanding of appropriate hormone receptor (ER, PR) cut-offs in EC, no such cut-offs are determined for EMT markers and Wnt markers in EC. IHC methods for assessment of molecular markers need broader criteria validation for assessment of EMT levels and Wnt marker cut-off values. Our explorative study has added to this understanding, but further evaluation is needed to test against specific cut-off values in subgroups of EC. Validation of potential biomarkers is an extensive process evaluating the rationale, mechanism and impact a certain molecule has on the process of carcinogenesis. Several recommendations suggest the use of archival samples and prospective samples as the first steps in the biomarker discovery process (60). These need to be followed or developed in parallel by translational validation using Western blot validation. This enables further protein identification and quantification and thus better understanding of the mechanism of action (61). Due to the limited resources, Western blot has not been performed in our study yet. Furthermore, in improving our mechanistic understanding of the topic, IHC is only the first step in Wnt signalling evaluation. Since we had very small group of tumours with *CTNNB1* mutations, we could not study the effects of alternative activation of Wnt pathway and its potential influence on EMT. Further studies are needed to address the different activation mechanisms of Wnt pathway and its connection to Wnt antagonists to evaluate the possible effects of guiding therapy for EC. Lastly, the results, due to its pilot nature, need to be cautiously evaluated due to a small number of cases reported. This cohort provides insight into the topic, yet larger subgroup analyses are needed to show utility for further translational understanding.

5 Conclusions

Our data indicates that Wnt signalling (nuclear β-catenin expression) in EC could be correlated to markers of EMT (N-cadherin), Wnt antagonist (Dkk1) and hormone receptors. Although this should be verified on larger population of EC patients, our data provides new insight into signalling pathways in EC. Correlation between expression of hormone receptors and other molecular markers affirms the connection between Wnt and EMT pathways in EC. Significant difference between expression of Dkk1 among ESGO-ESTRO-ESP patient risk assessment categories contributes to a better understanding of its role in EC with further implications for research of potential target immunotherapy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

Conceptualization: ŽL, MS, and JK. Methodology: ŽL and MS. Formal analysis: DS, MH, TB, MS, ŽL, and JK. Resources: JK, MS, and UP. Data curation: DS, MH, TB, MS, ŽL, and JK. Writing—original draft preparation: ŽL and MS. Writing—review and editing: ŽL, MS, and JK. Visualization: ŽL. Supervision: JK and UP. Project administration: MS. Funding acquisition: JK. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: An overview. *Int J Cancer* (2021) 149(4):778–89. doi: 10.1002/ijc.33588

2. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer* (2021) 31(1):12–39. doi: 10.1136/ijgc-2020-002230

3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

4. Imboden S, Nastic D, Ghaderi M, Rydberg F, Siegenthaler F, Mueller MD, et al. Implementation of the 2021 molecular ESGO/ESTRO/ESP risk groups in endometrial cancer. *Gynecol Oncol* (2021) 162(2):394–400. doi: 10.1016/j.ygyno.2021.05.026

5. Editorial Board WC of T. WHO classification of tumours female genital tumours. In: Herrington CS, editor. *WHO female genital tumours, 5th ed*, vol. Volume 4. International Agency for Research on Cancer (2020). p. 632 p.

6. Rubeša-Mihaljević R, Babarović E, Vrdoljak-Mozetič D, Štemberger-Papić S, Klarić M, Krašević M, et al. The immunohistochemical pattern of epithelial-mesenchymal transition markers in endometrial carcinoma. *Appl Immunohistochem Mol Morphol* (2019) 28(5):339–46. doi: 10.1097/PAI.0000000000000754

7. Koskas M, Amant F, Mirza MR, Creutzberg CL. Cancer of the corpus uteri: 2021 update. *Int J Gynecol Obstet* (2021) 155(S1):45–60. doi: 10.1002/ijgo.13866

8. Nero C, Ciccarone F, Pietragalla A, Duranti S, Daniele G, Scambia G, et al. Adjuvant treatment recommendations in early-stage endometrial cancer: What changes with the introduction of the integrated molecular-based risk assessment. *Front Oncol* (2021) 11. doi: 10.3389/fonc.2021.612450

9. Markowska A, Pawałowska M, Lubin J, Markowska J. Signalling pathways in endometrial cancer. *Wspolczesna Onkolog* (2014) 18:143–8. doi: 10.5114/wo.2014.43154

10. Kasoha M, Dernektsi C, Seibold A, Bohle RM, Takacs Z, Ioan-Iulian I, et al. Crosstalk of estrogen receptors and wnt/β-catenin signaling in endometrial cancer. *J Cancer Res Clin Oncol* (2020) 146(2):315–27. doi: 10.1007/s00432-019-03114-8

11. McMellen A, Woodruff ER, Corr BR, Bitler BG, Moroney MR. Wnt signaling in gynecologic malignancies. *Int J Mol Sci* (2020) 21:1–21. doi: 10.3390/ijms21124272

12. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene* (2017) 36:1461–73. doi: 10.1038/onc.2016.304

13. Taank Y, Agnihotri N. Understanding the regulation of β – catenin expression and activity in colorectal cancer carcinogenesis : beyond destruction complex. *Clin Trans Oncol* (2021) 23:2448–2459. doi: 10.1007/s12094-021-02686-7

14. Gao C, Wang Y, Broaddus R, Sun L, Xue F, Zhang W. Exon 3 mutations of CTNNB1 drive tumorigenesis: a review. *Oncotarget* (2018) 9(4):5492. doi: 10.18632/ONCOTARGET.23695

15. Ledinek Ž, Sobočan M, Knez J. The role of CTNNB1 in endometrial cancer. *Dis Markers* (2022), 1–9. doi: 10.1155/2022/1442441

16. Park MH, Sung E-A, Sell M, Chae W-J. Dickkopf1: An immunomodulator in tissue injury, inflammation, and repair. *Immunohorizons.* (2021) 5(11):898–908. doi: 10.4049/immunohorizons.2100015

17. Zhu G, Song J, Chen W, Yuan D, Wang W, Chen X, et al. Expression and role of dickkopf-1 (Dkk1) in tumors: From the cells to the patients. *Cancer Manage Res* (2021) 13:659–75. doi: 10.4049/immunohorizons.2100015

18. Chu HY, Chen Z, Wang L, Zhang ZK, Tan X, Liu S, et al. Dickkopf-1: A promising target for cancer immunotherapy. *Front Immunol* (2021) 12. doi: 10.3389/fimmu.2021.658097

19. Fatima I, Barman S, Rai R, Thiel KW, Chandra V. Targeting wnt signaling in endometrial cancer. *Cancers* (2021) 13(10):23. doi: 10.3390/CANCERS13102351

20. Bian Y, Chang X, Liao Y, Wang J, Li Y, Wang K, et al. Promotion of epithelial-mesenchymal transition by Frizzled2 is involved in the metastasis of endometrial cancer. *Oncol Rep* (2016) 36(2):803–10. doi: 10.3892/or.2016.4885

21. Wang Y, Hanifi-Moghaddam P, Hanekamp EE, Kloosterboer HJ, Franken P, Veldscholte J, et al. Progesterone inhibition of wnt/β-catenin signaling in normal endometrium and endometrial cancer. *Clin Cancer Res* (2009) 15(18):5784–93. 10.1158/1078-0432.CCR-09-0814

22. Hashmi AA, Hussain ZF, Qadri A, Irfan M, Ramzan S, Faridi N, et al. Androgen receptor expression in endometrial carcinoma and its correlation with clinicopathologic features. *BMC Res Notes* (2018) 11(1):289. doi: 10.1186/S13104-018-3403-9

23. Kamal AM, Bulmer JN, Decruze SB, Stringfellow HF, Martin-Hirsch P, Hapangama DK. Androgen receptors are acquired by healthy postmenopausal endometrial epithelium and their subsequent loss in endometrial cancer is associated with poor survival. *Br J Cancer* (2016) 114(6):688. doi: 10.1038/BJC.2016.16

24. Gibson DA, Simitsidellis I, Collins F, Saunders PTK. Evidence of androgen action in endometrial and ovarian cancers. *Endocr Relat Cancer* (2014) 21(4):T203-218. doi: 10.1530/ERC-13-0551

25. Tangen IL, Onyango TB, Kopperud R, Berg A, Halle MK, Øyan AM, et al. Androgen receptor as potential therapeutic target in metastatic endometrial cancer. *Oncotarget* (2016) 7(31):49289. doi: 10.18632/ONCOTARGET.10334

26. Shorning BY, Dass MS, Smalley MJ, Pearson HB. The PI3K-AKT-mTOR pathway and prostate cancer: At the crossroads of AR, MAPK, and WNT signaling. *Int J Mol Sci* (2020) 21(12):1–47. doi: 10.3390/IJMS21124507

27. Jahan N, Jones C, Rahman RL. Androgen receptor expression in breast cancer: Implications on prognosis and treatment, a brief review. *Mol Cell Endocrinol* (2021), 531. doi: 10.1016/J.MCE.2021.111324

28. Sobočan M, Smolle MA, Schatz C, Haybaeck J. The interplay of tumor stroma and translational factors in endometrial cancer. *Cancers* (2020) 12(8):2074. doi: 10.3390/cancers12082074

29. Lewczuk Ł, Pryczynicz A, Guzińska-Ustymowicz K. Expression level of e-, n- and p-cadherin proteins in endometrial cancer. *Oncol Lett* (2021) 21(4):9. doi: 10.3892/ol.2021.12522

30. Kaszak I, Witkowska-Piłaszewicz O, Niewiadomska Z, Dworecka-Kaszak B, Toka FN, Jurka P. Role of cadherins in cancer–a review. *Int J Mol Sci* (2020) 21:1–17. doi: 10.3390/ijms21207624

31. Chiu HC, Li CJ, Yiang GT, Tsai A, Wu MY. Epithelial to mesenchymal transition and cell biology of molecular regulation in endometrial carcinogenesis. *J Clin Med* (2019) 8(4):439. doi: 10.3390/ijms21207624

32. Giannone G, Attademo L, Scotto G, Genta S, Ghisoni E, Tuninetti V, et al. Endometrial cancer stem cells: Role, characterization and therapeutic implications. *Cancers (Basel)* (2019) 11(11):17. doi: 10.1097/PAI.0000000000000754

33. Yang N, Chen H, Huang Y, Song X, Yang P, Zhang S, et al. The role and significance of Wnt5a in regulating epithelial-mesenchymal transition in endometrioid adenocarcinoma. *Cancer Manag Res* (2021) 13:6527–35. doi: 10.3390/jcm8040439

34. Scheel C, Eaton EN, Li SHJ, Chaffer CL, Reinhardt F, Kah KJ, et al. Paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast. *Cell* (2011) 145(6):926. doi: 10.3390/cancers11111820

35. Knez J, Sobocan M, Belak U, Kavalar R, Zupin M, Büdefeld T, et al. Pre-treatment risk assessment of women with endometrial cancer: Differences in outcomes of molecular and clinical classifications in the Slovenian patient cohort. *Radiol Oncol* (2021) 13:6527–6535. doi: 10.2147/CMAR.S319346

36. Florescu MM, Pirici D, Simionescu CE, Stepan AE, Mărgăritescu C, Tudorache Ş, et al. E-cadherin and β-catenin immunoexpression in endometrioid endometrial carcinoma. *Roman J Morphol Embryol* (2016) 57(4):1235–40. doi: 10.1016/J.CELL.2011.04.029

37. Buchynska LG, Naleskina L, Nesina IP. Morphological characteristics and expression of adhesion markers in cells of low differentiated endometrial carcinoma. *Exp Oncol* (2020) 41(4):335–41. doi: 10.3389/fimmu.2021.658097

38. Xie X, Zheng X, Wang J, Chen L. Clinical significance of twist, e-cadherin, and n-cadherin protein expression in endometrioid adenocarcinoma. *J Cancer Res Ther* (2017) 13(5):817–22. doi: 10.2478/RAON-2021-0036

39. Singh M, Darcy KM, Brady WE, Clubwala R, Weber Z, Rittenbach JV, et al. Cadherins, catenins and cell cycle regulators: Impact on survival in a gynecologic oncology group phase II endometrial cancer trial. *Gynecol Oncol* (2011) 123(2):320–8. doi: 10.1016/j.ygyno.2011.07.005

40. Yi N, Liao QP, Li T, Xiong Y. Novel expression profiles and invasiveness-related biology function of DKK1 in endometrial carcinoma. *Oncol Rep* (2009) 21(6):1421–7. doi: 10.32471/exp-oncology.2312-8852.vol-41-no-4.13965

41. Park JY, Hong DG, Chong GO, Park JY. Tumor budding is a valuable diagnostic parameter in prediction of disease progression of endometrial endometrioid carcinoma. *Pathol Oncol Res* (2019) 25(2):723–30. doi: 10.1007/s12253-018-0554-x

42. Kim G, Kurnit KC, Djordjevic B, Singh C, Munsell MF, Wang WL, et al. Nuclear β-catenin localization and mutation of the CTNNB1 gene: a context-dependent association. *Modern Pathol* (2018) 31(10):1553–9. doi: 10.1016/j.ygyno.2011.07.005

43. Travaglino A, Raffone A, Saccone G, De Luca C, Mollo A, Mascolo M, et al. Immunohistochemical nuclear expression of β-catenin as a surrogate of CTNNB1 exon 3 mutation in endometrial cancer. *Am J Clin Pathol* (2019) 151(5):529–38. doi: 10.3892/or_00000370

44. Łapińska-Szumczyk SM, Supernat AM, Majewska HI, Gulczyński J, Biernat W, Wydra D, et al. Immunohistochemical characterisation of molecular subtypes in endometrial cancer. *Int J Clin Exp Med* (2015) 8(11):21981. doi: 10.1007/s12253-018-0554-x

45. Wang C, Tran DA, Fu MZ, Chen W, Fu SW, Li X. Estrogen receptor, progesterone receptor, and HER2 receptor markers in endometrial cancer. *J Cancer* (2020) 11(7):1693. doi: 10.1038/s41379-018-0080-0

46. Guan J, Xie L, Luo X, Yang B, Zhang H, Zhu Q, et al. The prognostic significance of estrogen and progesterone receptors in grade I and II endometrioid endometrial adenocarcinoma: Hormone receptors in risk stratification. *J Gynecol Oncol* (2019) 30 (1):529-38. doi: 10.1093/ajcp/aqy178

47. Tomica D, Ramić S, Danolić D, Šušnjar L, Perić-Balja M, Puljiz M. Impact of oestrogen and progesterone receptor expression in the cancer cells and myometrium on survival of patients with endometrial cancer. *J Obstet Gynaecol* (2018) 38(1):96–102. doi: 10.1080/01443615.2017.1328591

48. van Weelden WJ, Reijnen C, Küsters-Vandevelde HVN, Bulten J, Bult P, Leung S, et al. The cutoff for estrogen and progesterone receptor expression in endometrial cancer revisited: a European network for individualized treatment of endometrial cancer collaboration study. *Hum Pathol* (2021) 109:80–91. doi: 10.1016/j.humpath.2020.12.003

49. Kim S, Jeong S. Mutation hotspots in the β-catenin gene: Lessons from the human cancer genome databases. *Mol Cells* (2019) 42(1):8–16. doi: 10.3802/jgo.2019.30.e13

50. Althubiti M. Mutation frequencies in endometrial cancer patients of different ethnicities and tumor grades: An analytical study. *Saudi J Med Med Sci* (2019) 7(1):16. doi: 10.4103/sjmms.sjmms_154_18

51. Igbinigie E, Guo F, Jiang SW, Kelley C, Li J. Dkk1 involvement and its potential as a biomarker in pancreatic ductal adenocarcinoma. *Clinica Chimica Acta* (2019) 488:226–34. doi: 10.1016/j.cca.2018.11.023

52. Coopes A, Henry CE, Llamosas E, Ford CE. An update of wnt signalling in endometrial cancer and its potential as a therapeutic target. *Endocrine-Related Cancer* (2018) 25:R647–62. doi: 10.1530/ERC-18-0112

53. Yi N, Liao QP, Li ZH, Xie BJ, Hu YH, Yi W, et al. RNA Interference-mediated targeting of DKK1 gene expression in ishikawa endometrial carcinoma cells causes increased tumor cell invasion and migration. *Oncol Lett* (2013) 6(3):756. doi: 10.3892/ol.2013.1439

54. Katoh M, Katoh M. Molecular genetics and targeted therapy of WNT-related human diseases (Review). *Int J Mol Med* (2017) 40(3):587–606. doi: 10.3892/ijmm.2017.3071

55. Stefansson IM, Salvesen HB, Akslen LA. Prognostic impact of alterations in p-cadherin expression and related cell adhesion markers in endometrial cancer. *J Clin Oncol* (2004) 22(7):1242–52. doi: 10.1200/JCO.2004.09.034

56. Mell LK, Meyer JJ, Tretiakova M, Khramtsov A, Gong C, Yamada SD, et al. Prognostic significance of e-cadherin protein expression in pathological stage I-III endometrial cancer. *Clin Cancer Res* (2004) 10(16):5546–53. doi: 10.1158/1078-0432.CCR-0943-03

57. Montserrat N, Mozos A, Llobet D, Dolcet X, Pons C, De Herreros AG, et al. Epithelial to mesenchymal transition in early stage endometrioid endometrial carcinoma. *Hum Pathol* (2012) 43(5):632–43. doi: 10.3892/IJMM.2017.3071/HTML

58. Farrag M, Anter A, Farrag N, Ibrahiem A. Switch of e-cadherin to n-cadherin expression in different molecular subtypes of breast invasive duct carcinomas and its correlation with clinicopathological features. *Indian J Pathol Microbiol* (2021) 64 (1):38–46. doi: 10.1200/JCO.2004.09.034

59. Quattrocchi L, Green AR, Martin S, Durrant L, Deen S. The cadherin switch in ovarian high-grade serous carcinoma is associated with disease progression. *Virchows Archiv* (2011) 459(1):21–9. doi: 10.1158/1078-0432.CCR-0943-03

60. Hayes DF. Biomarker validation and testing. *Mol Oncol* (2015) 9(5):960–6. doi: 10.1016/j.humpath.2011.06.021

61. Signore M, Manganelli V, Hodge A. Antibody validation by Western blotting. *Methods Mol Biol* (2017) 1606:51–70. doi: 10.1007/978-1-4939-6990-6_4

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EDITED BY
Emanuele Perrone,
Department of Women's Health, Children's Health and Public Health (IRCCS), Italy

REVIEWED BY
Ilaria Capasso,
Agostino Gemelli University Polyclinic (IRCCS), Italy
Matteo Loverro,
Unit of Cancer Gynaecology (IRCCS), Italy

*CORRESPONDENCE
Andrea Romano
a.romano@maastrichtuniversity.nl
Tea Lanišnik Rižner
tea.lanisnik-rizner@mf.uni-lj.si

†These authors share first authorship

‡BioEndoCar Consortium includes partners of the Transcan2 ERA-Net project, 2018-2021

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Endometrial cancer diagnostic and prognostic algorithms based on proteomics, metabolomics, and clinical data: a systematic review

Andrea Romano[1,2*†], Tea Lanišnik Rižner[3*†], Henrica Maria Johanna Werner[1,2], Andrzej Semczuk[4], Camille Lowy[5], Christoph Schröder[5], Anne Griesbeck[5], Jerzy Adamski[3,6,7], Dmytro Fishman[8,9] and Janina Tokarz[10,11] on behalf of BIOENDOCAR[‡]

[1]Department of Gynaecology, Maastricht University Medical Centre (MUMC), Maastricht, Netherlands, [2]GROW – School for Oncology and Reproduction, Maastricht University, Maastricht, Netherlands, [3]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [4]Department of Gynaecology, Lublin Medical University, Lublin, Poland, [5]Sciomics GmbH, Heidelberg, Germany, [6]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, [7]Institute of Experimental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany, [8]Institute of Computer Science, University of Tartu, Tartu, Estonia, [9]Quretec Ltd., Tartu, Estonia, [10]Institute for Diabetes and Cancer, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany, [11]German Center for Diabetes Research (DZD), Neuherberg, Germany

Endometrial cancer is the most common gynaecological malignancy in developed countries. Over 382,000 new cases were diagnosed worldwide in 2018, and its incidence and mortality are constantly rising due to longer life expectancy and life style factors including obesity. Two major improvements are needed in the management of patients with endometrial cancer, i.e., the development of non/minimally invasive tools for diagnostics and prognostics, which are currently missing. Diagnostic tools are needed to manage the increasing number of women at risk of developing the disease. Prognostic tools are necessary to stratify patients according to their risk of recurrence pre-preoperatively, to advise and plan the most appropriate treatment and avoid over/under-treatment. Biomarkers derived from proteomics and metabolomics, especially when derived from non/minimally-invasively collected body fluids, can serve to develop such prognostic and diagnostic tools, and the purpose of the present review is to explore the current research in this topic. We first provide a brief description of the technologies, the computational pipelines for data analyses and then we provide a systematic review of all published studies using proteomics and/or metabolomics for diagnostic and prognostic biomarker discovery in endometrial cancer. Finally, conclusions and recommendations for future studies are also given.

KEYWORDS

endometrial cancer, proteomics, metabolomics, biomarker, machine learning

1 Introduction

1.1 Endometrial cancer – The need for minimally invasive diagnostic and prognostic biomarkers

Endometrial cancer (EC) is the most common gynaecological neoplasm in developed countries, and over 382,000 new cases were diagnosed worldwide in 2018 (1). In general, EC is diagnosed in postmenopausal women (85% of cases) and its incidence is rising due to longer life expectancy and life style associated risk factors. Women with BMI above 35 have an odds ratio of 5.7 for developing EC, with an increase of 1.1 odds ratio per BMI unit (2, 3). Exposure to unopposed estrogens or tamoxifen or genetic aberrations associated with Lynch syndrome confer a cumulative risk up to 70% (4, 5). Finally, endocrine disruptors and other environmental pollutants can also increase EC risk (6, 7). Therefore, an alarmingly high number of women in the general population is exposed to risk factors for developing EC.

In this context, screening programs would be extremely beneficial for these women, but, unfortunately, no minimally- or non-invasive diagnostic tool for EC exists today, and diagnosis relies on invasive endometrial biopsy and pathology investigation.

A second unmet clinical need in EC is the necessity to accurately stratify patients. EC is diagnosed at an early FIGO stage in 80% of the cases, and the five-year survival of FIGO stage 1a is around 95%. However, a proportion of women diagnosed with early-stage EC develop recurrent disease, which dramatically decreases survival rates (8). This represents a challenge as recent projections indicate that the worldwide EC mortality will increase by 70% by 2040 (Global Cancer Observatory, World Health Organisation - https://gco.iarc.fr).

Therefore, prognostic biomarkers to reliably predict patient prognosis are needed, both prior to any intervention - to decide on the most appropriate treatment and if needed optimally plan the surgical procedure - as well as post-operatively, to define the most appropriate adjuvant treatment, and avoid over-treatment and under-treatment. A number of prognostic markers like histological assessment of tumour type and grade, hormone receptor status, PTEN expression, mismatch repair proteins (MLH1, PMS2, MSH2, MSH6), *POLE* exon 3 mutation, *CTTNB1* mutation, L1CAM overexpression, and *TP53* aberrations allow stratification of patients according to their risk of recurrence (9–20). In particular, the recent introduction of The Cancer Genome Atlas (TCGA) molecular classification improved the risk stratification at the postsurgical (21, 22), but also improved the concordance between presurgical biopsy and pathology assessment at hysterectomy (23), which has been a problem in the past (24). This classification groups EC patients in four clusters with distinct prognosis and a number of studies demonstrated the reliability and the clinical applicability of this classification using surrogate analyses (i.e., IHC and *POLE* gene mutation analyses). Patients with *POLE* mutated tumours have the best prognosis, followed by mismatch repair deficient tumours and with the final groups having an intermediate and the worse prognosis (no specific molecular profile and p53 mutated, respectively) (14). Recently, also classification methods fully based on IHC, hence applicable also in centres with limited access to molecular infrastructures, showed robustness and reliability (19).

Nevertheless, these methods require invasive biopsies, and women consider the presurgical biopsy procedures discomforting and painful (25). Therefore, non- or minimally-invasive prognostic tools applicable presurgically are urgently needed.

Proteomic and metabolomic profiles are attractive approaches for identifying biomarkers that can be detected in tissues or body fluids obtained *via* non-, minimaly or semi–invasive procedures. The purpose of the present review is to explore the current research on the use of proteomics and metabolomics in the context of EC. This review provides a brief introduction to the wet-lab technologies, the computational pipelines for data analyses and a systematic review of all published studies aimed at using proteomics and/or metabolomics for diagnostic and prognostic biomarker discovery in EC. This is followed by conclusions and recommendations for future studies.

1.2 Proteomic and metabolomic approaches for biomarker discovery

Proteomics and metabolomics represent fields that have grown significantly in the last decades, thanks to the important technological advances that allow accurate and sensitive analyses. Both approaches have been extensively used for biomarker discovery in various disorders (26–31).

1.2.1 Targeted and non-targeted proteomics

Large-scale proteomics mainly relies on two different methodological approaches, namely immune-based, targeted protein microarrays and (non-targeted and targeted) mass spectrometry (MS). Making use of antibody-protein specific binding, protein microarrays can be seen as miniaturized conventional assays, thereby allowing multiplexing and high throughput. Protein microarrays relevant for biomarker discovery fall into three categories: analytical microarrays, reverse phase protein array (RPPA), and bead-based microarrays. Analytical protein microarrays are also called capture or antibody microarray because proteins from complex protein lysates are captured by antibodies or aptamers, which have been previously immobilized on the surface of an array. Conversely, RPPA is based on the immobilization of complex samples on a surface and subsequent probing by pre-selected antibodies. Bead-based microarrays use capture antibodies immobilised on microbeads combined with secondary, detection antibodies. Protein microarrays are highly sensitive and highly specific assays, which allow relative quantification among different clinical sample groups. While multiplexity is usually higher in analytical microarrays compared to RPPA and bead-based microarrays, all methods share simple sample processing allowing high throughput. A further immune-based method, Olink technology, uses antibodies that are labelled with ssDNA and detect proteins in a sample by

proximity extension assay (PEA). Pairwise antibodies are linked with complementary ssDNA which upon binding the target protein are hybridized and extended using a DNA polymerase. Despite being targeted hypothesis-driven approaches, antibody-based technologies like protein arrays are solid and promising tools for biomarker discovery and verification (32–34).

Mass spectrometry measures mass-to-charge ratios of ionized peptides in order to analyse proteins. Ionization of proteins can be achieved by electrospray ionization (ESI) or by matrix-assisted laser desorption/ionization (MALDI). ESI allows the creation of ions in solution, while in MALDI, ions are created by laser light pulsing on matrix embedded proteins. A variation of MALDI is SELDI (surface-enhanced laser desorption/ionization), where the proteins are applied on a modified matrix surface allowing binding of specific proteins or proteins classes (35). Mass analysis of proteins is primarily conducted using TOF (time-of-flight) or quadrupoles. Sample preparation for mass spectrometry is a complex process. Upon cellular lysis, it includes subcellular fractionation, depletion of highly abundant proteins, enrichment of target proteins, denaturation and protein digestion. Resolving and denaturation of proteins can also be achieved by SDS polyacrylamide gel electrophoresis, 1D or 2D polyacrylamide gel electrophoresis (PAGE) or difference gel electrophoresis (DIGE). Mass spectrometry is not inherently quantitative but different types of labelling (isobaric tags for relative and absolute quantitation - iTRAQ; isotope-coded affinity tag - ICAT, stable isotope labelling by amino acids in cell culture - SILAC) allow relative and absolute quantification. Label-free quantification, based on signal intensity, is an alternative, cost-efficient option but with a relatively low throughput (36).

Non-targeted mass spectrometry is widely used for biomarker discovery because of its suitability for hypothesis-free approaches. Due to the complexity of the workflow, the number of samples analysed in a discovery setting is usually quite limited, especially when plasma samples are used. Furthermore, fractionation, depletion of high abundant proteins or digestion could bias the results and limit the sensitivity in the untargeted approach. In general, only a small number of candidates undergo clinical validation using orthogonal platforms and even fewer are tested in clinical studies (37, 38) as these studies need first the transition of MS data into immunobased assays to analyse a sufficiently large and statistically relevant number of samples. In this regard, protein microarrays for discovery present the advantage that such translation is not necessary (33).

Proteomics displays a large panel of different tools, which can be combined for discovery and validation phases and subsequently integrated in multi-omics approaches (39).

1.2.2 Targeted and non-targeted metabolomics

Metabolomics is the most recent 'omics'-technology and strives to measure ideally all metabolites in a given biological sample (40). Since metabolites are final downstream products of all cellular processes, metabolomics is closest to the phenotype compared to the other 'omics'-techniques.

Similar to proteomics, two approaches with different objectives are used (41), namely non-targeted and targeted metabolomics. Non-targeted metabolomics (profiling metabolomics) is a hypothesis-free approach, which aims to detect simultaneously as many metabolites as possible. Depending on the analytical platform, non-targeted metabolomics reveals metabolites from a wide range of metabolite classes (42), which are annotated after the measurement. Thus, the detection of unknown metabolites not yet annotated in metabolite databases is common in non-targeted metabolomics. Although being comprehensive, non-targeted metabolomics does not allow absolute quantification, but can provide at best only semiquantitative results (42).

Targeted metabolomics is hypothesis-driven and aims to quantify the absolute concentrations of a predefined set of metabolites (42). Since all measured metabolites are pre-selected, a standard calibration curve for accurate quantification can be prepared for each metabolite. Stable-isotope labelled internal standards are added at known concentrations to all samples, allowing compensation for any analytical interferences. With its advantages such as validated analytical performance and the results delivered in absolute concentrations, targeted metabolomics is often used for biomarker validation (42). However, the limited number of simultaneously quantified metabolites in targeted metabolomics increases the risk of missing relevant biological processes.

Metabolomic approaches usually use MS or nuclear magnetic resonance spectroscopy (NMR). While MS offers high mass accuracy, high resolution, high dynamic range and high sensitivity (43), NMR is less sensitive but is superior in terms of structural information content, robustness, and reproducibility (44, 45). However, current analytical methods are not able to cover the entire metabolome (46). To achieve a high metabolite coverage combined with quantitative data, the integration of different metabolomic techniques (multiplatform approaches) is necessary (46, 47).

1.3 Bioinformatics and statistical approaches for constructing diagnostic and prognostic algorithms

Data from proteomic and metabolomic experiments can reveal molecules that can possibly serve as diagnostic or prognostic markers. However, even if well-designed and executed, experiments often result in noisy, biased and incomplete data due to a multitude of uncontrollable factors. Therefore, thorough data analysis needs to be performed to eliminate technical noise, while preserving genuine biological variation between samples. A set of computational methods used to analyse data are typically bundled together into one unified data analysis pipeline (Figure 1), which treats raw data files as an input while providing the list of potential biomarkers as the output.

Proteomic and metabolomic raw data is first processed by background correction, signal transformation, outlier detection, and normalisation. Pre-processing is essential to minimise unwanted technical bias and enable comparisons of samples. Further, integration of clinical information enables comparisons of average metabolite and protein signals between phenotypic groups of interest (48–50).

Background correction addresses different effects in proteomics and metabolomics. In protein microarrays, it is challenging to

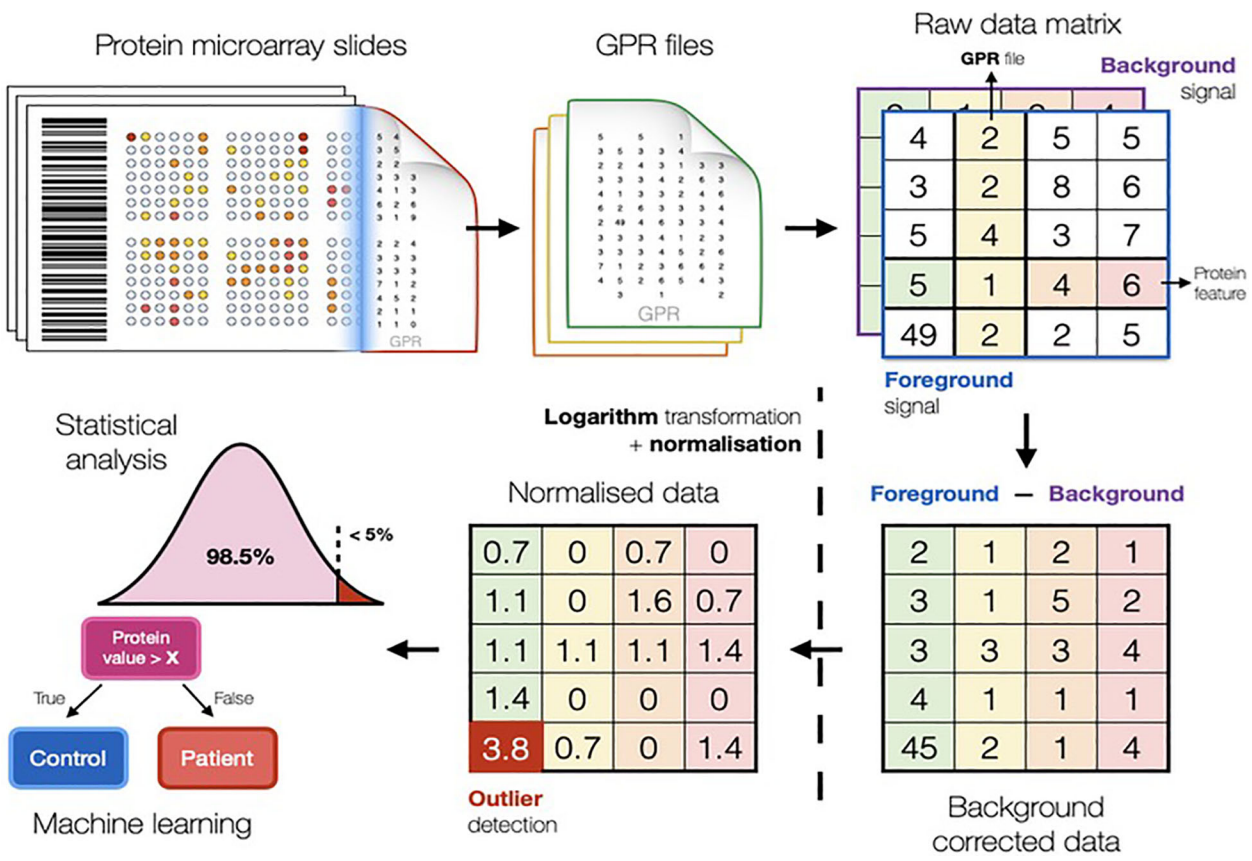

FIGURE 1
Scheme illustrating the typical computational methods used in biomarker discovery studies to analyse data. The pipeline in figure refers to the conventional protein microarray analysis (Fishman et al. https://arxiv.org/abs/2201.06074).

correctly quantify the fluorescent signal produced by the biological reaction avoiding local residuary background (51–53). In metabolomics, background (or baseline) correction is used to eliminate low frequency artefacts and differences generated by the measuring instrument (54). Data log-transformation is common practice, as this renders fold changes symmetric around zero, reduces potential skew in the data and provides a good approximation for the normal distribution, which is a prerequisite for most computational methods (52, 55), especially for linear models. Following background correction, outlier detection is performed by the three standard deviations technique and subsequently removed or replaced.

In large-scale studies based on MS proteomics or metabolomics, samples will be distributed into several analytical batches, which may introduce instrumental variabilities into the data set. Such batch effects can be very destructive as they render comparison between phenotypic groups ineffective. Normalisation strategies for metabolomic and proteomic experiments make use of control samples and control molecules (52, 56, 57), which are usually assumed to exhibit constant signal levels. Any differences in signal values are considered to be technogenic and thus, corrected for. The most popular normalisation strategies are global scaling, quantile normalisation, cyclic loess and the ones involving linear models (56).

After appropriate pre-processing, the data is used for statistical analysis, where a large number of techniques are available. Characteristics of data and the research question determine the choice of the statistical method. In biomarker discovery studies, molecules that can reliably distinguish between two (or more) groups, like disease versus controls, are referred to as significantly differential and can be used as biomarker candidates. The process of identifying such molecules is termed differential analysis (52, 58). Classical univariate statistics such as Student's t-test (requiring normal data distribution) or Mann-Whitney U test (non-parametric test that does not rely on parameterized data distribution) can be used for differential analyses. Differential analyses have a low probability (usually less than 5%) to deliver significant results by mistake; however, if repeated multiple times, as for omics studies, can result in the generation of false significant hits. Therefore, the number of tests performed needs to be taken into account. The simplest and one of the most popular methods for multiple testing correction is 'Bonferroni correction' (59), which adjusts the p-value threshold by dividing it by the number of tests. This is a conservative approach that may result in a high number of potential biomarkers being ignored, hence, less stringent methods can be considered (like Benjamini and Hochberg False Discovery Rate correction) that keeps the number of falsely significant results at a predefined level (e.g., 5%).

While classical statistical methods analyse the significance of each molecule of interest independently (60), machine learning algorithms are able to efficiently assess the predictive performance of multiple proteins, metabolites, features and even their combinations. Machine learning is a field of computer science that studies algorithms capable of learning valuable relationships from data without being explicitly programmed. Myriads of machine learning algorithms have been developed over the past years (61) and are frequently used in biology to discover biomarkers for various diseases (49, 62). The most popular machine learning methods are decision tree (63), support vector machine, random forest (64) and gradient boosting machines (65).

It can be challenging to build reliable machine learning models, because most model algorithms can learn random patterns that can only explain data these models were exposed to. This phenomenon is known as overfitting and might cause models to report completely irrelevant biomarkers and thus, render the entire study obsolete. In order to account for potential overfitting and keep its influence at minimum, various strategies have been proposed (66). One of the most important techniques is k-fold cross validation. By using only one part of the data to build a model (training set) and the remaining part to assess its performance (test set), researchers can be confident that the biomarkers identified by the model are not random fluctuations in the training data.

2 Methods

2.1 Study design

With this systematic review we aimed to respond to the following question: Can proteomics and metabolomics contribute to identification of biomarkers for diagnostics and prognostics in EC? The review was conducted according to the PRISMA guidelines (67) and is registered at the 'International prospective register of systematic reviews' (PROSPERO, Registration number CRD42022245880).

2.2 Search strategy, data extraction and quality assessment

We performed a systematic search of the literature in the PubMed® and OVID® Embase databases on July 20, 2022 using the search terms listed in Supplementary Table S1. We focused on proteomic and metabolomic studies performed in physiological fluids and tissue samples. There was no restriction on publication date. Reports were retrieved, and titles/abstracts were screened according to the inclusion and exclusion criteria (Table 1) independently by authors AR and TLR. Disagreements were discussed and consensus was reached. The search strategy is provided in Table 2.

The selected reports were read in detail and the following relevant data was extracted (when applicable): author and year of publication, country, fundings; sample: tissue (kind), plasma, serum or other body-fluid; study design; methods: omics approach, targeted/nontargeted proteomics/metabolomics; analytical methods; patient selection: case/control, stratification of patients according to reference test; patient characteristics: number of patients, characteristics of the enrolled patients with EC (e.g., mean age, body mass index [BMI], type of EC, histological differentiation, FIGO stage, menopausal status) and control patients or healthy women (e.g., mean age, BMI, diagnosis, menopausal status); study phase and statistical methods: discovery, validation phase, machine learning approaches used; differentially abundant proteins and metabolites in the study groups; diagnostic characteristics (e.g., sensitivity, specificity, area under the curve [AUC], positive predictive value [PPV], negative predictive value [NPV]) or prognostic characteristics (overall survival [OS], disease-free survival [DFS]), and hazard ratios [HR]; diagnostic or prognostic models; disclosures: affiliations with industry, industrial funds, patents.

Reporting was performed under the guidance of the PRISMA diagnostic test accuracy checklist (67). The risk of bias and quality of individual diagnostic accuracy studies were assessed following the QUADOMICS tool, an adaptation of QUADAS (68) that was designed specifically for omics studies (69) (Supplementary Table S2). This tool focuses on study design, patient selection, index test, reference standards, flow of timing, pre-analytical and analytical procedures, and statistical analysis and nine questions per study were specifically answered (Supplementary Tables S2–S4). Additional potential financial, commercial and conflict of interest biases were further examined (Supplementary Tables S5).

3 Results

Systematic literature search led to the identification of 52 studies in EC, 23 on proteomics and 29 on metabolomics (Figure 2).

3.1 Evaluation of the quality of published studies

The quality of the studies included was assessed systematically according to the QUADOMICS tool (Supplementary Tables S2–S4; Figure 3). We evaluated study design and pre-analytical, analytical, and post-analytical bias of all included studies. The majority of the studies described the criteria for patient selection (question 1) in

TABLE 1 Inclusion and exclusion criteria.

Inclusion criteria	research papers, papers in English, studies in humans, blood (plasma or serum), urine, other physiological fluids, tissue samples, at least 10 subjects per study group.
Exclusion criteria	abstracts, review papers, papers in other languages, studies in animals, studies in cell lines, studies including only unidentified metabolites, epidemiological studies, studies evaluating drug effects.

TABLE 2 Search strategy for identification of manuscripts in Pubmed and OVID Embase.

Search Query	Search results	Selected manuscripts	Additional manuscripts	Included
Endometrial cancer and proteomics*	**746 total** 570 PubMed 176 OVID **275 duplicates** **471 total** **224 removed/titles** **247 total** 175 removed/abstract **Total 72**	**23** 49 removed after full-text reading	**0**	**23**
Endometrial cancer and metabolomics*	**214 total** 89 PubMed 125 OVID **43 duplicates** **171 total** **70 removed/titles** **101 total** 70 removed/abstract **Total 31**	**31** 4 removed after full-text reading	**2**	**29**

*Search strategy is provided as Supplementary Table S1.

appropriate detail. Approximately 50% of metabolomic and 20% of proteomic studies did not reflect the real clinical setting, because they compared patients with healthy women, who are not likely to need a diagnostic test (see discussion - question 2). The assessment of pre-analytical bias (questions 3A and 3B) revealed that only a fraction of all studies reported appropriate descriptions of the samples, including the procedures for sample collection and processing (e.g., centrifugation time, type of blood tube). Furthermore, the majority of the studies did not report any information about the time of sample collection, the time between blood draw and centrifugation, the time between sample acquisition and storage, and the number of freeze/thaw cycles. In 75% of the metabolomic and 51% of the proteomic studies sufficient information on the clinical and physiological factors that can affect -omics data was not provided (question 4; e.g., BMI, menopausal status, menstrual phase cycle, fasting status). Approximately 70% of the included studies reported detailed descriptions on sample storage and metabolite extraction (question 5). Almost all samples were stored at -80°C or in liquid nitrogen, but several studies failed to report this information. The time between the reference standard

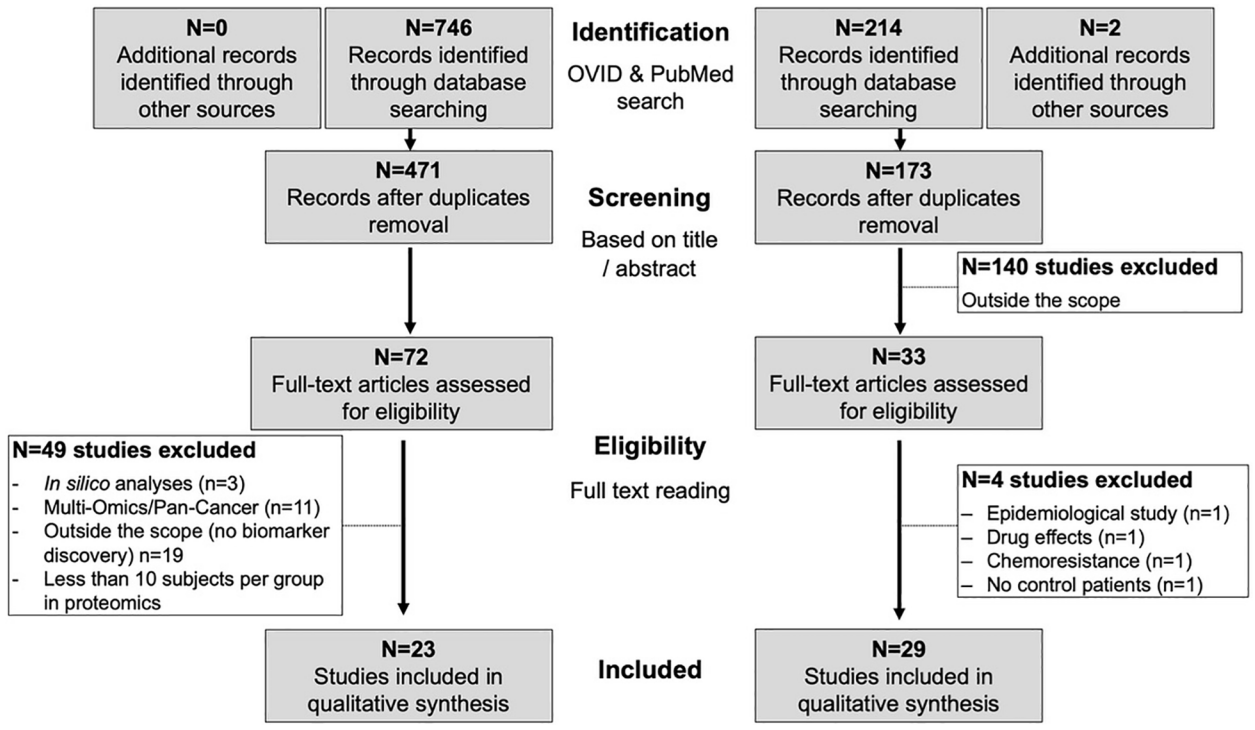

FIGURE 2
Workflow of the systematic search and paper selection.

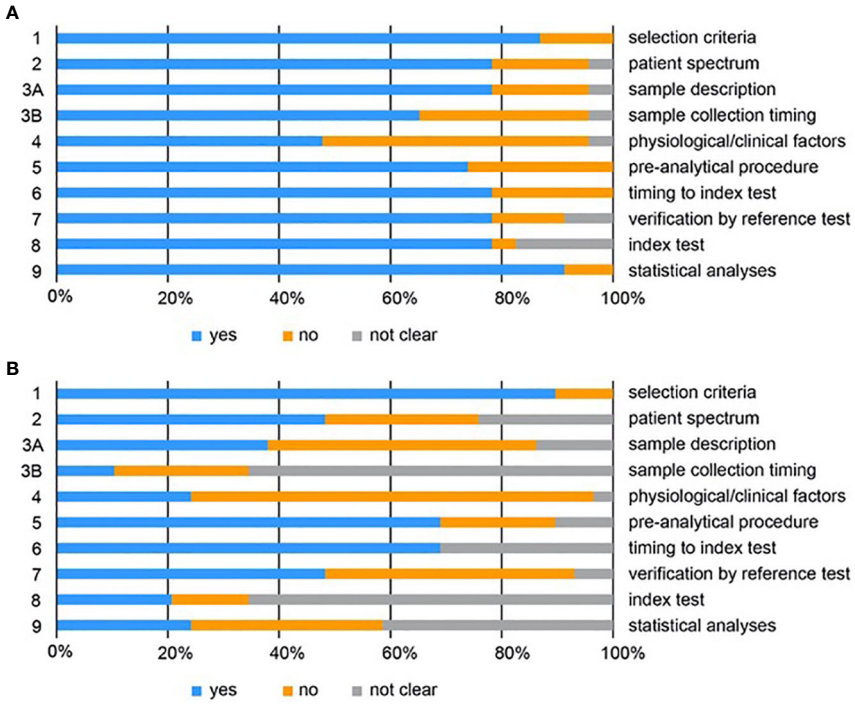

FIGURE 3
QUADOMICS scoring of all studies included for proteomics **(A)** and metabolomics **(B)**. Proportion of studies with answers "yes", "no", or "not clear" to each of the signalling questions. Each signalling question is numbered on the left and a short description of each question is given on the right. The detailed scoring is given in Supplementary Tables S3 and S4.

and the index test (metabolomics or proteomics) was not clear for 31% of the included metabolomic and 21% of the proteomic studies (question 6), while in 52% of the metabolomic and 22% of the proteomic studies, the verification by reference test was not performed in all patients (question 7). With respect to analytical biases, we observed that only 21% of all studies provided a detailed description of the metabolomic analysis (question 8). Most studies did not provide information on sample randomisation for MS-based metabolomics, for the use and type of quality control samples, and occasionally, important MS parameters were not given. In proteomics, 78% of all included studies described the index test in sufficient detail. Regarding post-analytical biases, we observed that 24% of the metabolomic studies described the statistical analysis in sufficient detail, while 42% of studies provided incomplete description. These studies failed to report information on missing value treatment, sample-to-sample normalization, data transformation and scaling and in one case also on model calculation and cross-validation. In proteomics, 91% of all included studies reported the statistical analysis in sufficient detail.

3.2 Disclosure of financial and other potential conflicts of interest

We also evaluated whether studies clearly stated the financial support, disclosed any potential conflict of interest, whether authors were affiliated to industry and whether the studies complied with the open science policy and deposited their data on public repositories (Supplementary Table S5). Although older studies tend not to report any information on the financial support or the presence of any conflict of interest, more recent studies provide this information. The source of funding was declared in 88% of the studies (19 out of 23 proteomic and 27 out of 29 metabolomic studies) and the presence of any potential conflict was declared in 77% of the studies (14 out of 23 proteomic and 26 out of 29 metabolomic studies). Three studies (6% of those declaring the source of funding) received industrial supports and four studies included authors affiliated to companies. Only 25% of the studies deposited the data in public repository (1 out of 23 proteomic and 12 out of 29 metabolomic studies) and four proteomic studies declare that data are available upon request.

3.3 Proteomics in endometrial cancer

From the systematic literature search, 72 research papers were selected based on title and abstract. From these, 19 papers were excluded because focusing on basic cell mechanisms of carcinogenesis with no further investigation on the diagnostic or prognostic potential (70–80), response to metformin (81), side effect to radiotherapy (82), racial disparities (83, 84), drug resistance (85–87), premenopausal endometrial physiology (88). Three studies were *in silico* analyses (89–91). Additionally, 11 papers (22, 92–101) were based on multi-omics approaches or focused on pan-cancer biomarkers and will be discussed in paragraph 3.4, thus resulting in 39 papers for review.

Papers that included less than 10 subjects per study group are not further discussed here (n=16; see Supplementary Table S6 for details). This resulted in a total of 23 papers focusing on proteomic biomarkers for diagnosis, prognosis, risk stratification or classification (Table 3 and Supplementary Table S7). Ten studies used blood (serum or plasma), two studies used uterine aspirate whereas eight studies used fresh frozen tissues and three used formalin-fixed-paraffin-embedded (FFPE) tissues. Various technologies were used, with the most common being 2D-DIGE/MS based methods. Although studies on tissue proteomics preceded chronologically those in body fluid, since this review focus of diagnostic/prognostic biomarkers where body fluids represent the most suitable biomaterial, we will start in the next paragraphs describing studies using body fluids for biomarker discovery.

3.3.1 Blood proteomics

Under the rationale that protein fragments/peptides are produced in the tissue microenvironment by proteolytic processes and released into the blood, the first proteomic studies based on blood (serum or plasma) were published during the first decade of 2000. Zhu and co-workers (102) performed a biomarker discovery study using SELDI-TOF-MS on 40 patients and 30 age-matched healthy controls and identified 13 m/z protein-peaks that were found in different levels between patients and healthy women. The sensitivity of each single peak ranged from 40-95%. The authors further built a decision tree-based algorithm that correctly identified 95.7% (70) of the samples (30/33 healthy women and 37/40 ECs; Supplementary Table S7). The same authors further improved the model using only four m/z protein peaks resulting in sensitivity and specificity of 100% and 92.3%, respectively, in the training set and 60% and 75%, respectively, in an independent validation cohort (104). The authors did not identify the proteins corresponding to the m/z spectra peaks (Supplementary Table S7).

A relatively large study including 199 serum samples from untreated EC patients (n=92), patients with prolapsed uterus (n=16), healthy women (n=17) (n=33), and uterine fibroids (n=74) identified 507 peaks with m/z values ranging from 2,000 to 30,000 by MALDI QTOF-MS (103). Based on predefined

TABLE 3 List of the 23 proteomic studies in endometrial cancer.

Study	Study aim	Samples *	Study design
Zhu, 2006 (102)	Diagnostic Biomarkers	Serum	Case - Control
Kikuchi, 2007 (103)	Diagnostic Biomarkers	Serum	Case - Control
Zhu, 2008 (104)	Diagnostic Biomarkers	Serum	Case - Control
Qiu, 2010 (105)	Diagnostic Biomarkers	Serum	Case - Control
Wang, 2011 (106)	Diagnostic Biomarkers	Serum	Cases only
Enroth, 2018 (107)	Diagnostic Biomarkers	Plasma	Case - Control
Tarney, 2019 (108)	Diagnostic Biomarkers	Serum	Nested case-control
Ura, 2021 (109)	Diagnostic Biomarkers	Serum	Case - Control
Celsi, 2022 (110)	Diagnostic Biomarkers	Serum	Case - Control
Ura, 2022 (111)	Diagnostic Biomarkers	Serum	Case - Control
Martinez-Garcia, 2016 (112)	Diagnostic Biomarkers	uterine aspirate	Case - Control
Martinez-Garcia, 2017 (113)	Diagnostic Biomarkers Prognostic Biomarkers	uterine aspirate	Case - Control
Yoshizaki, 2005 (114)	Diagnostic Biomarkers	Frozen tissue	Case - Control
DeSouza, 2007 (115)	Diagnostic Biomarkers	Frozen tissue	Case - Control
Voisin, 2011 (116)	Diagnostic, Prognostic, Therapeutic Biomarkers	Frozen tissue	Case - Control
Shan, 2016 (117)	Diagnostic Biomarkers	Frozen tissue	Cases only
Ceylan, 2020 (118)	Diagnostic Biomarkers	Frozen tissue	Case - Control
Mauland, 2017 (119)	Prognostic Biomarker associated with obesity	Frozen tissue	Cases only
Akkour, 2022 (120)	Diagnostic Biomarkers	Frozen tissue	Case - Control
Kurimchak, 2020 (121)	Prognostic Biomarkers	Frozen tissue	Cases only
DeSouza, 2010 (122)	Diagnostic Biomarkers	FFPE tissue	Case - Control
Aboulouard, 2021 (123)	Prognostic Biomarkers	FFPE tissue	Case - Control
Janacova, 2020 (124)	Prognostic Biomarkers in the tamoxifen users	FFPE tissue	Cases only

See Supplementary Table S7 for further details.
* FFPE: Formalin fixed paraffin embedded.

stringent criteria (P < 0.00001, AUC value > 0.80) three peaks were differentially abundant between the case and the control groups and showed sensitivity and specificity of 65.2% and 93.9%, respectively (Supplementary Table S7). Surgical stage of patients could not be discriminated by the selected m/z peaks but patients with EC and patients with uterine fibroids could be distinguished (Supplementary Table S7).

Qiu and co-workers (105) performed proteomics on 30 EC patients and 30 control patients on serum collected pre-operatively and identified 147 differential peaks. They further used different algorithms based on various peaks (from two to 10) and reported specificities and sensitivities up to 97% and 100%, respectively (Supplementary Table S7).

In another study, Wang and colleagues (106) performed a pilot study to compare the serum proteomics in patients with distinct stages of endometrial disease, from simple endometrial hyperplasia (n=6), complex hyperplasia (n=4), hyperplasia with atypia (n=4) and with early-stage EC (n=6). The authors identified 74 proteins including potential biomarkers (Supplementary Table S7), but the number of samples included was very limited.

A large nested case-control study aiming at identifying early detection biomarkers for EC was based on the UK Prostate, Lung, Colorectal, and Ovarian cancer screening trial (n =78,216 subjects), including 112 incident EC cases and 112 matched postmenopausal controls (108). Among cases who received an EC diagnosis less than two years after inclusion (n=31), 1,100 total proteins were identified, 565 of which were co-quantified across all patient samples and 47 proteins resulted altered compared with controls. Six candidate protein biomarkers were used to build a diagnostic algorithm with over 45% sensitivity and 96% specificity (Supplementary Table S7). A recent study employed PEA proteomics (PCR-based) and Olink Multiplex assays to search for candidate diagnostic biomarkers in gynaecologic malignancies, including EC (107). The authors compared malignant cases with both a group of healthy controls and with a group of women with benign tumours. The abundance of 441 unique proteins in plasma was first evaluated in a discovery phase that resulted in 16 potential protein biomarkers. The diagnostic value of nine out of these 16 proteins was validated in a replication cohort and resulted in sensitivities and specificities above 64% and 67%, respectively, to distinguish EC from healthy women or from patients with benign tumours (Supplementary Table S7).

Three proteomics studies using serum (109–111) were performed by an Italian group. In 2021, the authors performed a pilot study using the serum of 15 EC patients and 15 [non-cancer patients (109)] and identified 16 proteins with diagnostic potential (Supplementary Table S7), four of which (ITIH4, CLU, SERPIN1, and C1R) were validated by western blotting. One year later, the study was extended to a larger cohort including 60 non-EC controls and 44 EC patients (110). Proteomic analyses was performed on 10 controls and 10 EC. It is not stated in the study whether the study population and the samples used for proteomics overlaps with the previous investigation from the same team (109). The authors further validated the observed downregulation of SBSN in serum of patients by western blotting and *in silico* analysis of the TCGA database. In a subsequent study, the authors included 44 EC cases and 44 non-oncologic patients (111) - the study does not specify whether this study population overlaps with the previously studied groups (109, 110). By using PEA on two distinct protein panels (Immuno-oncology panel and Target 96 Oncology III panel), the authors identified several differentially expressed proteins and proposed different models resulting in AUCs up to 0.96 (Supplementary Table S7).

3.3.2 Other body fluids

For diagnostics, another potentially interesting minimally invasively obtained body fluid is the uterine aspirate, which has the advantage to capture the tumour heterogeneity better than a presurgical biopsy (current standard diagnostic method). Uterine aspirate for proteomic biomarker discovery was used by Martinez-Garcia and collaborators (112, 113). Since the LC-PRM targeted proteomics technique allows the quantification of a limited number of predefined proteins, the authors adopted a sequential workflow: 506 candidate biomarkers were first extracted from a literature search. Subsequently, the authors determined the presence of these biomarkers in uterine aspirates by LC-MS/MS and confirmed the presence of 158 proteins. After method optimisation, a list of 52 candidate biomarkers was selected for PRM design/development and 26 proteins were differentially expressed between cases and controls (112). The same set of 52 proteins was subsequently tested on an independent prospective cohort of 116 women entering the EC diagnostic workup due to EC suspicion (113). A diagnosis of EC was confirmed in 69 women and 28 proteins elevated in EC *versus* controls had an AUC >0.75. Various tests and combinations of the five best individual biomarkers were assessed, resulting in diagnostic and prognostic models with sensitivities and specificities above 89% and 83%, respectively (Table 3).

3.3.3 Tissue proteomics

3.3.3.1 Frozen tissue

Pioneering studies were conducted as early as 2005 using iTRAQ. After determining the feasibility and comparing the performance of iTRAQ and cICAT for proteomics (authors used less than 10 samples per group; Supplementary Table S6, (125), the authors used iTRAQ to analyse 40 frozen tissue samples including proliferative, secretory endometrium and EC (115). Over 1,000 proteins were identified among which six candidate markers (PK, PIGR, CPN10, MIF, AAT, CKB, and TAGLN) were confirmed as differentially expressed from their previous pilot investigation (125). Fourteen proteins were selected for further analyses and after assessing the associations of each individual protein with malignant or benign status using the two-sample t-test (p<0.005), four proteins (PK, CPN10, AAT and CKB) were selected to build a prediction model. Although these proteins used as single markers reached maximum AUC of 0.95 (sensitivity: 85%; specificity: 90%; PV: 87%; PPV: 89%), the use of three markers (AAT, PK and CPN10) resulted in improved performance and an AUC of 0.96 (Table 3). The validity of these biomarkers was further confirmed by two-thirds/one-third cross-validation, and also by using dot-blot and IHC on a panel of independent samples (115). In a subsequent study, the authors verified five of the identified markers (CPN10,

S100A8, PIGR, PK-M2 and AAT) and one additional marker (TIMP-1) by IHC on a tissue microarray (TMA), including 148 samples (two simple hyperplasia; eight complex hyperplasia; 39 endometrioid EC; 13 serous papillary/clear cell or Type II EC; one carcinosarcoma: 85 benign endometrium samples of which 25 proliferative, 25 secretory, 25 atrophic, and 10 menstrual). They further showed that CPN10 and PK-M2 could distinguish hyperplasia and EC cases *versus* controls (sensitivity and specificity of 77% and 87%, respectively), whereas the combination of AAT, CPN10, and PK-M2 resulted in sensitivity and specificity of 85% and 93%, respectively, in distinguishing EC *versus* control patients (126). The same authors subsequently performed a pilot study on 10 EC patients and 10 control patients using a novel strategy (drill-down coupled to iTRAQ) to improve their ability to detect novel proteins and identified 1,529 proteins, among which 40 candidate biomarkers. The PPV and AUC of these proteins used as single diagnostic biomarkers ranged between 62%-100% and 0.60-1.00, respectively (Supplementary Table S7) (116).

In parallel to these studies based on iTRAQ, the first studies analysing fresh/frozen EC tissues using 2D gel separation followed by MS were published in 2005. By applying SELDI-TOF-MS to 19 cases of EC and 20 control patients, the authors identified one peak (m/z 9,600) consistently upregulated and a second peak (m/z 11,300) consistently downregulated in case group versus control group (114).

Additional biomarker discovery studies were published in subsequent years, with most of them being pilot or feasibility in nature and including less than 40 samples. Shan and co-workers (117) compared EC *versus* adjacent normal tissue in 10 cases with iTRAQ-based proteomics and identified 1,266 proteins, 103 of which were upregulated and 30 downregulated in cancer *versus* control tissue (Supplementary Table S7). Results were confirmed by western blotting, qRT-PCR and functional studies using cell lines. Ceylan and co-workers (118) also performed a diagnostic biomarker discovery study based on 2D-DIGE-MALDI-TOF and compared controls (pre- and post-menopausal women), hyperplasia and EC. Several proteins were differentially expressed between controls and EC, controls and hyperplasia (Supplementary Table S7), or were associated with advanced-stage disease (CAH1, PPIB, K2C8, and UAP56).

Mauland and colleagues (119) explored the levels of 163 proteins using RPPA in relation to prognosis and obesity. The authors used patient cohorts from different geographical regions: a group of samples collected in Norway in two different periods served as training (n=272 collected between 2001-2013) and validation (n=68 collected between 2011-2015) cohorts and a third cohort collected in Texas (USA) was used as extra validation (n=178 collected between 2000-2009). Beside correlation with BMI, several proteins were associated with patient prognosis, including proteins indicative of a low PI3K activation in non-obese early-stage ER-positive tumours. Data was further validated by RNA (correlation) and IHC (Table 3). Akkour and colleagues (120) used 2D-DIGE to analyse tissues from patients with hyperplasia (n=12), EC (n=12) and age-matched control patients (n=12) and identified 87 differentially expressed proteins (26 between controls and hyperplasia and 32 between EC and hyperplasia; Table 3). Further modelling was not performed. In a recent study, Kurimchak et al. (121) used an innovative approach based on Multiplexed Inhibitor Beads (MIB) and MS to chart the kinase network in EC (n=20) and adjacent normal tissues (n=16). The MIB binding value was measured for 347 kinases, 300 of which were quantitated by both LFQ and s-SILAC, whereas 37 and 10 by LFQ and s-SILAC, respectively. These analyses showed that SRPK1 was overexpressed in cancer tissue (Table 3). IHC on TMAs (39 serous and 18 endometrioid and 12 normal endometrial tissues), functional/loss of function studies *in vitro* and the TGCA and CPTAC datasets confirmed that SRPK1 is associated with EC and with poor patient survival.

3.3.3.2 Formalin-Fixed-Paraffin-Embedded (FFPE) tissue

A number of studies investigated the potential use of FFPE material for proteomics (Supplementary Table 6), but only three of them met our selection criteria (Table 3; Supplementary Table S). DeSouza and colleagues confirmed the feasibility of mTRAQ targeted proteomics using FFPE tissues (122). The authors laser-capture-microdissected tissue and examined the tissue of interest from 10 ECs and 15 proliferative endometrium samples and detected 13 out of the 17 targeted proteins across 12 samples (Table 3; Supplementary Table 7).

Janacova and colleagues (124) explored archival material from 36 EC patients, 15 of whom had received tamoxifen adjuvant treatment for breast cancer, whereas 21 were never exposed to tamoxifen previously. The authors explored with LC–MS/MS in SWATH-MS mode 34 tumour samples (each from one subject) and 11 myometrial tissues adjacent to the tumours. The proteomic approach targeted over 1,100 different proteins, of which over 900 were consistently identified. The authors compared clinical features in the tamoxifen *versus* tamoxifen naïve patients and identified six upregulated and 22 downregulated proteins. The expression of CAPS and STMN1 was confirmed with IHC and STMN1 was also associated with poor patient prognosis (Table 3). Using a very innovative approach (123), Aboulouard and coworkers compared the proteome profile in EC and sentinel-lymph-node SLN tissues (Table 3; Supplementary Table 7). Regions of interest were first microdissected, then analysed with NanoLC-ESI-MS and a number of potential biomarkers indicative of lymph node disease were identified.

3.4 Metabolomics in endometrial cancer

Our literature search identified 29 studies using metabolomics in EC (Table 4 and Supplementary Table S8), with the majority evaluating the metabolic profiles in blood samples (10 serum, seven plasma, one serum and plasma, one dried blood). Seven studies focused on endometrial tissue samples, one on cervical lavage, one on endometrial brushing and one study on urine samples. Most studies aimed to identify diagnostic and/or prognostic biomarkers, better understanding the mechanisms of carcinogenesis (studies in

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TABLE 4 List of the 29 metabolomic studies in endometrial cancer.

Study	Study aim	Samples	Study design
Ihata, 2014 (127)	Diagnostic Biomarkers	Plasma	Case-control
Knific, 2018 (128)	Diagnostic Biomarkers Prognostic Biomarkers	Plasma	Case-control
Strand, 2019 (129)	Prognostic Biomarkers	Plasma	Cases only
Njoku, 2021 (130)	Diagnostic Biomarkers Prognostic Biomarkers	Plasma	Case-control
Kliemann, 2021 (131)	Association	Plasma & serum	Nested case-control
Dossus, 2021 (132)	Association	Plasma	Nested case-control
Breeur, 2022 (133)	Association	Plasma & serum	Case-control study
Audet-Delage, 2018 (134)	Diagnostic Biomarkers Prognostic Biomarkers	Serum	Case-control
Audet-Delage, 2018 (135)	Diagnostic Biomarkers Prognostic Biomarkers	Serum	Case-control
Troisi, 2018 (136)	Diagnostic Biomarkers Prognostic Biomarkers	Serum	Case-control
Shi, 2018 (137)	Exploratory	Serum	Case-control
Bahado-Singh, 2017 (138)	Diagnostic Biomarkers	Serum	Case –control
Lunde, 2020 (139)	Exploratory	Serum	Cases only
Kozar, 2021 (140)	Exploratory	Serum	Prospective observational study
Gu, 2021 (141)	Diagnostic Biomarkers Prognostic Biomarkers	Serum	Case-control
Yan, 2022 (142)	Diagnostic Biomarkers Prognostic Biomarkers	Serum	Case-control
Schuhn, 2022 (143)	Diagnostic Biomarkers	Serum	Case-control
Troisi, 2020 (144)	Diagnostic Biomarkers	Dried blood samples	Multicenter prospective cohort study
Shao, 2016 (145)	Diagnostic Biomarkers	Urine	Case-control
Cheng, 2019 (146)	Diagnostic Biomarkers	Cervicovaginal fluid	Case-control
Jove, 2016 (147)	Diagnostic Biomarkers	Tissue	Case-control
Altadill, 2017 (148)	Diagnostic Biomarkers	Tissue	Case-control
Trousil, 2014 (149)	Diagnostic Biomarkers	Tissue	Case-control
Cummings, 2019 (150)	Diagnostic Biomarkers	Tissue	Case-control
Skorupa, 2021 (151)	Diagnostic Biomarkers	Tissue	Case-control
Arda Düz, 2022 (152)	Diagnostic Biomarkers	Tissue	Case-control
Gatius, 2022 (153)	Diagnostic Biomarkers	Tissue from Biobank	Cases only
Shafiee, 2020 (154)	Diagnostic Biomarkers	Plasma & tissue	Cros-sectional study
Yi, 2022 (101)	Diagnostic Biomarkers	Tissue & Urine	Case-control

See Supplementary Table S8 for further details.

tissue samples), and also to determine associations between metabolic profiles and EC (131, 132). Plasma, serum and urine represent appropriate sources for discovery of diagnostic/prognostic biomarkers (155). However, also metabolic profiles of cervical lavage, brushing endometrial samples, and tissue samples (if obtained as pre-surgical biopsy) may be of clinical relevance. Non-targeted metabolomics was more commonly applied (20 studies) as compared to targeted metabolomics (10 studies). Only six studies used NMR analysis, and there was one study that combined NMR with the most commonly used LC-MS/MS (138). The majority of the targeted metabolomic studies focused on lipids and amino acids.

3.4.1 Blood metabolomics

Metabolomic studies on serum samples from EC patients have been performed from 2017 and identified a series of metabolites in differential concentrations between study groups (Supplementary Table S8). Audet-Delage and co-workers (134) reported that the levels of 115 acylcholines, monoacylglycerols, and acylcarnitines were increased while the levels of 22 free fatty acids were decreased in 26 postmenopausal EC patients (type I and II, recurrent and non-recurrent) *versus* 18 patients with benign conditions. The authors identified a series of metabolites specific for recurrent EC, where bile acids were increased in type I and sphingolipids in type II recurrent EC. The authors constructed a diagnostic model (including the levels of spermine, isovalerate, glycylvaline and gamma-glutamyl-2-aminobutyrate) with an AUC of 0.92 and a prognostic model (including 2-oleoylglycerol and TAG 42.2-GA12:0) that separated between recurrent and non-recurrent EC with an AUC of 0.90. Troisi and colleagues (136) used a GC-MS approach and determined the metabolic profiles in 118 EC patients and 130 healthy women and control patients. Using several machine learning approaches and distinct patient cohorts, they constructed and validated a diagnostic model (EC *versus* healthy women) with accuracy of 0.99 and a prognostic model (type I/type II) with accuracy of 0.93. The first was based on increased levels of lactic acid, homocysteine, 3-hydroxybutyrate, and decreased levels of linoleic acid, stearic acid, myristic acid, threonine, valine and progesterone, whereas the latter on increased levels of progesterone and decreased levels of lactic acid, cystine, serine, malate, glutamate and homo-cysteine. Bahado-Singh and co-workers (138) performed NMR analysis in 56 stage I-IV EC patients and 60 healthy women, divided in discovery (33 ECs and 36 healthy women) and validation phase (23 EC and 24 healthy women) and constructed several diagnostic logistic regression models based on lipid levels with an AUC above 0.8. The highest AUC (0.83) was reported for the combination of C14:2, PCae C38:1 and 3-hydroxybutyric acid. This model separated also between stage I-II EC and healthy women (AUC = 0.82). An exploratory MS analysis by Kozar et al. in 15 EC and 21 control patients (140) reported a Random Forest model including Cer 34:1;2, Cer 40:1;2, AC 16:1-OH and 1-methyladenosine with AUC of 0.92, but reported no validation. Yan et al. performed a MS-based study (142) which included 23 EC patients, 30 healthy women, 30 patients with endometrial polyps and 12 patients with endometrial hyperplasia in the discovery phase and 50 EC patients (stage I-IV) and 195 healthy women, 171 polyps and 40 hyperplasia patients in validation phase. Their logistic regression models for separation between EC and endometrial polyps included 6-keto PGF1α, PA(37:4), LysoPC (20:1) and PS (36:0) and showed good characteristics with AUC > 0.90. A recent MS/MS targeted metabolomic study by Schuhn et al. performed in 20 EC patients, 157 healthy women and 14 control patients (143) reported that individual metabolites (carnitines and amino acids) allow stratification between EC and healthy women, and EC and control patients, with AUCs of 0.82 and 0.85 for malonylcarnitine and threonine, respectively (Supplementary Table S8). The study by Lunde et al. (139) performed NMR analysis in serum samples from 78 EC patients stored in Danish Cancer Biobank to determine metabolic profiles that allow identification of patients with chronic pelvic pain after hysterectomy. Using different machine learning approaches on metabolites with different levels in the two groups (Supplementary Table S8), several models were built, with the best diagnostic characteristics (AUC of 0.87) seen for linear support vector model.

Due to potential variability in the composition of serum, plasma represents the preferred source for biomarker discovery. However, so far a minority of the studies on blood metabolomics were performed using plasma. The first study by Ihata et al. (127) used MS to analyse plasma from 80 EC patients (stages I-IV), 122 patients with benign gynaecological diseases and 240 healthy women using training (40 EC and 120 healthy women) and validation sets (40 EC and 120 healthy women and 122 control patients). The authors built logistic regression diagnostic models based on panels of amino acids (histidine, isoleucine, valine and proline) that separated EC from healthy women (AUC > 0.91) and EC from control patients (AUC = 0.83; Supplementary Table S8). In a study by Knific et al. (128), 61 EC patients and 65 patients with benign uterine conditions were included. By employing LC-MS/MS analysis in training and test sets, the authors constructed diagnostic logistic regression models to separate EC from controls (AUC = 0.84) and prognostic models that allowed stratification of patients with lymphovascular invasion (LVI; AUC = 0.94) and myometrial invasion (AUC = 0.86). These were the first diagnostic and prognostic models of EC that included metabolite ratios. Strand et al. (129) used the same methodological approach but focused on prognostic biomarkers to identify metabolic differences between 20 EC patients with long *versus* 20 EC patients with short survival, where patients were matched for stage, grade, age, and BMI. Using Partial Least-Squares Discriminant Analysis (PLS-DA), three models with AUC up to 0.96 were constructed but none has been validated yet (Supplementary Table S8). Another MS-based study in plasma samples (130) focused on diagnosis of EC in obese patients (BMI > 30) and included 67 EC patients and 69 control patients (test and training sets). RF algorithms including 20 metabolites separated all EC patients from controls (AUC = 0.95) and showed even better characteristics for separation of stage I EC from control patients (AUC = 0.98). Individual metabolites showed potential as prognostic biomarkers and separated EC patients with/without LVI (AUC = 0.83; Supplementary Table S8). Other studies on serum/plasma metabolome in EC patients reported only different levels of metabolites in EC patients (133, 135, 137, 141) and associations of individual metabolites with EC (131, 132).

A well-designed GC-MS discovery study analysed dried blood samples analysed 50 postmenopausal EC patients and 70 patients without EC and validated prospectively the results among 1,430 postmenopausal women including 16 incident EC patients (Supplementary Table S8). Their ensemble machine-learning algorithm included 10 different classification models with accuracy of 99.9% (144). Among studies reporting serum and/or plasma metabolic profiles in EC patients, only few diagnostic/prognostic models have been validated in large multicenter studies, and the majority still awaits appropriate validation.

3.4.2 Metabolomics in other physiological fluids

Only two studies searched for biomarkers of endometrial cancer in urine samples (Supplementary Table S8). Shao et al. (145) used nontargeted metabolomics to determine differences in urine metabolic profiles from 25 EC patients, 25 healthy women and 10 endometrial hyperplasia patients and constructed PLS-DA and Support Vector Machine models, but provided no diagnostic characteristics. Yi et al. (101) analysed urine, tissue samples, and brushing endometrial samples and identified 285 metabolites in differential levels in urine samples from 10 EC patients compared with 10 control patients. PLS-DA based on the top 100 metabolites showed an AUC of 0.81. The cervicovaginal fluid from 21 EC patients and 33 non-EC controls was analysed by NMR using training and test sets (146). The levels of 29 metabolites differed between groups and RF and SVM models with accuracy up to 0.78 were constructed (Supplementary Table S8). These studies in urine samples and cervicovaginal fluid included a small number of samples thus future attempts for biomarker discovery should include respective metabolomics profiles from larger group of EC and control patients.

3.4.3 Tissue metabolomics

Seven studies explored the metabolic profiles in EC tissue. The first study was published in 2014 (149) and included 10 ECs and 10 control patients. NMR analysis revealed deviated concentration of of several amino acids, phosphocholine, glutathione, scyllo-inositol, myo-inositol, and inosine/adenosine in EC tissue (Supplementary Table S8) and the authors built a PLS-DA model with an AUC of 0.99. Arda Düz et al. (152) employed NMR to analyse tissues from 17 ECs and 18 control patients, and reported a number of candidate metabolite biomarkers e.g., lactate, alanina, phenylalanine and ratios glutamate/glutamine/methionine and leucine/isoleucine with AUCs up to 0.88 (Supplementary Table S8). A recent non-targeted NMR analysis on 64 EC tissue (patients with different grades of disease) and 10 tissues from patients with benign uterine diseases, identified using OPLS-DA the levels of a number of metabolites differentiating the patient groups (151). The concentrations of dimethylsulfone and phosphocholine were higher whereas the concentrations of glycerophosphocholine and glutamine were lower in low grade EC. In grade 1/2 EC, the levels of myoinositol were decreased and in grade 3 there were higher levels of 3-hydroxybutyrate, alanine, and betaine. The models constructed based on individual metabolites allowed separation between different grades of tumours with AUCs above 0.90 (Supplementary Table S8).

Other studies that investigated the metabolic profiles in cancerous tissues contributed mainly to a better understanding of the pathophysiology of EC, as these studies reported differential metabolites and dysregulated pathways in EC. Jove et al. (147) analysed 27 EC tissue samples and 15 normal endometrium samples by MS/MS and identified 44 differential metabolites including increased levels of stearamide, monoolein, hypoxanthine, 1,2-dihexadecanoyl-sn-glycerol (Supplementary Table S8). Comparison of cancer tissue of different grades identified 26 metabolites with increased levels of taurine and erythriol and decreased levels of oleamide. Importantly, this nontargeted metabolomic study used a novel approach as the authors examined the differences between surface EC and the myometrial invasion front and reported 104 differential metabolites (147). Altadil et al. (148) used non-targeted MS/MS to analyse 39 EC tissues and 17 control samples from postmenopausal women with stage I-III EC and benign diseases, respectively. Eighty metabolites, with 42 exhibiting differential levels, were identified with increased levels of taurine and erythriol and decreased levels of oleamide (Supplementary Table S8). Specific metabolites had different levels between cancers and controls (glutamate-phenylalanine-arginine-tryptophan, palmic amide, stearamide, oleamide, 2-phosphatidylserine, phosphatidylglycerol, inosine, and picolinic acid) or between stage I/II and stage III disease (phosphatidylcholines, phosphatidylethanolamines, and arachidonic acid; (Supplementary Table S8). Cummings et al. (150) performed a targeted metabolomic study on 108 cancer tissues, 53 samples of normal endometrium, 33 atrophic endometrium and 31 samples of atypical hyperplasia. The authors showed decreased concentrations of a number of metabolites including 13,14-dihydro-15-keto PGE_2 in type 1 and 2 EC versus normal endometrium and 12-HETE in EC type 2 versus type 1; Supplementary Table S8). Shafiee et al. (154) focused on the pathophysiology of EC and compared 34 cancerous tissues with 34 control endometrial tissues from patients with polycystic ovarian syndrome (PCOS). Their nontargeted MS-based analysis revealed changes mainly among lipids. Yi et al. (101) performed nontargeted metabolomics in urine, intrauterine brushing, and tissues from 24 EC patients and 18 control patients where PLS-DA identified 74 metabolites of which 47 were found in higher levels and 27 in lower levels. Comparison of metabolic profiles in tissue samples to urine and brushing samples showed that 49 of 74 metabolites were also detected in urine samples and 21 of 74 metabolites in intrauterine brushing samples, which supports the potential of urine metabolomics profiles for non-invasive diagnostic/prognosis (Supplementary Table S8). A recent study explored the metabolic profiles in biobanked tissue samples from endometrioid (n=20) and serous EC (n=11) (153). Using non-targeted MS analysis, 232 metabolic differences could be characterised (Supplementary Table S8).

Three metabolomic studies using tissue samples (149, 151, 152) identified individual metabolites and constructed diagnostic models with promising AUC values; however, these studies included very limited number of patients.

3.5 Combined metabolomics/proteomics

There was only one study that employed a combined omics approach (101) and performed nontargeted metabolomics on 24 cancer and 20 control tissue samples (Supplementary Table S7) and also nontargeted proteomics on a subset of 12 cancer and 9 control tissue samples by LC-MS/MS. The authors identified 1,445 proteins significantly up- or down-regulated in the EC group compared with the control group (adj. $p<0.05$, $FC<1.5$). To further characterise any relation between the metabolic and proteomic profiles, the authors performed network analysis that showed 28 metabolites and 135 proteins with 212 connections. Glutamine, dopamine, noradrenaline, adenosine-5-monophospate, and guanosine-5'-monophosphate were the major centres of sub-networks showing differences in amino acid and nucleotide metabolism.

3.6 Additional multi-omics or pan-cancer studies

A number of studies explored the proteome in patients with EC, but such analyses were part of a larger multi-omics approach, or part of systems-biology/pan-cancer approaches to study human disease or to establish databases and repositories. Most of these studies did not use proteomics for biomarker discovery, but as tools to understand the pathophysiological processes.

Five studies aiming at improved patient classification were performed within the TCGA consortium and used RPPA as proteomic method (22, 92, 94, 95, 99). Two studies demonstrated the utility of functional proteomics based on RPPA next to genomics and transcriptomics (92) and further created the bioinformatic resource 'The Cancer Proteome Atlas' (TCPA; (94)). The study of Kandoth and co-workers (13) explored a cohort of 373 EC patients, including 307 endometrioid and 53 serous or mixed histology cases to assess somatic mutations, copy number alterations, RNA expression, protein expression, DNA methylation and micro-RNA expression. With regard to proteomics, 293 samples were analysed by RPPA and several differentially expressed proteins were associated with other specific molecular tumour features (22). One of the latter two TCGA studies did not use proteomics (99), whereas the other study (95) explored 57 carcinosarcomas and -also in this case-, protein analyses were used to confirm other features identified with the molecular analyses (like EMT transitions, PI3K/AKT pathway activation, low steroid hormone receptor signalling). Similar to the TCPA (initiated within TCGA), the Clinical Proteomic Tumor Analysis Consortium (CTPAC) generated large proteomic datasets across various tumour types, and further characterised the proteogenomic landscape in EC (100). A pan-cancer study (98) aimed to characterise the actionable mutations across different solid tumours (including EC) and used RPPA to demonstrate PI3K and MAPK signalling pathways, whereas one study was focussed on human diseases other than cancer (97).

4 Discussion

In this study, we systematically reviewed all papers that explored the proteome or the metabolome in search for candidate biomarkers for prognostic or diagnostic purposes in EC. After screening the retrieved publications, we included 23 studies on proteomics (serum, plasma, uterine aspirate or tissue) and 29 studies on metabolomics (serum, plasma, urine, intra-uterine brushing, dried blood, cervicovaginal fluid or tissue).

Proteomic studies on body fluids and tissues (first fresh frozen then FFPE) have been published from second half of 2000. Initial studies were pilot in nature, and enrolled only a few patients (Supplementary Table S6). The first metabolomic studies were published one decade later, and study populations were in general larger than those used for proteomics. Seven out of 23 proteomic and 14 out of 19 metabolomic studies reported the performance of the models as AUCs, and several candidate biomarkers show great potential with AUC values above 0.8. However, the majority of the reported proteins or metabolites and corresponding models represent biomarker candidates that still require validation. The models developed need evaluation of their statistical performance by splitting the data into training and test sets (so called "statistical validation") and further experimental validation on independent cohort is essential (156).

With regard to statistical validation, this was performed by two proteomic studies (two-thirds/one-third cross-validation (115); leave-one-out-cross-validation (113) and four metabolomic studies (127, 128, 136, 142). Data on differentially expressed proteins was also confirmed experimentally using alternative methodologies like dot-blot (115), IHC (119, 121), RNA/qRT-PCR (117, 119), western blotting (110, 117), *in vitro* functional studies (117, 121) or using existing databases and repositories (119, 121).

In the context of proteomics, four studies only validated their data using independent sample cohorts (104, 107, 113, 119), and one study (115), validated their proteomic-based model in a subsequent publication using TMAs (126). Three metabolomic studies performed similar validations in independent cohorts (138, 142, 144), and one study in particular successfully validated the metabolic profiles identified in serum samples (136) also using dried blood and reported excellent diagnostic characteristics (144). For validation, the authors adopted a prospective design on a very large study cohort (over 1,000 subjects), therefore, candidate biomarkers identified in this study bear great potential for translation into clinical practice. Of interest, these biomarkers include steroids, which were also selected as candidate molecules in other studies (135, 157)

A caveat in a number of the included studies on proteomics (102, 104, 108) or metabolomics (136, 138, 142, 143) is the use of healthy (not age/comorbidity matched) women, likely resulting in an overestimated diagnostic accuracy. Also, if pre-menopausal controls are included, this may induce biases as EC is predominantly a postmenopausal disease (114–116, 122, 147, 149, 154). Additionally, biomarker discovery preferably includes a relevant population needing diagnostic tests such as women with postmenopausal uterine bleeding, or high-risk women, e.g., patients treated with tamoxifen or with Lynch syndrome, but also women with PCOS and obesity. Some proteomic studies focussed on these target groups, like obese subjects (119), or women with previous exposure to tamoxifen (124). One metabolomic study was performed in an obese population, and reported a Random Forest-based diagnostic model combining the top 10 performing metabolites that stratified stage I EC from other obese patients with an AUC of 0.98 (130), whereas the second metabolomic study included patients with PCOS (154).

A final relevant confounder is the ethnic background, known to affect the proteome profiles (83, 84). However, only one study used a cohort from a different geographical background, although still Caucasian to validate their data (119).

In a diagnostic/preoperative workup, ideal biomarkers should be present in easily and minimally invasively obtained body specimens. The proteomic studies included in this review predominantly used blood (serum of plasma) or uterine aspirate. The pilot studies using urine, although possibly the ideal body fluid

for biomarker detection, were too small to be included (Supplementary Table S6). Also in the context of metabolomics, only a few studies were performed on physiological fluids other than blood. One study explored cervicovaginal fluid (146) and two studies used urine samples, both of which have important limitations as they either used healthy women as controls (145) or included a few samples only (10 EC patients and 10 control patients) (101). Despite these limitations, these studies are also promising and future attempts for biomarker discovery in urine or cervicovaginal fluids using larger group of EC and control patients are warranted.

Importantly, although research groups were often able to validate their own candidate biomarkers in subsequent studies, rarely data was confirmed by independent authors/researchers, most probably due to methodological issues and also the abovementioned biases associated with geographical locations/ethnicity and lifestyles. Of note, there were candidate biomarkers that were validated in independent studies, and these represent highly promising molecules. Besides lipids, phospholipids and steroids, candidate proteins were reported as well. ANXA1 is described upregulated by three independent groups in EC tissues, uterine aspirates, and lymph nodes contaminated with EC cells (112, 113, 118, 123). ANXA1, annexin A1, plays an important role in immunity and inflammation and is associated with various diseases and cancers (158). HSPB1 was found upregulated in EC tissues and uterine aspirates by two teams (112, 113, 118). The *HSPB1* gene encodes for Heat Shock Protein Family B (Small) Member 1, a protein that is associated with gynaecological cancers (159). In addition, SERPINC1, APOA4, APOE and ITIH4 are described deviated in the serum of hyperplasia or cancer patients by two teams (106, 109). In metabolomic studies, both molecule levels but also the ratio between levels of molecules proved to be good biomarkers, and a number of studies included metabolite ratios in modelling/analyses (128, 130, 132, 148, 152).

Overall, the major limitations of the studies published up to data are: i) the use of small study cohorts; ii) the diagnostic or prognostic accuracy was seldom compared with other known biomarkers or reference molecules (e.g., CA-125). iii) the 95% confidence intervals for AUC values, sensitivity and specificity were rarely reported; iv) as outlined above, validation in independent cohorts was done by a few studies only; specifically in metabolomic studies, validation using other technologies was never performed.

4.1 Strengths and limitations of the present study

The limitations reported above related to the papers retrieved and reviewed are reflected also in the present study, i.e., small study cohorts, potenial pre-analytical and analytical bias, potential bias due to ethnic background, lifestyle; lack of validations; no comparison with a reference or gold standard. This, in combination with the heterogeneity in study designs and in the technologies adopted precluded us making any meta-analyses of identified candidate biomarkers, whose potential can be assessed at a qualitative level only. This implies the impossibility to make any clinically relevant recommendation or conclusion at this moment. Nevertheless, the strength of the present study is that, due to the rigorous systematic approach we adopted, it offers a balanced and realistic view of the potential of these technologies for the future. It sets the milestones in proteomic and metabolomic biomarker discovery research, and indicates the path to follow in the future (see paragraph 4.2. Recommendations for further biomarker discovery).

It should be also noted that recently systematic reviews focused on metabolomic and/or proteomic biomarkers for diagnosis of EC (160), and liquid biomarkers for diagnosis of EC (161). However, our systematic review has additional unique strengths: we did not limit our analyses on one biospecimen only, but focused on metabolomics and proteomics in different biospecimens; we rigorously assessed the study quality using QUADOMICS and analysed additional potential conflicts of interest. The study quality was also assessed by Karkia and co-workers (161), however, these authors included only studies published in the two years prior to the publication, whereas we did not set a time limit for publication. Additionally, we used strict inclusion and exclusion criteria, which were defined and deposited in the PROSPERO repository prior to the start of our work. We finally provide comprehensive tables with all available diagnostic accuracy data (AUC, sensitivity, specificity).

4.2 Recommendations for further biomarker discovery

As thoroughly discussed, body fluids and liquid biopsies represent the most suitable material for diagnostic and prognostic biomarkers (162, 163), as they capture the disease heterogeneity better than a biopsy and they are non- or minimally invasive, thus create less anxiety in patients. In the context of the most appropriate body material, plasma preparation is less prone to technical (pre-analytical) biases than serum. Therefore, plasma represents the preferred source at least for the discovery phase of non-invasive diagnostic/prognostic biomarkers. Urine also represents an important clinical sample for non-invasive diagnostics (155), calling for further biomarker discovery studies. However, urine poses a challenge for biobanking, as large sample volumes are needed for analyses (this applies not only if 24-hour urine needs to be collected, but also for morning urine, which is common in biomarker discovery studies).

In terms of methodology, statistical and experimental validation in independent cohorts should be an intrinsic part of biomarker discovery studies, and inclusion of study populations with distinct lifestyles, geographical regions of origin and ethnic backgrounds is essential to identify candidate biomarkers truly associated with diseases. Strict standard operation procedures for sample collection and processing should be prepared by experts and rigorously followed (164). The importance of adhering to quality standards is also emphasized in a recent narrative review on biomarker discovery in EC (165).

5 Conclusions and future prospects

Clinically, there is a great need for non/minimally invasive biomarkers of EC that could serve as replacement test for endometrial biopsy or a triage test to select patients for further invasive diagnosis. Tissue biomarkers are also needed to allow preoperative stratification of patients and further individualised treatment. Recent advances in analytical technologies and computational approaches that can handle increasingly larger numbers of features offer unprecedented potentials to develop diagnostic and prognostic tools. The studies performed so far were in most cases pilot or explorative in nature, and heterogeneous in terms of study design, technology, and methodologies. These aspects need harmonisation for the future, and the study quality should be scrupulously monitored by journals, reviewers, and stakeholders in order to ensure translationability of the discoveries.

BIOENDOCAR

Members of the consortium besides the authors: Špela Smrkolj, Luka Roškar, Boštjan Pirš, Department of Gynecology, University Medical Centre Ljubljana, Slovenia; Aneta Adamiak-Godlewska, Sara Wawrysiuk and Ola Kaminska, Department of Gynaecology, Lublin Medical University, Poland. External collaborators: Jure Knez, Monika Sobočan and Iztok Takač, University Medical Centre Maribor, Slovenia; Fabio Barra, Department of Obstetrics and Gynaecology, IRCCS Ospedale Sacro Cuore - Don Calabria, Genoa, Italy; Simone Ferrero, Academic Unit of Obstetrics and Gynaecology, IRCCS Ospedale Policlinico San Martino; Vit Weinberger and Petra Vinklerova, Department of Gynecology and Obstetrics, University Hospital Brno, Czechia.

Author contributions

AR, TR, HW, AS, CL, CS, AG, JA, DF, and JT contributed to design the study, AR and TR to the literature search, AR, TR, HW, AS, CL, AG, DF and JT to writing the manuscript and issuing the figures. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Authors CL, CS, and AG are or were employed by Sciomics GmbH. Author DF is employed by Quretec Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1120178/full#supplementary-material

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2018) 68(6):394–424. doi: 10.3322/caac.21492

2. Renehan AG, Soerjomataram I, Leitzmann MF. Interpreting the epidemiological evidence linking obesity and cancer: a framework for population-attributable risk estimations in Europe. *Eur J Cancer* (2010) 46(14):2581–92. doi: 10.1016/j.ejca.2010.07.052

3. Rota M, Rumi F, Bagnardi V, Dal Maso L, Zucchetto A, Levi F, et al. Modelling body mass index and endometrial cancer risk in a pooled-analysis of three case-control studies. *BJOG* (2016) 123(2):285–92. doi: 10.1111/1471-0528.13717

4. Vasen HF, Blanco I, Aktan-Collan K, Gopie JP, Alonso A, Aretz S, et al. Revised guidelines for the clinical management of lynch syndrome (Hnpcc): recommendations by a group of European experts. *Gut* (2013) 62(6):812–23. doi: 10.1136/gutjnl-2012-304356

5. Schmeler KM, Lynch HT, Chen LM, Munsell MF, Soliman PT, Clark MB, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the lynch syndrome. *N Engl J Med* (2006) 354(3):261–9. doi: 10.1056/NEJMoa052627

6. Mallozzi M, Leone C, Manurita F, Bellati F, Caserta D. Endocrine disrupting chemicals and endometrial cancer: An overview of recent laboratory evidence and epidemiological studies. *Int J Environ Res Public Health* (2017) 14(3):334. doi: 10.3390/ijerph14030334

7. Adani G, Filippini T, Wise LA, Halldorsson TI, Blaha L, Vinceti M. Dietary intake of acrylamide and risk of breast, endometrial, and ovarian cancers: a systematic review

and dose-response meta-analysis. *Cancer Epidemiol Biomarkers Prev* (2020) 29 (6):1095–106. doi: 10.1158/1055-9965.EPI-19-1628

8. Amant F, Mirza MR, Koskas M, Creutzberg CL. Cancer of the corpus uteri. *Int J Gynaecol Obstet* (2018) 143 Suppl 2:37–50. doi: 10.1002/ijgo.12612

9. Liu Y, Patel L, Mills GB, Lu KH, Sood AK, Ding L, et al. Clinical significance of Ctnnb1 mutation and wnt pathway activation in endometrioid endometrial carcinoma. *J Natl Cancer Inst* (2014) 106(9). doi: 10.1093/jnci/dju245

10. Stelloo E, Nout RA, Osse EM, Jurgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, et al. Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the portec cohorts. *Clin Cancer Res* (2016) 22(16):4215–24. doi: 10.1158/1078-0432.CCR-15-2878

11. Karnezis AN, Leung S, Magrill J, McConechy MK, Yang W, Chow C, et al. Evaluation of endometrial carcinoma prognostic immunohistochemistry markers in the context of molecular classification. *J Pathol Clin Res* (2017) 3(4):279–93. doi: 10.1002/cjp2.82

12. Kurnit KC, Kim GN, Fellman BM, Urbauer DL, Mills GB, Zhang W, et al. Ctnnb1 (Beta-catenin) mutation identifies low grade, early stage endometrial cancer patients at increased risk of recurrence. *Mod Pathol* (2017) 30(7):1032–41. doi: 10.1038/modpathol.2017.15

13. Kommoss FK, Karnezis AN, Kommoss F, Talhouk A, Taran FA, Staebler A, et al. L1cam further stratifies endometrial carcinoma patients with no specific molecular risk profile. *Br J Cancer* (2018) 119(4):480–6. doi: 10.1038/s41416-018-0187-6

14. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, et al. Final validation of the promise molecular classifier for endometrial carcinoma in a Large population-based case series. *Ann Oncol* (2018) 29(5):1180–8. doi: 10.1093/annonc/mdy058

15. Moroney MR, Davies KD, Wilberger AC, Sheeder J, Post MD, Berning AA, et al. Molecular markers in recurrent stage I, grade 1 endometrioid endometrial cancers. *Gynecol Oncol* (2019) 153(3):517–20. doi: 10.1016/j.ygyno.2019.03.100

16. De Leo A, de Biase D, Lenzi J, Barbero G, Turchetti D, Grillini M, et al. Arid1a and Ctnnb1/Beta-catenin molecular status affects the clinicopathologic features and prognosis of endometrial carcinoma: implications for an improved surrogate molecular classification. *Cancers (Basel)* (2021) 13(5). doi: 10.3390/cancers13050950

17. Guo M, Gong H, Nie D, Li Z. High L1cam expression predicts poor prognosis of patients with endometrial cancer: a systematic review and meta-analysis. *Med (Baltimore)* (2021) 100(13):e25330. doi: 10.1097/MD.0000000000025330

18. Vrede SW, van Weelden WJ, Visser NCM, Bulten J, van der Putten LJM, van de Vijver K, et al. Immunohistochemical biomarkers are prognostic relevant in addition to the esmo-Esgo-Estro risk classification in endometrial cancer. *Gynecol Oncol* (2021) 161(3):787–94. doi: 10.1016/j.ygyno.2021.03.031

19. Perrone E, De Felice F, Capasso I, Distefano E, Lorusso D, Nero C, et al. The immunohistochemical molecular risk classification in endometrial cancer: a pragmatic and high-reproducibility method. *Gynecol Oncol* (2022) 165(3):585–93. doi: 10.1016/j.ygyno.2022.03.009

20. Travaglino A, Raffone A, Raimondo D, Reppuccia S, Ruggiero A, Arena A, et al. Prognostic significance of Ctnnb1 mutation in early stage endometrial carcinoma: a systematic review and meta-analysis. *Arch Gynecol Obstet* (2022) 306(2):423–31. doi: 10.1007/s00404-021-06385-0

21. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, Marnitz S, et al. Esgo/Estro/Esp guidelines for the management of patients with endometrial carcinoma. *Int J Gynecol Cancer* (2021) 31(1):12–39. doi: 10.1136/ijgc-2020-002230

22. Cancer Genome Atlas Research N, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. *Nature* (2013) 497(7447):67–73. doi: 10.1038/nature12113

23. Talhouk A, Hoang LN, McConechy MK, Nakonechny Q, Leo J, Cheng A, et al. Molecular classification of endometrial carcinoma on diagnostic specimens is highly concordant with final hysterectomy: earlier prognostic information to guide treatment. *Gynecol Oncol* (2016) 143(1):46–53. doi: 10.1016/j.ygyno.2016.07.090

24. Visser NCM, Reijnen C, Massuger L, Nagtegaal ID, Bulten J, Pijnenborg JMA. Accuracy of endometrial sampling in endometrial carcinoma: a systematic review and meta-analysis. *Obstet Gynecol* (2017) 130(4):803–13. doi: 10.1097/AOG.0000000000002261

25. Narice BF, Delaney B, Dickson JM. Endometrial sampling in low-risk patients with abnormal uterine bleeding: a systematic review and meta-synthesis. *BMC Fam Pract* (2018) 19(1):135. doi: 10.1186/s12875-018-0817-3

26. Messner CB, Demichev V, Wang Z, Hartl J, Kustatscher G, Mulleder M, et al. Mass spectrometry-based high-throughput proteomics and its role in biomedical studies and systems biology. *Proteomics* (2022) e2200013. doi: 10.1002/pmic.202200013

27. Aerqin Q, Wang ZT, Wu KM, He XY, Dong Q, Yu JT. Omics-based biomarkers discovery for alzheimer's disease. *Cell Mol Life Sci* (2022) 79(12):585. doi: 10.1007/s00018-022-04614-6

28. Neagu AN, Jayathirtha M, Whitham D, Mutsengi P, Sullivan I, Petre BA, et al. Proteomics-based identification of dysregulated proteins in breast cancer. *Proteomes* (2022) 10(4). doi: 10.3390/proteomes10040035

29. Agregan R, Pateiro M, Kumar M, Franco D, Capanoglu E, Dhama K, et al. The potential of proteomics in the study of processed meat products. *J Proteomics* (2023) 270:104744. doi: 10.1016/j.jprot.2022.104744

30. Li W, Shao C, Zhou H, Du H, Chen H, Wan H, et al. Multi-omics research strategies in ischemic stroke: a multidimensional perspective. *Ageing Res Rev* (2022) 81:101730. doi: 10.1016/j.arr.2022.101730

31. Zou Z, Sun W, Xu Y, Liu W, Zhong J, Lin X, et al. Application of multi-omics approach in sarcomas: a tool for studying mechanism, biomarkers, and therapeutic targets. *Front Oncol* (2022) 12:946022. doi: 10.3389/fonc.2022.946022

32. Ren AH, Diamandis EP, Kulasingam V. Uncovering the depths of the human proteome: Antibody-based technologies for ultrasensitive multiplexed protein detection and quantification. *Mol Cell Proteomics* (2021) 20:100155. doi: 10.1016/j.mcpro.2021.100155

33. Hoheisel JD, Alhamdani MS, Schroder C. Affinity-based microarrays for proteomic analysis of cancer tissues. *Proteomics Clin Appl* (2013) 7(1-2):8–15. doi: 10.1002/prca.201200114

34. Solier C, Langen H. Antibody-based proteomics and biomarker research - current status and limitations. *Proteomics* (2014) 14(6):774–83. doi: 10.1002/pmic.201300334

35. Tang N, Tornatore P, Weinberger SR. Current developments in seldi affinity technology. *Mass Spectrom Rev* (2004) 23(1):34–44. doi: 10.1002/mas.10066

36. Bantscheff M, Schirle M, Sweetman G, Rick J, Kuster B. Quantitative mass spectrometry in proteomics: a critical review. *Anal Bioanal Chem* (2007) 389(4):1017–31. doi: 10.1007/s00216-007-1486-6

37. Anderson NL. The clinical plasma proteome: A survey of clinical assays for proteins in plasma and serum. *Clin Chem* (2010) 56(2):177–85. doi: 10.1373/clinchem.2009.126706

38. Makawita S, Diamandis EP. The bottleneck in the cancer biomarker pipeline and protein quantification through mass spectrometry-based approaches: current strategies for candidate verification. *Clin Chem* (2010) 56(2):212–22. doi: 10.1373/clinchem.2009.127019

39. Torres S, Bartolome RA, Mendes M, Barderas R, Fernandez-Acenero MJ, Pelaez-Garcia A, et al. Proteome profiling of cancer-associated fibroblasts identifies novel proinflammatory signatures and prognostic markers for colorectal cancer. *Clin Cancer Res* (2013) 19(21):6006–19. doi: 10.1158/1078-0432.CCR-13-1130

40. Tweeddale H, Notley-McRobb L, Ferenci T. Effect of slow growth on metabolism of escherichia coli, as revealed by global metabolite pool ("Metabolome") analysis. *J Bacteriol* (1998) 180(19):5109–16. doi: 10.1128/JB.180.19.5109-5116.1998

41. Patti GJ, Yanes O, Siuzdak G. Innovation: Metabolomics: the apogee of the omics trilogy. *Nat Rev Mol Cell Biol* (2012) 13(4):263–9. doi: 10.1038/nrm3314

42. Tokarz J, Haid M, Cecil A, Prehn C, Artati A, Moller G, et al. Endocrinology meets metabolomics: achievements, pitfalls, and challenges. *Trends Endocrinol Metab* (2017) 28(10):705–21. doi: 10.1016/j.tem.2017.07.001

43. Hocher B, Adamski J. Metabolomics for clinical use and research in chronic kidney disease. *Nat Rev Nephrol* (2017) 13(5):269–84. doi: 10.1038/nrneph.2017.30

44. Kostidis S, Addie RD, Morreau H, Mayboroda OA, Giera M. Quantitative nmr analysis of intra- and extracellular metabolism of mammalian cells: a tutorial. *Anal Chim Acta* (2017) 980:1–24. doi: 10.1016/j.aca.2017.05.011

45. Schripsema J. Application of nmr in plant metabolomics: techniques, problems and prospects. *Phytochem Anal* (2010) 21(1):14–21. doi: 10.1002/pca.1185

46. Naz S, Vallejo M, Garcia A, Barbas C. Method validation strategies involved in non-targeted metabolomics. *J Chromatogr A* (2014) 1353:99–105. doi: 10.1016/j.chroma.2014.04.071

47. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, et al. The human serum metabolome. *PloS One* (2011) 6(2):e16957. doi: 10.1371/journal.pone.0016957

48. Zheng Y. Study design considerations for cancer biomarker discoveries. *J Appl Lab Med* (2018) 3(2):282–9. doi: 10.1373/jalm.2017.025809

49. Ottas A, Fishman D, Okas TL, Pussa T, Toomik P, Martson A, et al. Blood serum metabolome of atopic dermatitis: Altered energy cycle and the markers of systemic inflammation. *PloS One* (2017) 12(11):e0188580. doi: 10.1371/journal.pone.0188580

50. Fishman D, Kisand K, Hertel C, Rothe M, Remm A, Pihlap M, et al. Autoantibody repertoire in apeced patients targets two distinct subgroups of proteins. *Front Immunol* (2017) 8:976. doi: 10.3389/fimmu.2017.00976

51. Duarte JG, Blackburn JM. Advances in the development of human protein microarrays. *Expert Rev Proteomics* (2017) 14(7):627–41. doi: 10.1080/14789450.2017.1347042

52. Abel L, Kutschki S, Turewicz M, Eisenacher M, Stoutjesdijk J, Meyer HE, et al. Autoimmune profiling with protein microarrays in clinical applications. *Biochim Biophys Acta* (2014) 1844(5):977–87. doi: 10.1016/j.bbapap.2014.02.023

53. Diez P, Dasilva N, Gonzalez-Gonzalez M, Matarraz S, Casado-Vela J, Orfao A, et al. Data analysis strategies for protein microarrays. *Microarrays (Basel)* (2012) 1 (2):64–83. doi: 10.3390/microarrays1020064

54. Xi Y, Rocke DM. Baseline correction for nmr spectroscopic metabolomics data analysis. *BMC Bioinf* (2008) 9:324. doi: 10.1186/1471-2105-9-324

55. Feng C, Wang H, Lu N, Chen T, He H, Lu Y, et al. Log-transformation and its implications for data analysis. *Shanghai Arch Psychiatry* (2014) 26(2):105–9. doi: 10.3969/j.issn.1002-0829.2014.02.009

56. Sboner A, Karpikov A, Chen G, Smith M, Mattoon D, Freeman-Cook L, et al. Robust-Linear-Model normalization to reduce technical variability in functional protein microarrays. *J Proteome Res* (2009) 8(12):5451–64. doi: 10.1021/pr900412k

57. Da Gama Duarte J, Goosen RW, Lawry PJ, Blackburn JM. Pma: Protein microarray analyser, a user-friendly tool for data processing and normalization. *BMC Res Notes* (2018) 11(1):156. doi: 10.1186/s13104-018-3266-0

58. DeLuca DS, Marina O, Ray S, Zhang GL, Wu CJ, Brusic V. Data processing and analysis for protein microarrays. *Methods Mol Biol* (2011) 723:337–47. doi: 10.1007/978-1-61779-043-0_21

59. Bonferroni CE. Teoria statistica delle classi e calcolo delle probabilita. *Pubblicazioni del R Istituto Superiore di Sci Economiche e Commericiali di Firenze* (1936) 8:3–62.

60. Zumbo BD. *Univariate tests*. Netherlands: Springer (2014) p. 6819–20.

61. Tarca AL, Carey VJ, Chen XW, Romero R, Draghici S. Machine learning and its applications to biology. *PloS Comput Biol* (2007) 3(6):e116. doi: 10.1371/journal.pcbi.0030116

62. Nagele E, Han M, Demarshall C, Belinka B, Nagele R. Diagnosis of alzheimer's disease based on disease-specific autoantibody profiles in human sera. *PloS One* (2011) 6(8):e23112. doi: 10.1371/journal.pone.0023112

63. Quinlan JR. Induction of decision trees. *Mach Learn* (1986) 1(1):81–106. doi: 10.1007/BF00116251

64. Breiman RF. Vaccines as tools for advancing more than public health: perspectives of a former director of the national vaccine program office. *Clin Infect Dis* (2001) 32(2):283–8. doi: 10.1086/318461

65. Wu X, Kumar V, Ross Quinlan J, Ghosh J, Yang Q, Motoda H, et al. Top 10 algorithms in data mining. *Knowledge Inf Syst* (2008) 14(1):1–37. doi: 10.1007/s10115-007-0114-2

66. Walsh I, Fishman D, Garcia-Gasulla D, Titma T, Pollastri GGroup EMLF, et al. Dome: Recommendations for supervised machine learning validation in biology. *Nat Methods* (2021) 18(10):1122–7. doi: 10.1038/s41592-021-01205-4

67. McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PMthe P-DTAG, et al. Preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies: the prisma-dta statement. *JAMA* (2018) 319(4):388–96. doi: 10.1001/jama.2017.19163

68. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. Quadas-2: A revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* (2011) 155(8):529–36. doi: 10.7326/0003-4819-155-8-201110180-00009

69. Lumbreras B, Porta M, Marquez S, Pollan M, Parker LA, Hernandez-Aguado I. Quadomics: an adaptation of the quality assessment of diagnostic accuracy assessment (Quadas) for the evaluation of the methodological quality of studies on the diagnostic accuracy of '-Omics'-Based technologies. *Clin Biochem* (2008) 41(16-17):1316–25. doi: 10.1016/j.clinbiochem.2008.06.018

70. Fata CR, Seeley EH, Desouki MM, Du L, Gwin K, Hanley KZ, et al. Are clear cell carcinomas of the ovary and endometrium phenotypically identical? A proteomic analysis. *Hum Pathol* (2015) 46(10):1427–36. doi: 10.1016/j.humpath.2015.06.009

71. Liu Z, Hong Z, Qu P. Proteomic analysis of human endometrial tissues reveals the roles of Pi3k/Akt/Mtor pathway and tumor angiogenesis molecules in the pathogenesis of endometrial cancer. *BioMed Res Int* (2020). doi: 10.1155/2020/5273969. (pagination).

72. Lopez-Janeiro A, Ruz-Caracuel I, Ramon-Patino JL, Rios VDL, Esparza MV, Berjon A, et al. Proteomic analysis of low-grade, early-stage endometrial carcinoma reveals new dysregulated pathways associated with cell death and cell signaling. *Cancers* (2021) 13(4):1–16. doi: 10.3390/cancers13040794

73. Mariscal J, Fernandez-Puente P, Calamia V, Abalo A, Santacana M, Matias-Guiu X, et al. Proteomic characterization of epithelial-like extracellular vesicles in advanced endometrial cancer. *J Proteome Res* (2019) 18(3):1043–53. doi: 10.1021/acs.jproteome.8b00750

74. Raglan O, Assi N, Nautiyal J, Lu H, Gabra H, Gunter MJ, et al. Proteomic analysis of malignant and benign endometrium according to obesity and insulin-resistance status using reverse phase protein array. *Transl Res* (2020) 218:57–72. doi: 10.1016/j.trsl.2019.12.003

75. Acland M, Arentz G, Mussared M, Whitehead F, Hoffmann P, Klingler-Hoffmann M, et al. Proteomic analysis of pre-invasive serous lesions of the endometrium and fallopian tube reveals their metastatic potential. *Front Oncol* (2020) 10:523989(no pagination). doi: 10.3389/fonc.2020.523989

76. Hiramatsu K, Yoshino K, Serada S, Yoshihara K, Hori Y, Fujimoto M, et al. Similar protein expression profiles of ovarian and endometrial high-grade serous carcinomas. *Br J Cancer* (2016) 114(5):554–61. doi: 10.1038/bjc.2016.27

77. Maxwell GL, Hood BL, Day R, Chandran U, Kirchner D, Kolli VS, et al. Proteomic analysis of stage I endometrial cancer tissue: identification of proteins associated with oxidative processes and inflammation. *Gynecol Oncol* (2011) 121(3):586–94. doi: 10.1016/j.ygyno.2011.02.031

78. Monge M, Doll A, Colas E, Gil-Moreno A, Castellvi J, Garcia A, et al. Subtractive proteomic approach to the endometrial carcinoma invasion front. *J Proteome Res* (2009) 8(10):4676–84. doi: 10.1021/pr900390t

79. Seo G, Han H, Vargas RE, Yang B, Li X, Wang W. Map4k interactome reveals Strn4 as a key stripak complex component in hippo pathway regulation. *Cell Rep* (2020) 32(1):107860. doi: 10.1016/j.celrep.2020.107860

80. Yoneyama K, Shibata R, Igarashi A, Kojima S, Kodani Y, Nagata K, et al. Proteomic identification of dihydrolipoamide dehydrogenase as a target of autoantibodies in patients with endometrial cancer. *Anticancer Res* (2014) 34(9):5021–7.

81. Bateman NW, Teng PN, Hope E, Hood BL, Oliver J, Ao W, et al. Jupiter Microtubule-associated homolog 1 (Jpt1): a predictive and pharmacodynamic biomarker of metformin response in endometrial cancers. *Cancer Med* (2020) 9(3):1092–103. doi: 10.1002/cam4.2729

82. Kodell RL, Haun RS, Siegel ER, Zhang C, Trammel AB, Hauer-Jensen M, et al. Novel use of proteomic profiles in a convex-Hull ensemble classifier to predict gynecological cancer patients' susceptibility to gastrointestinal mucositis as side effect of radiation therapy. *J Proteomics Bioinform* (2015) 8(7):149–54. doi: 10.4172/jpb.1000363

83. Javadian P, Xu C, Sjoelund V, Borden LE, Garland J, Benbrook DM. Identification of candidate biomarker and drug targets for improving endometrial cancer racial disparities. *Int J Mol Sci* (2022) 23(14). doi: 10.3390/ijms23147779

84. Bateman NW, Dubil EA, Wang G, Hood BL, Oliver JM, Litzi TA, et al. Race-specific molecular alterations correlate with differential outcomes for black and white endometrioid endometrial cancer patients. *Cancer* (2017) 123(20):4004–12. doi: 10.1002/cncr.30813

85. Lin ST, Chou HC, Chang SJ, Chen YW, Lyu PC, Wang WC, et al. Proteomic analysis of proteins responsible for the development of doxorubicin resistance in human uterine cancer cells. *J Proteomics* (2012) 75(18):5822–47. doi: 10.1016/j.jprot.2012.07.047

86. Lin ST, Lo YW, Chang SJ, Wang WC, Chang MD, Lyu PC, et al. Redox-proteomic analysis of doxorubicin resistance-induced altered thiol activity in uterine carcinoma. *J Pharm BioMed Anal* (2013) 78-79:1–8. doi: 10.1016/j.jpba.2013.01.028

87. Lo YW, Lin ST, Chang SJ, Chan CH, Lyu KW, Chang JF, et al. Mitochondrial proteomics with sirna knockdown to reveal Acat1 and Mdh2 in the development of doxorubicin-resistant uterine cancer. *J Cell Mol Med* (2015) 19(4):744–59. doi: 10.1111/jcmm.12388

88. Casado-Vela J, Rodriguez-Suarez E, Iloro I, Ametzazurra A, Alkorta N, Garcia-Velasco JA, et al. Comprehensive proteomic analysis of human endometrial fluid aspirate. *J Proteome Res* (2009) 8(10):4622–32. doi: 10.1021/pr9004426

89. Monsivais D, Vasquez YM, Chen F, Zhang Y, Chandrashekar DS, Faver JC, et al. Mass-Spectrometry-Based proteomic correlates of grade and stage reveal pathways and kinases associated with aggressive human cancers. *Oncogene* (2021) 40(11):2081–95. doi: 10.1038/s41388-021-01681-0

90. Lai J, Xu T, Yang H. Protein-based prognostic signature for predicting the survival and immunotherapeutic efficiency of endometrial carcinoma. *BMC Cancer* (2022) 22(1):325. doi: 10.1186/s12885-022-09402-w

91. Zhang Q, Xia T, Qi C, Du J, Ye C. High expression of S100a2 predicts poor prognosis in patients with endometrial carcinoma. *BMC Cancer* (2022) 22(1). doi: 10.1186/s12885-022-09180-5

92. Akbani R, Ng PKS, Werner HMJ, Shahmoradgoli M, Zhang F, Ju Z, et al. A pan-cancer proteomic perspective on the cancer genome atlas. *Nat Commun* (2014) 5(3887). doi: 10.1038/ncomms4887

93. Berger AC, Korkut A, Kanchi RS, Hegde AM, Lenoir W, Liu W, et al. A comprehensive pan-cancer molecular study of gynecologic and breast cancers. *Cancer Cell* (2018) 33(4):690–705. doi: 10.1016/j.ccell.2018.03.014

94. Chen MJM, Li J, Wang Y, Akbani R, Lu Y, Mills GB, et al. Tcpa V3.0: An integrative platform to explore the pan-cancer analysis of functional proteomic data. *Mol Cell Proteomics* (2019) 18(8):S15–25. doi: 10.1074/mcp.RA118.001260

95. Cherniack AD, Shen H, Walter V, Stewart C, Murray BA, Bowlby R, et al. Integrated molecular characterization of uterine carcinosarcoma. *Cancer Cell* (2017) 31(3):411–23. doi: 10.1016/j.ccell.2017.02.010

96. Huang W, Chen J, Weng W, Xiang Y, Shi H, Shan Y. Development of cancer prognostic signature based on pan-cancer proteomics. *Bioengineered* (2020) 11(1):1368–81. doi: 10.1080/21655979.2020.1847398

97. Pietzner M, Wheeler E, Carrasco-Zanini J, Cortes A, Koprulu M, Worheide MA, et al. Mapping the proteo-genomic convergence of human diseases. *Science* (2021) 374(6569). doi: 10.1126/science.abj1541

98. Toomey S, Carr A, Mezynski MJ, Elamin Y, Rafee S, Cremona M, et al. Identification and clinical impact of potentially actionable somatic oncogenic mutations in solid tumor samples. *J Transl Med* (2020) 18(1):99. doi: 10.1186/s12967-020-02273-4

99. Iijima M, Banno K, Okawa R, Yanokura M, Iida M, Takeda T, et al. Genome-wide analysis of gynecologic cancer: The cancer genome atlas in ovarian and endometrial cancer. *Oncol Lett* (2017) 13(3):1063–70. doi: 10.3892/ol.2017.5582

100. Dou Y, Kawaler EA, Cui Zhou D, Gritsenko MA, Huang C, Blumenberg L, et al. Proteogenomic characterization of endometrial carcinoma. *Cell* (2020) 180(4):729–48.e26. doi: 10.1016/j.cell.2020.01.026

101. Yi R, Xie L, Wang X, Shen C, Chen X, Qiao L. Multi-omic profiling of multi-biosamples reveals the role of amino acid and nucleotide metabolism in endometrial cancer. *Front Oncol* (2022) 12:861142. doi: 10.3389/fonc.2022.861142

102. Zhu LR, Zhang WY, Yu L, Zheng YH, Zhang JZ, Liao QP. Serum proteomic features for detection of endometrial cancer. *Int J Gynecol Cancer* (2006) 16(3):1374–8. doi: 10.1111/j.1525-1438.2006.00561.x

103. Kikuchi S, Honda K, Handa Y, Kato H, Yamashita K, Umaki T, et al. Serum albumin-associated peptides of patients with uterine endometrial cancer. *Cancer Sci* (2007) 98(6):822–9. doi: 10.1111/j.1349-7006.2007.00458.x

104. Zhu LR, Zhang WY, Yu L, Zheng YH, Hu J, Liao QP. Proteiomic patterns for endometrial cancer using seldi-Tof-Ms. *J Zhejiang Univ Sci B* (2008) 9(4):286–90. doi: 10.1631/jzus.B0710589

105. Qiu F, Gao YH, Jiang CG, Tian YP, Zhang XJ. Serum proteomic profile analysis for endometrial carcinoma detection with maldi-tof Ms. *Arch Med Sci* (2010) 6(2):245–52. doi: 10.5114/aoms.2010.13903

106. Wang YS, Cao R, Jin H, Huang YP, Zhang XY, Cong Q, et al. Altered protein expression in serum from endometrial hyperplasia and carcinoma patients. *J Hematol Oncol* (2011) 4:15. doi: 10.1186/1756-8722-4-15

107. Enroth S, Berggrund M, Lycke M, Lundberg M, Assarsson E, Olovsson M, et al. A two-step strategy for identification of plasma protein biomarkers for endometrial and ovarian cancer. *Clin Proteomics* (2018) 15:38. doi: 10.1186/s12014-018-9216-y

108. Tarney CM, Wang G, Bateman NW, Conrads KA, Zhou M, Hood BL, et al. Biomarker panel for early detection of endometrial cancer in the prostate, lung, colorectal, and ovarian cancer screening trial. *Am J Obstet Gynecol* (2019) 221 (5):472.e1–.e10. doi: 10.1016/j.ajog.2019.06.005

109. Ura B, Biffi S, Monasta L, Arrigoni G, Battisti I, Di Lorenzo G, et al. Two dimensional-difference in gel electrophoresis (2d-dige) proteomic approach for the identification of biomarkers in endometrial cancer serum. *Cancers* (2021) 13(14). doi: 10.3390/cancers13143639

110. Celsi F, Monasta L, Arrigoni G, Battisti I, Licastro D, Aloisio M, et al. Gel-based proteomic identification of suprabasin as a potential new candidate biomarker in endometrial cancer. *Int J Mol Sci* (2022) 23(4). doi: 10.3390/ijms23042076

111. Ura B, Capaci V, Aloisio M, Di Lorenzo G, Romano F, Ricci G, et al. A targeted proteomics approach for screening serum biomarkers observed in the early stage of type I endometrial cancer. *Biomedicines* (2022) 10(8). doi: 10.3390/biomedicines10081857

112. Martinez-Garcia E, Lesur A, Devis L, Campos A, Cabrera S, van Oostrum J, et al. Development of a sequential workflow based on lc-prm for the verification of endometrial cancer protein biomarkers in uterine aspirate samples. *Oncotarget* (2016) 7 (33):53102–15. doi: 10.18632/oncotarget.10632

113. Martinez-Garcia E, Lesur A, Devis L, Cabrera S, Matias-Guiu X, Hirschfeld M, et al. Targeted proteomics identifies proteomic signatures in liquid biopsies of the endometrium to diagnose endometrial cancer and assist in the prediction of the optimal surgical treatment. *Clin Cancer Res* (2017) 23(21):6458–67. doi: 10.1158/1078-0432.Ccr-17-0474

114. Yoshizaki T, Enomoto T, Nakashima R, Ueda Y, Kanao H, Yoshino K, et al. Altered protein expression in endometrial carcinogenesis. *Cancer Lett* (2005) 226 (2):101–6. doi: 10.1016/j.canlet.2004.11.053

115. DeSouza LV, Grigull J, Ghanny S, Dube V, Romaschin AD, Colgan TJ, et al. Endometrial carcinoma biomarker discovery and verification using differentially tagged clinical samples with multidimensional liquid chromatography and tandem mass spectrometry. *Mol Cell Proteomics* (2007) 6(7):1170–82. doi: 10.1074/mcp.M600378-MCP200

116. Voisin SN, Krakovska O, Matta A, DeSouza LV, Romaschin AD, Colgan TJ, et al. Identification of novel molecular targets for endometrial cancer using a drill-down lc-Ms/Ms approach with itraq. *PloS One* (2011) 6(1):e16352. doi: 10.1371/journal.pone.0016352

117. Shan N, Zhou W, Zhang S, Zhang Y. Identification of Hspa8 as a candidate biomarker for endometrial carcinoma by using itraq-based proteomic analysis. *Onco Targets Ther* (2016) 9:2169–79. doi: 10.2147/ott.S97983

118. Ceylan Y, Akpinar G, Doger E, Kasap M, Guzel N, Karaosmanoglu K, et al. Proteomic analysis in endometrial cancer and endometrial hyperplasia tissues by 2d-dige technique. *J Gynecol Obstet Hum Reprod* (2020) 49(2):101652. doi: 10.1016/j.jogoh.2019.101652

119. Mauland KK, Ju Z, Tangen IL, Berg A, Kalland KH, Oyan AM, et al. Proteomic profiling of endometrioid endometrial cancer reveals differential expression of hormone receptors and mapk signaling proteins in obese versus non-obese patients. *Oncotarget* (2017) 8(63):106989–7001. doi: 10.18632/oncotarget.22203

120. Akkour K, Alanazi IO, Alfadda AA, Alhalal H, Masood A, Musambil M, et al. Tissue-based proteomic profiling in patients with hyperplasia and endometrial cancer. *Cells* (2022) 11(13). doi: 10.3390/cells11132119

121. Kurimchak AM, Kumar V, Herrera-Montavez C, Johnson KJ, Srivastava N, Davarajan K, et al. Kinome profiling of primary endometrial tumors using multiplexed inhibitor beads and mass spectrometry identifies Srpk1 as candidate therapeutic target. *Mol Cell Proteomics* (2020) 19(12):2068–89. doi: 10.1074/mcp.RA120.002012

122. DeSouza LV, Krakovska O, Darfler MM, Krizman DB, Romaschin AD, Colgan TJ, et al. Mtraq-based quantification of potential endometrial carcinoma biomarkers from archived formalin-fixed paraffin-embedded tissues. *Proteomics* (2010) 10 (17):3108–16. doi: 10.1002/pmic.201000082

123. Aboulouard S, Wisztorski M, Duhamel M, Saudemont P, Cardon T, Narducci F, et al. In-depth proteomics analysis of sentinel lymph nodes from individuals with endometrial cancer. *Cell Rep Med* (2021) 2(6). doi: 10.1016/j.xcrm.2021.100318

124. Janacova L, Faktor J, Capkova L, Paralova V, Pospisilova A, Podhorec J, et al. Swath-Ms analysis of ffpe tissues identifies stathmin as a potential marker of endometrial cancer in patients exposed to tamoxifen. *J Proteome Res* (2020) 19 (7):2617–30. doi: 10.1021/acs.jproteome.0c00064

125. DeSouza L, Diehl G, Rodrigues MJ, Guo J, Romaschin AD, Colgan TJ, et al. Search for cancer markers from endometrial tissues using differentially labeled tags itraq and cicat with multidimensional liquid chromatography and tandem mass spectrometry. *J Proteome Res* (2005) 4(2):377–86. doi: 10.1021/pr049821j

126. Dube V, Grigull J, DeSouza LV, Ghanny S, Colgan TJ, Romaschin AD, et al. Verification of endometrial tissue biomarkers previously discovered using mass spectrometry-based proteomics by means of immunohistochemistry in a tissue microarray format. *J Proteome Res* (2007) 6(7):2648–55. doi: 10.1021/pr070087o

127. Ihata Y, Miyagi E, Numazaki R, Muramatsu T, Imaizumi A, Yamamoto H, et al. Amino acid profile index for early detection of endometrial cancer: Verification as a novel diagnostic marker. *Int J Clin Oncol* (2014) 19(2):364–72. doi: 10.1007/s10147-013-0565-2

128. Knific T, Vouk K, Smrkolj Š, Prehn C, Adamski J, Rižner TL. Models including plasma levels of sphingomyelins and phosphatidylcholines as diagnostic and prognostic biomarkers of endometrial cancer. *J Steroid Biochem Mol Biol* (2018) 178:312–21. doi: 10.1016/j.jsbmb.2018.01.012

129. Strand E, Tangen IL, Fasmer KE, Jacob H, Halle MK, Hoivik EA, et al. Blood metabolites associate with prognosis in endometrial cancer. *Metabolites* (2019) 9 (12):302. doi: 10.3390/metabo9120302

130. Njoku K, Campbell AE, Geary B, MacKintosh ML, Derbyshire AE, Kitson SJ, et al. Metabolomic biomarkers for the detection of obesity-driven endometrial cancer. *Cancers (Basel)* (2021) 13(4). doi: 10.3390/cancers13040718

131. Kliemann N, Viallon V, Murphy N, Beeken RJ, Rothwell JA, Rinaldi S, et al. Metabolic signatures of greater body size and their associations with risk of colorectal and endometrial cancers in the European prospective investigation into cancer and nutrition. *BMC Med* (2021) 19(1):101. doi: 10.1186/s12916-021-01970-1

132. Dossus L, Kouloura E, Biessy C, Viallon V, Siskos AP, Dimou N, et al. Prospective analysis of circulating metabolites and endometrial cancer risk. *Gynecol Oncol* (2021) 162(2):475–81. doi: 10.1016/j.ygyno.2021.06.001

133. Breeur M, Ferrari P, Dossus L, Jenab M, Johansson M, Rinaldi S, et al. Pan-cancer analysis of pre-diagnostic blood metabolite concentrations in the European prospective investigation into cancer and nutrition. *BMC Med* (2022) 20(1):351. doi: 10.1186/s12916-022-02553-4

134. Audet-Delage Y, Villeneuve L, Grégoire J, Plante M, Guillemette C. Identification of metabolomic biomarkers for endometrial cancer and its recurrence after surgery in postmenopausal women. *Front Endocrinol* (2018) 9:87. doi: 10.3389/fendo.2018.00087

135. Audet-Delage Y, Grégoire J, Caron P, Turcotte V, Plante M, Ayotte P, et al. Estradiol metabolites as biomarkers of endometrial cancer prognosis after surgery. *J Steroid Biochem Mol Biol* (2018) 178:45–54. doi: 10.1016/j.jsbmb.2017.10.021

136. Troisi J, Sarno L, Landolfi A, Scala G, Martinelli P, Venturella R, et al. Metabolomic signature of endometrial cancer. *J Proteome Res* (2018) 17(2):804–12. doi: 10.1021/acs.jproteome.7b00503

137. Shi K, Wang Q, Su Y, Xuan X, Liu Y, Chen W, et al. Identification and functional analyses of differentially expressed metabolites in early stage endometrial carcinoma. *Cancer Sci* (2018) 109(4):1032–43. doi: 10.1111/cas.13532

138. Bahado-Singh RO, Lugade A, Field J, Al-Wahab Z, Han B, Mandal R, et al. Metabolomic prediction of endometrial cancer. *Metabolomics Off J Metabolomic Soc* (2017) 14(1):6. doi: 10.1007/s11306-017-1290-z

139. Lunde S, Nguyen HT, Petersen KK, Arendt-Nielsen L, Krarup HB, Søgaard-Andersen E. Chronic postoperative pain after hysterectomy for endometrial cancer: A metabolic profiling study. *Mol Pain* (2020) 16:1744806920923885. doi: 10.1177/1744806920923885

140. Kozar N, Kruusmaa K, Dovnik A, Bitenc M, Argamasilla R, Adsuar A, et al. Identification of novel diagnostic biomarkers in endometrial cancer using targeted metabolomic profiling. *Adv Med Sci* (2021) 66(1):46–51. doi: 10.1016/j.advms.2020.12.001

141. Gu M, Chen X, Sun Y, Wang L, Shu H, Qian C. A metabolomic signature of figo stage I and ii endometrial cancer. *Neoplasma* (2021) 68(6):1283–91. doi: 10.4149/neo_2021_210306N288

142. Yan X, Zhao W, Wei J, Yao Y, Sun G, Wang L, et al. A serum lipidomics study for the identification of specific biomarkers for endometrial polyps to distinguish them from endometrial cancer or hyperplasia. *Int J Cancer* (2022) 150(9):1549–59. doi: 10.1002/ijc.33943

143. Schuhn A, Tobar TW, Gahlawat AW, Hauke J, Baumann L, Okun JG, et al. Potential of blood-based biomarker approaches in endometrium and breast cancer: A case-control comparison study. *Arch Gynecol Obstet* (2022) 306(5):1623–32. doi: 10.1007/s00404-022-06482-8

144. Troisi J, Raffone A, Travaglino A, Belli G, Belli C, Anand S, et al. Development and validation of a serum metabolomic signature for endometrial cancer screening in postmenopausal women. *JAMA Network Open* (2020) 3(9):e2018327. doi: 10.1001/jamanetworkopen.2020.18327

145. Shao X, Wang K, Liu X, Gu C, Zhang P, Xie J, et al. Screening and verifying endometrial carcinoma diagnostic biomarkers based on a urine metabolomic profiling study using uplc-Q-Tof/Ms. *Clinica chimica acta; Int J Clin Chem* (2016) 463:200–6. doi: 10.1016/j.cca.2016.10.027

146. Cheng SC, Chen K, Chiu CY, Lu KY, Lu HY, Chiang MH, et al. Metabolomic biomarkers in cervicovaginal fluid for detecting endometrial cancer through nuclear magnetic resonance spectroscopy. *Metabolomics Off J Metabolomic Soc* (2019) 15 (11):146. doi: 10.1007/s11306-019-1609-z

147. Jove M, Gatius S, Yeramian A, Portero-Otin M, Eritja N, Santacana M, et al. Metabotyping human endometrioid endometrial adenocarcinoma reveals an

implication of endocannabinoid metabolism. *Oncotarget* (2016) 7(32):52364–74. doi: 10.18632/oncotarget.10564

148. Altadill T, Dowdy TM, Gill K, Reques A, Menon SS, Moiola CP, et al. Metabolomic and lipidomic profiling identifies the role of the rna editing pathway in endometrial carcinogenesis. *Sci Rep* (2017) 7(1):8803. doi: 10.1038/s41598-017-09169-2

149. Trousil S, Lee P, Pinato DJ, Ellis JK, Dina R, Aboagye EO, et al. Alterations of choline phospholipid metabolism in endometrial cancer are caused by choline kinase alpha overexpression and a hyperactivated deacylation pathway. *Cancer Res* (2014) 74 (23):6867–77. doi: 10.1158/0008-5472.CAN-13-2409

150. Cummings M, Massey KA, Mappa G, Wilkinson N, Hutson R, Munot S, et al. Integrated eicosanoid lipidomics and gene expression reveal decreased prostaglandin catabolism and increased 5-lipoxygenase expression in aggressive subtypes of endometrial cancer. *J Pathol* (2019) 247(1):21–34. doi: 10.1002/path.5160

151. Skorupa A, Ponski M, Ciszek M, Cichon B, Klimek M, Witek A, et al. Grading of endometrial cancer using ^{1}H hr-mas nmr-based metabolomics. *Sci Rep* (2021) 11 (1):18160. doi: 10.1038/s41598-021-97505-y

152. Arda Düz S, Mumcu A, Doğan B, Yılmaz E, İnci Çoşkun E, Sarıdogan E, et al. Metabolomic analysis of endometrial cancer by high-resolution magic angle spinning nmr spectroscopy. *Arch Gynecol Obstet* (2022) 306(6):2155–66. doi: 10.1007/s00404-022-06587-0

153. Gatius S, Jove M, Megino-Luque C, Alberti-Valls M, Yeramian A, Bonifaci N, et al. Metabolomic analysis points to bioactive lipid species and acireductone dioxygenase 1 (Adi1) as potential therapeutic targets in poor prognosis endometrial cancer. *Cancers (Basel)* (2022) 14(12). doi: 10.3390/cancers14122842

154. Shafiee MN, Ortori CA, Barrett DA, Mongan NP, Abu J, Atiomo W. Lipidomic biomarkers in polycystic ovary syndrome and endometrial cancer. *Int J Mol Sci* (2020) 21(13). doi: 10.3390/ijms21134753

155. Rizner TL. Noninvasive biomarkers of endometriosis: Myth or reality? *Expert Rev Mol Diagn* (2014) 14(3):365–85. doi: 10.1586/14737159.2014.899905

156. Xia J, Broadhurst DI, Wilson M, Wishart DS. Translational biomarker discovery in clinical metabolomics: an introductory tutorial. *Metabolomics Off J Metabolomic Soc* (2013) 9(2):280–99. doi: 10.1007/s11306-012-0482-9

157. Tangen IL, Fasmer KE, Konings G, Jochem A, Delvoux B, Xanthoulea S, et al. Blood steroids are associated with prognosis and fat distribution in endometrial cancer. *Gynecol Oncol* (2019) 152:46–52. doi: 10.1016/j.ygyno.2018.10.024

158. Lim LH, Pervaiz S. Annexin 1: the new face of an old molecule. *FASEB J* (2007) 21(4):968–75. doi: 10.1096/fj.06-7464rev

159. Wyciszkiewicz A, Kalinowska-Lyszczarz A, Nowakowski B, Kazmierczak K, Osztynowicz K, Michalak S. Expression of small heat shock proteins in exosomes from patients with gynecologic cancers. *Sci Rep* (2019) 9(1):9817. doi: 10.1038/s41598-019-46221-9

160. Raffone A, Troisi J, Boccia D, Travaglino A, Capuano G, Insabato L, et al. Metabolomics in endometrial cancer diagnosis: a systematic review. *Acta Obstet Gynecol Scand* (2020) 99(9):1135–46. doi: 10.1111/aogs.13847

161. Karkia R, Wali S, Payne A, Karteris E, Chatterjee J. Diagnostic accuracy of liquid biomarkers for the non-invasive diagnosis of endometrial cancer: a systematic review and meta-analysis. *Cancers (Basel)* (2022) 14(19). doi: 10.3390/cancers14194666

162. Muinelo-Romay L, Casas-Arozamena C, Abal M. Liquid biopsy in endometrial cancer: New opportunities for personalized oncology. *Int J Mol Sci* (2018) 19(8). doi: 10.3390/ijms19082311

163. Pineiro-Perez R, Abal M, Muinelo-Romay L. Liquid biopsy for monitoring ec patients: Towards personalized treatment. *Cancers (Basel)* (2022) 14(6). doi: 10.3390/cancers14061405

164. 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. doi: 10.1016/j.jsbmb.2018.09.017

165. Njoku K, Sutton CJ, Whetton AD, Crosbie EJ. Metabolomic biomarkers for detection, prognosis and identifying recurrence in endometrial cancer. *Metabolites* (2020) 10(8). doi: 10.3390/metabo10080314

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Glossary

AUC	area under the curve
BMI	body mass index
CPTAC	Clinical Proteomic Tumor Analysis Consortium
Da	Dalton
DFS	disease-free survival
DIGE	difference gel electrophoresis
EC	Endometrial cancer
ELISA	enzyme-linked immunosorbent assay
ER	estrogen receptor
ESI	electrospray ionization
FFPE	formalin-fixed-paraffin-embedded
FIGO	International federation of gynaecologic oncology
GC	Gas chromatography
HER2/Neu	Human Epidermal growth factor Receptor 2
HR	hazard ratios
iCAT	Isotope-coded affinity tag
IHC	immunohistochemistry
iTRAQ	Isobaric tags for relative and absolute quantitation
LC	Liquid chromatography
LFQ	Label free quantification (LFQ)
LVI	lymphovascular invasion
MALDI	matrix-assisted laser desorption/ionization
MIB	Multiplexed Inhibitor Beads
MRM	multiple-reaction monitoring
MS	mass spectrometry
MSI	mass spectrometry imaging
NMR	nuclear magnetic resonance spectroscopy
NPV	negative predictive value
OS	overall survival
PAGE	polyacrylamide gel electrophoresis
PCOS	Poly Cystic Ovarian Syndrome
PEA	proximity extension assay
PLS-DA	Partial Least-Squares Discriminant Analysis
PPV	positive predictive value
PR	progesterone receptor
PRM	parallel reaction monitoring
PTEN	Phosphatase and tensin homolog
RPPA	reverse phase protein microarray
SD	Standard Deviation
SDS	sodium dodecyl sulphate
SELDI	surface-enhanced laser desorption/ionization
SILAC	Stable isotope labelling by amino acids in cell culture
SLN	sentinel-lymph-node
SRM	Selected-reaction monitoring
TCGA	The Cancer Genome Atlas
TCGA	The Cancer Proteome Atlas
TMA	tissue microarray
TOF	time-of-flight

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EDITED BY
Tea Lanisnik Rizner,
University of Ljubljana, Slovenia

REVIEWED BY
Emanuele Perrone,
(IRCCS), Italy
Anthony Magliocco,
Protean BioDiagnostics Inc., United States

*CORRESPONDENCE
Francesco Alessandrino
falessandrino@med.miami.edu

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Uterine serous carcinoma: assessing association between genomics and patterns of metastasis

Francesco Alessandrino[1]*, Nicole Goncalves[2], Sarah Wishnek Metalonis[3], Cibele Luna[1], Matthew M. Mason[2], Jiangnan Lyu[3] and Marilyn Huang[4]

[1]Department of Radiology, University of Miami, Miami, FL, United States, [2]University of Miami Miller School of Medicine, Miami, FL, United States, [3]Division of Biostatistics, Department of Public Health Science, University of Miami, Miami, FL, United States, [4]Division of Gynecologic Oncology, Sylvester Comprehensive Cancer Center, University of Miami, FL, United States

Background: Uterine serous carcinoma (USC) is an aggressive subtype of endometrial carcinoma which has been increasing at alarming rates, particularly among Asian, Hispanic and Black women. USC has not been well characterized in terms of mutational status, pattern of metastases and survival.

Objective: To investigate the association between sites of recurrence and metastases of USC, mutational status, race, and overall survival (OS).

Methods: This single-center retrospective study evaluated patients with biopsy-proven USC that underwent genomic testing between January 2015 and July 2021. Association between genomic profile and sites of metastases or recurrence was performed using $\chi 2$ or Fisher's exact test. Survival curves for ethnicity and race, mutations, sites of metastasis/recurrence were estimated using the Kaplan-Meier method and compared with log-rank test. Cox proportional hazard regression models were used to examine the association between OS with age, race, ethnicity, mutational status, and sites of metastasis/recurrence. Statistical analyses were performed using SAS Software Version 9.4.

Results: The study included 67 women (mean age 65.8 years, range 44-82) with 52 non-Hispanic women (78%) and 33 Black women (49%). The most common mutation was *TP53* (55/58 women, 95%). The peritoneum was the most common site of metastasis (29/33, 88%) and recurrence (8/27, 30%). PR expression was more common in women with nodal metastases (p=0.02) and non-Hispanic women (p=0.01). *ERBB2* alterations were more common in women with vaginal cuff recurrence (p=0.02), while *PIK3CA* mutation was more common in women with liver metastases (p=0.048). *ARID1A* mutation and presence of recurrence or metastases to the liver were associated with lower OS (Hazard Ratio (HR): 31.87; 95%CI: 3.21, 316.9; p<0.001 and HR: 5.66; 95%CI: 1.2, 26.79; p=0.01, respectively). In the bivariable Cox model, the presence of metastasis/recurrence to the liver and/or the peritoneum were both independent significant predictors of OS (HR: 9.8; 95%CI: 1.85-52.7; p=0.007 and HR: 2.7; 95%CI: 1.02-7.1; p=0.04, respectively).

Conclusions: *TP53* is often mutated in USC, which most commonly metastasize and recur in the peritoneum. OS was shorter in women with *ARID1A* mutations and with metastasis/recurrence to the liver. The presence of metastasis/recurrence to liver and/or peritoneum were independently associated with shorter OS.

KEYWORDS

uterine serous cancer, next generation sequencing, endometrial cancer, somatic mutations, recurrence, metastases

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in the United States (1, 2) and is increasing at an alarming rate. Based on clinical and histological variables, EC have been divided into two types. Type I tumors, or endometrioid EC, represent approximately 85% of cases, and are usually low-grade with favorable outcomes. Type II tumors, or non-endometrioid EC, typically arise in postmenopausal patients, and are frequently of high-grade thus contributing to a relatively poor prognosis (3, 4). Type II EC is largely comprised of uterine serous carcinomas (USC), which represents less than 10% of all endometrial cancers, yet accounts for more than half of deaths attributed to EC (4–6). While type I are estrogen-dependent, the role of estrogens in type II EC is less clear, although studies have shown that the pathogenesis of type II EC may at least partially depend on the level of estrogens, differently from what was previously believed (7).

Pathological reporting of EC has limitations due to poor reproducibility of tumor typing and to accurately identify patients at risk for recurrence or metastatic disease (8). The identification of the underlying molecular background of EC has resulted in the development of new molecular based classifications of EC, namely The Cancer Genome Atlas (TCGA), which stratifies EC into four distinct clusters with prognostic significance: polymerase ε (POLE) ultramutated, copy-number low, and microsatellite instability (MSI) hypermutated, and copy-number high (9, 10).

The genomic characterization of USC is distinct from endometrioid EC, with USC exhibiting a high frequency of genetic aberrations involving *TP53*, *FBXW7*, *PPP2R1A* and *ARID1A*.USC are mostly classified as copy-number high according to TCGA (10, 11). In contrast, endometrioid EC typically demonstrates a higher frequency of microsatellite instability, frequent activation of WNT/CTNNB1 signaling, and mutations of *POLE*, *KRAS*, and *CTNNB1*. Endometrioid EC are mostly classified as copy-number low according to TCGA (9–11).

Although EC is typically diagnosed early and associated with a high 5-year survival of 80-90%, USCs have higher recurrence rates and carry a poor prognosis with significantly lower survival (4, 12). USCs have high risk of recurrence (up to 80%) and are associated with an increased incidence of extrauterine disease on presentation (5, 12–15). Hence, the ability to reduce mortality of EC largely depends on developing tailored therapy and management for recurrent and advanced USC (16).

Patterns of recurrence and metastasis may provide prognostic information for EC. For example, patients with single-site local or nodal recurrence of EC have been associated with improved survival compared to those with pelvic recurrence or distant metastasis (17, 18), while patients with peritoneal carcinomatosis or multiple sites of recurrence have significantly worse post-relapse survival rates (18).

Comprehensive genomic analysis of USC provides a clearer understanding of the molecular pathways involved in oncogenesis (19). Knowledge of the somatic mutations may help predict patterns of metastases and recurrence in various cancers, including urothelial cancer, where patients with *TP53* mutations are at a higher risk of lymph nodes metastases (12, 20–22).

The primary objective of this study is to investigate the association of somatic mutations occurring in a diverse patient population diagnosed with USC to the patterns of metastases, recurrence, overall survival (OS) and recurrence free survival (RFS).

Materials and methods

Patients and histopathologic data

This institutional review board (IRB)-approved, Health Insurance Portability and Accountability Act (HIPAA)-compliant retrospective study was performed at a single institution on consecutive patients diagnosed with USC who underwent somatic molecular testing between January 2015 and July 2021. Patients were excluded for 1) non-serous or mixed histology, or 2) data on recurrence or metastasis was not available in the electronic medical records (EMR).

Clinical and histopathologic data

Medical records in the EMR were reviewed to extract clinicopathologic data of the patients by (C.L.) (M.M.) (N.G.) (M.H.). The collected information included demographics; date and stage at diagnosis; histopathology and genomic testing results; initial treatment (systemic therapy, radiotherapy, or surgery); date

and sites of metastatic and recurrent disease; treatment modality for recurrent disease; progression date; and date of death or last follow-up.

Imaging review

Cross-sectional images (Computed tomography (CT) of the chest, abdomen, and pelvis; FDG- Positron emission tomography (PET)/CT, and Magnetic resonance Imaging (MRI)) and reports were reviewed initially by a cancer imaging fellow (C.L.) and separately by a fellowship-trained cancer imaging radiologist with 5 years of experience (F.A.). Discrepancies were resolved by consensus between the two radiologists. The date of the first imaging showing metastasis or recurrence was recorded. Reports and images, when available, were analyzed to record sites of metastatic or recurrent disease, including pelvis, lung, liver, pleura, lymph nodes, peritoneum, bones, brain, and muscle for all patients. Lymph node involvement was determined by short-axis diameter ≥1.0 cm. Any new lesion identified at follow-up imaging after curative treatment was defined as recurrence based on pathologic confirmation whenever possible, or if it showed unequivocal growth on follow-up imaging, defined as >20% increase in the sum of diameters compared to baseline or nadir (with an absolute increase of at least 5 mm) according to RECIST 1.1 (23). Any extrauterine lesion present before curative treatment was performed was considered metastatic, based on pathologic confirmation whenever possible, or if it showed unequivocal growth on follow-up imaging exams according to RECIST 1.1 (23).

Molecular testing

Molecular profiling was performed with immunohistochemistry (IHC) and next-generation sequencing (NGS) on either the primary tissue at diagnosis or from tissue obtained at recurrence. A board-certified gynecologic oncologist (M.H.) assigned a TCGA cluster based on the mutational profile (10). IHC and molecular testing was performed using either Caris Life Sciences (Caris Life Sciences, Phoenix, AZ, USA) or FoundationOne CDx (Foundation medicine inc., Cambridge, MA, USA) genomic profiling assays.

The IHC assays were performed using FDA-approved companion diagnostic or FDA-cleared tests consistent with the manufacturer's instructions: ALK (Ventana ALK (D5F3) CDxAssay; ER (confirm anti-estrogen receptor ER, SP1, Ventana; FOLR1 (Ventana FOLR1-2.1 RxDx, Ventana; PR (confirm anti-progesterone PR (1E2), Ventana); HER2/neu (pathway anti-HER-2/neu (4B5), Ventana; PD-L1 22c3. pharmDx, Dako; Mismatch repair (MMR) proteins (MLH1, MSH2, MSH6, and PMS2; Ventana MMR RxDx Panel, Ventana). For ER/PR, staining intensity was classified as 0, 1+, 2+, 3+. The intensity thresholds for a positive test for ER were >/= 2+ with >/= 75% or >/=3+ with >/=50% of cells stained, for PR were >/=1+ and >/= 10% of cells stained.

Regarding molecular testing, details of specific NGS testing are available from Caris Life Sciences or FoundationOne CDx (24, 25). Among the genes covered, we focused our mutational analysis on the 12 genes most frequently mutated in this cohort.

Statistical analysis

Categorical variables were summarized using frequencies and percentages and the continuous variable, age, was summarized using mean and standard deviation. Different genetic aberrations involving the same gene were grouped under that gene. The association of mutational status with the location of metastases or recurrence was assessed using Chi-square tests or Fisher's exact tests when 20% or more of the frequency cells had expected counts less than 5.

OS was measured from the date of initial diagnosis of USC to death from any cause, censored at the date of the last follow-up in alive patients. Stratified Kaplan-Meier survival curves were generated, and univariable Cox proportional hazards regression was used to examine the association of ethnicity, race, mutational status, sites of recurrence or metastases with OS. OS curves were computed in all patients. Statistical significance was determined through the log-rank test. A p-value <0.05 was considered statistically significant.

Multivariable Cox proportional hazard regression with backward stepwise selection was performed with OS as response. The variables that had log-rank p-values<0.20 and sample size > 50 were used as the predictors in the model. Likelihood Ratio tests were used to test model predictability and test significance for additional individual and/or collection of variables. Hazards ratios and 95% CIs were calculated in each model to determine association and significant predictors of survival. Statistical computations were performed, and output was generated using SAS Software Version 9.4 (The SAS Institute, Cary, NC).

Results

Patient characteristics

From a total of 134 patients with EC who underwent genomic profiling, 67 patients with USC were included in the final study analysis. Fifty-eight patients with endometrioid histology, 5 patients with clear cell histology, 3 patients with mixed histology, and 1 patient with no data on recurrence or metastasis were excluded. Characteristics of the included subjects are summarized in Table 1. The average age was 65.8 years (Standard Deviation=8.3 years). Fifty-two patients were non-Hispanic (78%), 33 patients were Black (49%), 32 were White (48%), 1 was Asian (1.5%), and 1 patient self-reported multi-racial (1.5%). Thirty-three patients had evidence of metastases at time of diagnosis (49.2%), 27 patients had evidence of recurrence during follow-up (40.3%), and 7 patients did not show

evidence of recurrence or metastasis during follow-up (10.5%). Median follow up was 21 months (range 20 days-10.2 years).

Molecular aberrations

Molecular profiling was performed on primary tissue at diagnosis on 45/67 patients (67%), on tissue obtained at recurrence in 20/67 patients (30%). In 2/67 patients (3%), molecular profiling was not performed. The most frequently mutated genes identified on NGS were *TP53* (55/58 patients; 94.8%), followed by *PIK3CA* (14/61 patients; 22.9%), and *ERBB2* (12/37 patients; 32%) (Table 1). A high tumor mutational burden (TMB) was identified in 14/51 patients (27.5%). On IHC, PTEN was expressed in 51/56 patients (91.1%), ER was expressed in 43/65 patients (66.2%), PR in 24/65 patients (36.9%). None of the patients expressed MMR gene mutation or microsatellite instability (MSI) (Table 1).

Fifty-three patients were classified as TCGA copy-number high (79%), six patients as TCGA copy-number low (9%), and 8 patients could not be classified (12%) based on mutational profile.

TABLE 1 Clinical characteristics of 67 patients with uterine serous carcinoma.

Characteristic	Number
Age (mean, standard deviation, in years), range	65.8 +/- 8.3, 44-82
Race	
Black	33 (49.2%)
White	32 (47.8%)
Asian	1 (1.5%)
More than one	1 (1.5%)
Ethnicity	
Hispanic	15 (22.4%)
Non-Hispanic	52 (77.6%)
Treatment	
Surgery	52 (78%)
TAH	*2*
" + *BSO*	*2*
" + *BSO + PLND*	*10*
" + *BSO + PLND + Omentectomy and debulking*	*38*
Initial chemotherapy	65 (97%)
Carboplatin	*2*
" + *Paclitaxel*	*55*
" + *Paclitaxel + Bevacizumab*	*4*
" + *Paclitaxel + Herceptin*	*1*
" + *Paclitaxel + Pembrolizumab*	*1*
" + *Taxotere*	*1*
" + *Taxotere + Bevacizumab*	*1*
Radiation therapy	23 (34.3%)
FIGO Stage at diagnosis	
I	13 (19.4%)
II	3 (4.5%)
III	18 (26.9%)
IV	33 (49.2%)
Metastatic disease	33 (49.2%)
Recurrent disease	27 (40.3%)
Mutational status	
TP53	55/58 (94.8%)
PIK3CA	14/61 (22.9%)
FBXW7	10/60 (15%)
ARID1A	3/32 (9.4%)
PTEN	5/57 (8.7%)
BRCA1	2/57 (3.5%)
BRCA2	2/57 (3.5%)
CTNNB1	1/58 (1.7%)
ERBB2 alterations	12/37 (32.4%)
AKT1,2,3	2/61 (3.3%)
KRAS	1/61 (1.6%)
Immunohistochemistry	
ER	43/65 (66.2%)
PR	24/65 (36.9%)
PTEN	51/56 (91.1%)
TOP2A	20/22 (90.9%)
TOP01	14/22 (63.6%)
RRM1	4/20 (20%)
MGMT	3/5 (60%)
TS	10/22 (45.5%)
TUBB3	11/22 (50%)

TAH, total hysterectomy; BSO, Bilateral salpingoophorectomy; PLND, pelvic lymph node dissection.

Location of metastases and recurrence

Thirty-three patients (49.2%) had evidence of metastases at time of diagnosis (7 on biopsy, 18 on CT, 1 on MRI and 7 on PET/CT), 27 patients (40.3%) had evidence of recurrence during follow-up (3 on biopsy, 13 on CT, and 11 on PET/CT), and 7 patients (10.5%) did not show evidence of metastasis or recurrence during follow-up (Table 1).

The sites of metastasis and recurrence are summarized in Figure 1. The most common sites of metastasis were the peritoneum (29/33, 88%), followed by lymph nodes (18/33, 55%) and in the pelvis (14/33, 42%). The most common site of recurrence was lymph nodes (18/27, 67%), followed by peritoneal implants (8/27, 30%) and vaginal cuff (6/27, 22%).

Association of mutational status with ethnicity, metastatic and recurrent disease with survival

PR was expressed on IHC more commonly in patients with lymph node metastases (p=0.02), and in non-Hispanic patients (p=0.01). *ERBB2* was more commonly mutated in patients with vaginal cuff recurrence (p=0.02), while *PIK3CA* was more commonly mutated in patients with liver metastases (p=0.048).

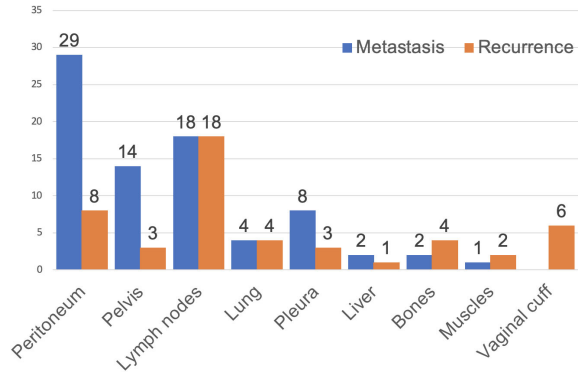

FIGURE 1
Sites of metastases (blue) and recurrence (orange) in women with USC.

Peritoneal metastases were significantly more common in Non-Hispanic patients (p=0.04). No other associations between mutations, ethnicity, sites of metastatic and recurrent disease were identified.

ARID1A mutation was associated with lower OS (mean OS: 10.2 months vs 60.9 months; Hazard Ratio (HR): 31.87; (95% CI: 3.21, 316.9)). Presence of recurrence or metastases to the liver was associated with lower OS (mean OS: 48.8 months vs 58.4 months; HR: 5.66; (95% CI: 1.2, 26.79)) (Figures 2, 3).

Predictive survival model

A Cox proportional hazards model was selected using a backward stepwise selection process starting with a fully adjusted model. The fully adjusted model included *AKT1,2,3* mutations, presence of recurrence or metastases to the liver, peritoneum, lymph nodes, lungs and a race dummy variable defined as Black or not Black. The predicters in the full model all had log rank p-values<0.20 except for race (p-value=0.59). The Full model was reduced 4 times based on the Wald test statistics for individual variables and the Likelihood Ratio tests (-2 Log Likelihood) resulting in a bivariable Cox model with a total of 60 cases. The final model included presence of recurrence or metastases to the liver, and presence of recurrence or metastases to the peritoneum as predictors (HR: 9.8; 95% CI: 1.85, 52.7; p=0.007 and HR: 2.7; 95% CI: 1.02, 7.1; p=0.04, respectively) (Table 2).

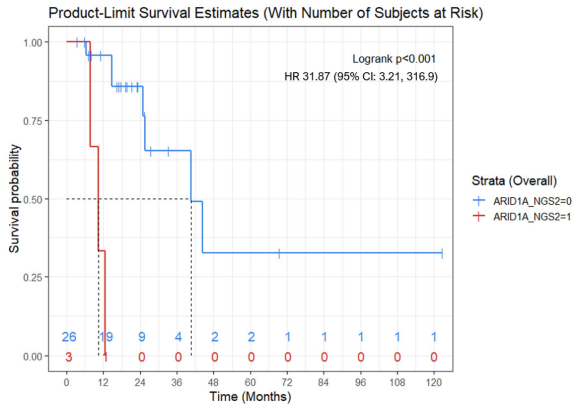

FIGURE 2
Kaplan-Meier OS curve for women with *ARID1A* mutations (red) versus women without *ARID1A* mutation (blue). ARID1A mutation was associated with shorter OS (mean, 10.2 months vs 60.9 months; Cox proportional hazard model - Hazard Ratio: 31.87; 95% CI: 3.21, 316.9; p<0.001). Numbers at the bottom of the graph refers to the number of women with *ARID1A* mutations (red) versus women without *ARID1A* mutation (blue) at risk at a determinate timepoint.

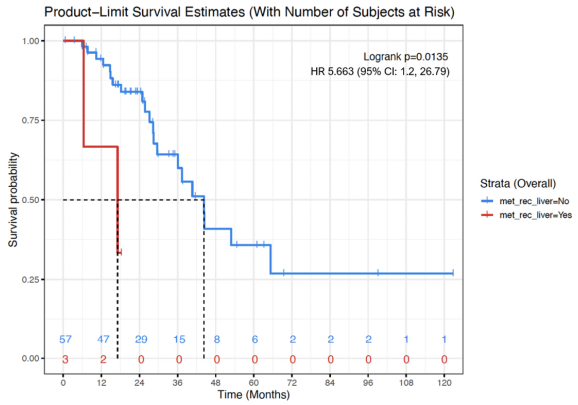

FIGURE 3
Kaplan-Meier OS curve for women with evidence of liver metastases/recurrence (red) versus women with no evidence or liver metastases or recurrence (blue). Presence of liver metastases/recurrence was associated with shorter OS (mean, 48.8 months vs 58.4 months; Cox proportional hazard model - Hazard Ratio: 5.66; 95% CI: 1.2, 26.79; p=0.01). Numbers at the bottom of the graph refers to the number of women with evidence of liver metastases/recurrence (red) versus women with no evidence or liver metastases or recurrence (blue) at risk at a determinate timepoint.

Discussion

With advances in genomic testing and improved understanding of cancer biology, an increasing number of cancer patients undergo mutational testing to identify potential targeted treatment options. As we gain more insight into the impact of the mutational make-up of cancer on prognosis, understanding its associations to known clinicopathologic factors is crucial. Our study cohort was largely comprised of minority Hispanic and Black women that were poorly represented in prior TCGA data and in the PanCancer Atlas (26). Furthermore, 40% of our patients had evidence of recurrence during follow-up while 49% of patients had metastatic disease at diagnosis. This is significantly higher than the TCGA cohort, where 32% presented with recurrence and only 11% had metastatic disease at diagnosis (10).

We showed that *TP53*, *PIK3CA* and *ERBB2* are often mutated in USC, consistent with TGCA data. In our cohort, 79% of cases were classified as copy-number high, consistent with TCGA data, where 77% of cases with serous histology were classified as such (10). In a recent study by Watanabe et al. on 100 EC cases, *TP53* mutations were associated with non-endometrioid histology (12). Several

TABLE 2 Univariable and multivariable Cox proportional hazard regression analysis of survival in patients with uterine serous carcinoma.

	Univariable Models		Full Model (n=50)		Reduced Model 1 (n=50)		Reduced Model 2 (n=60)		Reduced Model 3 (n=60)		Reduced Model 4 (n=60)	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Recurrence/Metastases Liver		**0.029**		0.365		0.366		0.100		0.091		**0.007**
	ref		ref		ref		ref		ref		ref	
	5.66 (1.20-26.79)		3.11 (0.27-36.27)		3.21 (0.26-40.44)		5.27 (0.73-38.22)		5.31 (0.77 -36.75)		**9.88 (1.85-52.75)**	
Recurrence/Metastases Peritoneum		0.098		**0.044**		**0.024**		**0.034**		**0.032**		**0.044**
	ref		ref		ref		ref		ref		ref	
	2.14 (0.87-5.25)		**3.53 (1.03-12.03)**		**3.91 (1.19-12.84)**		**2.88 (1.09-7.66)**		**2.89 (1.10-7.62)**		**2.7 (1.03-7.10)**	
Recurrence/Metastases Lung		0.099		0.268		0.325		0.208		0.207		
	ref		ref		ref		ref		ref			
	3.13 (0.81-12.10)		2.92 (0.44-19.42)		2.61 (0.39-17.73)		2.95 (0.55-15.89)		2.95 (0.55-15.83)			
Black		0.587		0.448		0.377		0.973				
	ref		ref		ref		ref					
	1.26 (0.55-2.88)		1.49 (0.53-4.19)		1.59 (0.57-4.44)		1.02 (0.43-2.38)					
AKT1,2,3		0.114		0.432		0.481						
	ref		ref		ref							
	5.46 (0.67-44.66)		4.34 (0.11-168.65)	3.65 (0.10-133.79)								
Recurrence/Metastases Lymph Nodes		0.130		0.473								
	ref		ref									
	0.52 (0.22-1.23)		0.68 (0.24-1.94)									
ARID1A		**0.003**										
	ref											
	31.87 (3.21-316.9)											

HR: Hazard Ratio; CI: Confidence Interval.
Bold values relate to the p value <0.05.

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studies have demonstrated that human epidermal growth factor receptor 2 (HER2) a tyrosine kinase increasing cell proliferation encoded by *ERBB2*, is frequently overexpressed in USC (27–29). HER2 overexpression has also been associated with advanced-stage disease, and poorer survival outcomes in USC (25). Few *ARID1A* gene mutations in were identified in our cohort, consistent with the findings of the PanCancer Atlas, where *ARID1A* gene mutations were identified in 13% of cases (26, 30). Mutations in *ARID1A* in EC have been associated with promoting tumor invasion and metastasis, which may shed light on the significant mortality among women diagnosed with USCs (30).

In our cohort, ER and PTEN are expressed in the majority of USC on IHC. A study on 1054 women with EC showed that ER, though more common in type I EC, was expressed in 72% of type II EC (31). A study on 56 high grade EC showed that majority of USC were positive for PTEN on IHC (32). A study on 221 women with EC, showed that loss of PTEN expression was associated with endometrioid histology and favorable survival (33).

In our study, *FBXW7* was mutated in 15% of USC. No association between *FBXW7* mutations, ethnicity and race was identified in our study. *FBXW7* was mutated in 29 out of 109 (27%) USC included in the PanCancer Atlas. In a study on 66 USC, *FBXW7* mutations were identified in 18.2% of cases (11). On the study by Watanabe et al., *FBWX7* mutations were associated with late-stage, vascular invasion, and lymph node metastasis (12).

In our cohort, USC most commonly metastasized to lymph nodes, peritoneum and pelvic organs, while recurrences were mostly nodal, peritoneal and at the vaginal cuff. These findings are similar to two prior studies: a study on 841 EC, which showed that the most common sites of recurrence of USC was the abdomen, including ascites, followed by the vaginal cuff and a study on 50 USC, which showed that the most common sites of metastases of USC were the lymph nodes, pelvic organs and peritoneum/omentum (13, 14).

In our study, PR expression was associated with non-Hispanic ethnicity and nodal metastases. A study on 99 endometrioid EC showed that nodal metastases correlated with negative PR status on IHC (34). This discrepancy may be related to the different histologies. The association between non-Hispanic ethnicity and PR expression is unclear.

We found that *ERBB2* alterations were more common in patients with vaginal cuff recurrence and *PIK3CA* mutation was more common in patients with liver metastases. *ERBB2* amplifications are associated with higher stage, chemoresistance, and lower survival, especially in Black patients (35, 36). Trastuzumab, a HER2/neu receptor inhibitor monoclonal antibody, demonstrated increased progression free survival when added to carboplatin paclitaxel, in patients with USC and ERBB2 overexpression (37). This highlights the significance of somatic tumor testing either at diagnosis or at recurrence to aid in prioritizing treatment options.

Women with presence of recurrence or metastases to the liver and *ARID1A* mutations had lower OS. A study on 86 recurrent EC, showed that recurrence to the liver was associated with lower OS, with an HR of 10 [3.72–26.81 95% CI] (14). A metanalysis on the prognostic significance of ARID1A in endometrium-related gynecological cancers showed that negative ARID1A expression predicted shorter progression free survival (38). *ARID1A* mutations affects multiple pathways, and may mediate resistance to platinum chemotherapy, possibly explaining the lower OS observed in our study (39). Therapies targeting the pathways affected by *ARID1A* mutations, such as poly(ADP-ribose) polymerase inhibitors, immune checkpoint inhibitors and mTOR inhibitors, have shown activity in preclinical models and in patient, and could be implemented in patients with *ARID1A*-mutated USC (40, 41).

Finally, on multivariable Cox proportional hazards regression analysis, the presence of recurrence or metastases to the liver and/or the peritoneum were independently associated with shorter OS regardless of their mutational status, race, ethnicity or other sites of recurrence or metastases. Various studies attempted to build predictive survival models for USC, including a study by Chen et al. on 110 women with USC which showed that a combination of mutated genes, a 4-gene signature, was predictive of OS (42). Differently from our study, their model did not include sites of metastases or survival as potential predictors. Knowledge of the associations between survival data and sites of recurrence or metastases of USC is valuable: it allows for a more accurate risk stratification and helps the oncologists and radiologists to potentially formulate more appropriate follow-up strategies (43).

Some limitations of this study should be noted. This is a retrospective study performed at a single institution with inherent selection bias. The relatively small patient cohort and the widely variable follow-up period may have limited the power in detection of some of the associations between mutations and patterns of metastases and recurrence, as well as the prognostic values of mutations. Furthermore, our sample included only patients with USC selected from a tertiary cancer center and may not be representative of patients treated at a community hospital. We grouped different genetic aberrations involving the same gene under that gene to facilitate analysis until additional data are available. This methodology has also been previously utilized and reported (11, 21).

One of the significant strengths of this study is the cohort of Hispanic and Black women diagnosed with USC with genomic testing. Our study showed that mutational status of USC had implications on pattern of metastases and survival, and that sites of recurrence and metastases influence survival. These findings should be assessed in larger studies, to confirm our findings and may be valuable for future trial design.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by University of Miami. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

MH: Supervision, ideation, and draft editing. SW: statistical analysis and draft editing. FA: ideation, statistical analysis, draft writing, and editing. CL: data collection and draft editing. NG: data collection MM: data collection and draft editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. US Surveillance, Epidemiology, and End Results (SEER). *Cancer stat facts - uterine cancer* (2022). Available at: https://www.seer.cancer.gov/statfacts/html/corp.html (Accessed January 5, 2022).

2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA: A Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654

3. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. *Lancet* (2016) 387(10023):1094–108. doi: 10.1016/S0140-6736(15)00130-0

4. Lu KH, Broaddus RR. Endometrial cancer. *New Engl J Med* (2020) 383(21):2053–64. doi: 10.1056/NEJMra1514010

5. del Carmen MG, Birrer M, Schorge JO. Uterine papillary serous cancer: a review of the literature. *Gynecol Oncol* (2012) 127(3):651–61. doi: 10.1016/j.ygyno.2012.09.012

6. Brooks RA, Fleming GF, Lastra RR, Lee NK, Moroney JW, Son CH, et al. Current recommendations and recent progress in endometrial cancer. *CA: A Cancer J Clin* (2019) 69(4):258–79. doi: 10.3322/caac.21561

7. Setiawan VW, Yang HP, Pike MC, McCann SE, Yu H, Xiang YB, et al. Type I and II endometrial cancers: have they different risk factors? *J Clin Oncol: Off J Am Soc Clin Oncol* (2013) 31(20):2607–18. doi: 10.1200/JCO.2012.48.2596

8. Luna C, Balcacer P, Castillo P, Huang M, Alessandrino F. Endometrial cancer from early to advanced-stage disease: an update for radiologists. *Abdominal Radiol (New York)* (2021) 46(11):5325–36. doi: 10.1007/s00261-021-03220-7

9. Arciuolo D, Travaglino A, Raffone A, Raimondo D, Santoro A, Russo D, et al. TCGA molecular prognostic groups of endometrial carcinoma: current knowledge and future perspectives. *Int J Mol Sci* (2022) 23(19):11684. doi: 10.3390/ijms231911684

10. Levine DA. Integrated genomic characterization of endometrial carcinoma. *Nature* (2013) 497(7447):67–73. doi: 10.1038/nature12113

11. Kuhn E, Wu RC, Guan B, Wu G, Zhang J, Wang Y, et al. Identification of molecular pathway aberrations in uterine serous carcinoma by genome-wide analyses. *J Natl Cancer Inst* (2012) 104(19):1503–13. doi: 10.1093/jnci/djs345

12. Watanabe T, Nanamiya H, Kojima M, Nomura S, Furukawa S, Soeda S, et al. Clinical relevance of oncogenic driver mutations identified in endometrial carcinoma. *Trans Oncol* (2021) 14(3):101010. doi: 10.1016/j.tranon.2021.101010

13. Goff BA, Kato D, Schmidt RA, Ek M, Ferry JA, Muntz HG, et al. Uterine papillary serous carcinoma: patterns of metastatic spread. *Gynecol Oncol* (1994) 54 (3):264–8. doi: 10.1006/gyno.1994.1208

14. Rosenberg P, Blom R, Högberg T, Simonsen E. Death rate and recurrence pattern among 841 clinical stage I endometrial cancer patients with special reference to uterine papillary serous carcinoma. *Gynecol Oncol* (1993) 51(3):311–5. doi: 10.1006/gyno.1993.1296

15. Sohaib SA, Houghton SL, Meroni R, Rockall AG, Blake P, Reznek RH. Recurrent endometrial cancer: patterns of recurrent disease and assessment of prognosis. *Clin Radiol* (2007) 62(1):28–34. doi: 10.1016/j.crad.2006.06.015

16. Sorbe B, Juresta C, Ahlin C. Natural history of recurrences in endometrial carcinoma. *Oncol Lett* (2014) 8(4):1800–6. doi: 10.3892/ol.2014.2362

17. Creutzberg CL, van Putten WL, Koper PC, Lybeert ML, Jobsen JJ, Wárlám-Rodenhuis CC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC study group. post operative radiation therapy in endometrial carcinoma. *Lancet* (2000) 355(9213):1404–11. doi: 10.1016/S0140-6736(00)02139-5

18. Legge F, Restaino S, Leone L, Carone V, Ronsini C, Di Fiore GLM, et al. Clinical outcome of recurrent endometrial cancer: analysis of post-relapse survival by pattern of recurrence and secondary treatment. *Int J Gynecol Cancer* (2019) 30(2):193–200. doi: 10.1136/ijgc-2019-000822

19. Ferriss JS, Erickson BK, Shih IM, Fader AN. Uterine serous carcinoma: key advances and novel treatment approaches. *Int J Gynecol Cancer* (2021) 31(8):1165–74. doi: 10.1136/ijgc-2021-002753

20. Uygur MC, Yaman I, Kutluay L, Altuğ U, Erol D. The relation between p53 overexpression and lymph node metastases in clinical stage t2 and t3a transitional cell bladder carcinoma. *J Exp Clin Cancer Res* (1999) 18(3):391–5.

21. Park DS, Lee YT, Lee JM. Prediction of lymph node metastasis based on p53 and nm23-H1 expression in muscle invasive grade III transitional cell carcinoma of bladder. *Adv Exp Med Biol* (2003) 539(Pt A):67–85. doi: 10.1007/978-1-4419-8889-8_6

22. Alessandrino F, Williams K, Nassar AH, Silverman SG, Sonpavde G, Shinagare AB. Muscle-invasive urothelial cancer: association of mutational status with metastatic pattern and survival. *Radiology* (2020) 295(3):572–80. doi: 10.1148/radiol.2020191770

23. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* (2009) 45(2):228–47. doi: 10.1016/j.ejca.2008.10.026

24. U.S. Food and Drug Administration. *FoundationOne CDx– P170019/S014 technical information*. Available at: https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019S006C.pdf (Accessed December 20, 2022).

25. Caris Life Sciences. *Comprehensive molecular profiling*. Available at: https://www.carislifesciences.com/products-and-services/molecular-profiling/ (Accessed December 20, 2022).

26. *Uterine corpus endometrial carcinoma (TCGA, PanCancer atlas)*. Available at: https://www.cbioportal.org/study/summary?id=ucec_tcga_pan_can_atlas_2018 (Accessed July 30, 2022).

27. Singh P, Smith CL, Cheetham G, Dodd TJ, Davy MLJ. Serous carcinoma of the uterus–determination of HER-2/neu status using immunohistochemistry, chromogenic *in situ* hybridization, and quantitative polymerase chain reaction techniques: its significance and clinical correlation. *Int J Gynecol Cancer* (2008) 18 (6):1344–51. doi: 10.1111/j.1525-1438.2007.01181.x

28. Slomovitz BM, Broaddus RR, Burke TW, Sneige N, Soliman PT, Wu W, et al. Her-2/neu overexpression and amplification in uterine papillary serous carcinoma. *J Clin Oncol* (2004) 22(15):3126–32. doi: 10.1200/JCO.2004.11.154

29. Villella JA, Cohen S, Smith DH, Hibshoosh H, Hershman D. HER-2/neu overexpression in uterine papillary serous cancers and its possible therapeutic implications. *Int J Gynecol Cancer* (2006) 16(5):1897–902. doi: 10.1111/j.1525-1438.2006.00664.x

30. Suryo Rahmanto Y, Shen W, Shi X, Chen X, Yu Y, Yu ZC, et al. Inactivation of Arid1a in the endometrium is associated with endometrioid tumorigenesis through transcriptional reprogramming. *Nat Commun* (2020) 11(1):2717. doi: 10.1038/s41467-020-16416-0

31. Shen F, Gao Y, Ding J, Chen Q. Is the positivity of estrogen receptor or progesterone receptor different between type 1 and type 2 endometrial cancer? *Oncotarget* (2016) 8(1):506–11. doi: 10.18632/oncotarget.13471

32. Gilks CB, Oliva E, Soslow RA. Poor interobserver reproducibility in the diagnosis of high-grade endometrial carcinoma. *Am J Surg Pathol* (2013) 37(6):874–81. doi: 10.1097/PAS.0b013e31827f576a

33. Akiyama-Abe A, Minaguchi T, Nakamura Y, Michikami H, Shikama A, Nakao S, et al. Loss of PTEN expression is an independent predictor of favourable survival in endometrial carcinomas. *Br J Cancer* (2013) 109(6):1703–10. doi: 10.1038/bjc.2013.455

34. Iwai. K, Fukuda. K, Hachisuga. T, Mori. M, Uchiyama. M, Iwasaka. T, et al. Prognostic significance of progesterone receptor immunohistochemistry for lymph node metastases in endometrial carcinoma. *Gynecol Oncol* (1999) 72(3):351–9. doi: 10.1006/gyno.1998.5286

35. Santin AD, Bellone S, Siegel ER, Palmieri M, Thomas M, Cannon MJ, et al. Racial differences in the overexpression of epidermal growth factor type II receptor (HER2/neu): a major prognostic indicator in uterine serous papillary cancer. *Am J Obstet Gynecol* (2005) 192(3):813–8. doi: 10.1016/j.ajog.2004.10.605

36. Santin AD, Bellone S, Van Stedum S, Bushen W, Palmieri M, Siegel ER, et al. Amplification of c-erbB2 oncogene. *Cancer* (2005) 104(7):1391–7. doi: 10.1002/cncr.21308

37. Fader AN, Roque DM, Siegel E, Buza N, Hui P, Abdelghany O, et al. Randomized phase II trial of carboplatin-paclitaxel versus carboplatin-Paclitaxel-Trastuzumab in uterine serous carcinomas that overexpress human epidermal growth factor receptor 2/neu. *J Clin Oncol: Off J Am Soc Clin Oncol* (2018) 36 (20):2044–51. doi: 10.1200/JCO.2017.76.5966

38. Liu G, Xu P, Fu Z, Hua X, Liu X, Li W, et al. Prognostic and clinicopathological significance of ARID1A in endometrium-related gynecological cancers: a meta-analysis. *J Cell Biochem* (2017) 118(12):4517–25. doi: 10.1002/jcb.26109

39. Mullen J, Kato S, Sicklick JK, Kurzrock R. Targeting ARID1A mutations in cancer. *Cancer Treat Rev* (2021) 100:102287. doi: 10.1016/j.ctrv.2021.102287

40. Samartzis EP, Noske A, Dedes KJ, Fink D, Imesch P. ARID1A mutations and PI3K/AKT pathway alterations in endometriosis and endometriosis-associated ovarian carcinomas. *Int J Mol Sci* (2013) 14(9):18824–49. doi: 10.3390/ijms140918824

41. Okamura R, Kato S, Lee S, Jimenez RE, Sicklick JK, Kurzrock R. ARID1A alterations function as a biomarker for longer progression-free survival after anti-PD-1/PD-L1 immunotherapy. *J Immunother Cancer* (2020) 8(1):e000438. doi: 10.1136/jitc-2019-000438

42. Chen H, Li L, Qin P, Xiong H, Chen R, Zhang M, et al. A 4-gene signature predicts prognosis of uterine serous carcinoma. *BMC Cancer* (2021) 21(1). doi: 10.1186/s12885-021-07834-4

43. Shinagare AB, Ip IK, Lacson R, Ramaiya NH, George S, Khorasani R. Gastrointestinal stromal tumor: optimizing the use of cross-sectional chest imaging during follow-up. *Radiology* (2015) 274(2):395–404. doi: 10.1148/radiol.14132456

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EDITED BY
Kari Ring,
University of Virginia, United States

REVIEWED BY
Andrea Giannini,
University of Pisa, Italy
Artur Rebelo,
University Hospital in Halle, Germany

*CORRESPONDENCE
Anouk A.S. van den Bosch
a.vdbosch@hotmail.com

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The impact of adipose tissue distribution on endometrial cancer: a systematic review

Anouk A. S. van den Bosch[1]*, Johanna M. A. Pijnenborg[2], Andrea Romano[1], Bjorn Winkens[3], Louis J. M. van der Putten[2], Roy F. P. M. Kruitwagen[1] and Henrica M. J. Werner[1]

[1]Department of Obstetrics and Gynecology, GROW-School for Oncology and Reproduction, Maastricht University Medical Centre, Maastricht, Netherlands, [2]Department of Obstetrics and Gynaecology, Radboudumc, Nijmegen, Netherlands, [3]Department of Methodology and Statistics, Maastricht University, Maastricht, Netherlands

Introduction: Endometrial cancer (EC) is the most common gynecological cancer with a rising incidence, attributed to advanced life expectancy and obesity. Adipose tissue (AT) is an important endocrine organ, and its metabolic activity is affected by the different anatomical distribution or locations. AT distribution influences a number of diseases. In EC, it remains unclear whether the type of AT distribution affects development or prognosis. This systematic review aimed to determine whether AT distribution is associated with patient characteristics, disease characteristics, and patient prognosis in EC.

Materials and methods: A search was conducted in Medline, MEDLINE EMBASE, and Cochrane Library. We included studies that enrolled patients with EC with any histological subtype and that distinguished between the visceral and subcutaneous AT compartment. In eligible studies, correlative analyses were performed for all outcome measures and AT distribution.

Results: Eleven retrospective studies were included, with a wide range of measurements for the visceral and subcutaneous AT compartments. AT distribution was found to be significantly correlated to a number of relevant (disease) characteristics including obesity measures, histological subtype, lymph node metastasis, and sex steroid levels. Five studies reported on survival parameters including overall survival, progression-free survival and disease-specific survival, and they found that increased VAT volume was statistically significantly associated with a worse survival.

Discussion/conclusion: This review demonstrates that there are significant correlations between AT distribution and prognosis, body mass index, sex steroid levels, and disease characteristics like histology. Well-designed, prospective, and larger-scale studies are needed to pinpoint these differences more specifically and understand how it can add in prediction and even therapy in EC.

KEYWORDS

endometrial cancer, adipose tissue distribution, prognosis, obesity, visceral adipose tissue, subcutaneous adipose tissue

1 Introduction

Endometrial cancer (EC) is the sixth most common cancer type in women worldwide with a rising incidence (1). Advanced life expectancy and obesity are the most important contributing factors for these increasing numbers (2). Obesity is defined as a body mass index (BMI) above 30 kg/m^2 (3). Obesity is linked to a number of diseases like cardiovascular disease (CVD), diabetes, and hypertension (4, 5). It is also a risk factor for the development of multiple cancer types, with the strongest association for EC (6). Every five BMI units above the normal range (18–25 kg/m^2) result in a 50% increase risk of developing EC (7). The association between obesity and EC is complex and only partially explained by the increased levels of circulating sex-steroid hormones in obese women. This may underlie that, despite this strong relationship of obesity with EC, the effects of obesity on EC characteristics and patient prognosis are still not fully understood. This includes the exact (molecular) mechanisms through which obesity facilitates EC development and understanding why (morbid) obesity does not cause EC in all women. In addition, it might clarify how obesity contributes to the rising incidence of non-endometrioid ECs, considered to be non-hormone sensitive (8). Furthermore, the impact of obesity on the prognosis of EC remains conflicting, as most patients with EC die because of CVD or other underlying comorbidities instead of EC (9). Three main hypotheses link obesity to cancer development: endogenous sex-steroid production, chronic hyperinsulinemia, and systemic inflammation (10, 11).

Adipose tissue (AT) is an endocrine organ that plays an important role in the production of a plethora of bioactive molecules with endocrine, paracrine, and autocrine functions (12). It has distinct metabolic activities depending on its anatomical locations. After menopause, circulating estrogens are produced predominantly in subcutaneous AT (SAT) through the conversion of androgens by aromatase (13). This mechanism of increased endogenous sex-steroid hormone production plays an important role in the development of EC, especially the endometrioid subtype. In contrast, visceral AT (VAT) plays a role in low-grade systemic inflammation and insulin resistance (14, 15), which have also been linked to cancer development.

Obesity is classified by the WHO as an abnormal or excess fat accumulation impairing health and includes any BMI ≥ 30 kg/m^2 (16). BMI is a simple and clinically easily applicable indicator; however, it neither does discriminate muscle from AT nor does give insight in the AT distribution. Magnetic resonance imaging (MRI) and computed tomography (CT) perform equally well in visualizing and measuring AT distribution, including in subcutaneous, visceral, and intramuscular compartments (17).

The relationship between AT distribution and prognosis of CVD and, e.g., (colo)rectal cancer has been studied (18–22). However, the impact of AT distribution on EC characteristics, like FIGO stage, histology, and patient' prognosis, is still unclear despite its tight relation with obesity. This systematic review aims to determine whether AT distribution is associated with patient characteristics (BMI and sex steroid levels), disease characteristics (FIGO, histopathology, and lymph node status), and patient prognosis.

Abbreviations: EC, endometrial cancer; BMI, body mass index; AT, adipose tissue; MRI, magnetic resonance imaging; CT, computed tomography; CVD, cardiovascular disease; NOS, Newcastle–Ottawa Scale; VAT%, visceral adipose tissue percentage; TAV, total adipose tissue volume; SAV, subcutaneous adipose tissue volume; VAV, visceral adipose tissue volume; A4, androstenedione; DHEAS, dehydroepiandrosteronsulfate; SATI, subcutaneous adipose tissue index; SFA, subcutaneous fat area; TFA, total fat area; OS, overall survival; PFS, progression-free survival; DSS, disease-specific survival; VAV%, visceral adipose volume percentage; VAT, visceral adipose tissue; V/S ratio, visceral/subcutaneous ratio; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; E2, estradiol.

2 Methods and materials

2.1 Study design and search strategy

We used the PRISMA 2020 checklist as a guideline to write this review (23). A search was conducted in Medline (1976 to May 2022), MEDLINE EMBASE (1951 to May 2022), and Cochrane Library, Database of Systematic Reviews for articles concerning this question (research question and search terms can be found in Supplementary File 1). The search strategy was constructed at the Maastricht University Medical Centre (MUMC+) by the primary researcher AvdB with support of a senior librarian of the Maastricht University.

Our search was finalized May 2022. As far as possible, search terms were identical in the three databases to ensure comparable output. The search resulted in 310 hits (see Figure 1).

2.2 Selection of studies

Articles were included if they met the following criteria: articles should investigate the relationship between EC and visceral/subcutaneous (V/S) AT and meet the search criteria.

For this review, we included primary research papers, both of prospective and retrospective nature. We included studies that enrolled patients with EC with any histological subtype that distinguished between the visceral AT and SAT compartment, either through CT or MRI. Studies were excluded if the language was other than English, Dutch, or German. From all relevant articles, full text could be obtained. Because of a lack of a gold standard, all levels of measuring AT distribution (L3 through S1) were accepted. If studies did not report on all outcomes, they were included for the reported outcomes only.

Exclusion criteria: conference papers

2.3 Quality assessment

To assess the risk of bias of the included studies, two different risks of bias tools were used to account for both cohort studies [Newcastle–Ottawa Scale (NOS)] and cross-sectional studies [appraisal tool for cross-sectional studies (AXIS)] (24, 25) (see Figures 2, 3). The NOS has thresholds to convert the study assessment into a categorical scale of "good", "fair", or "poor". The

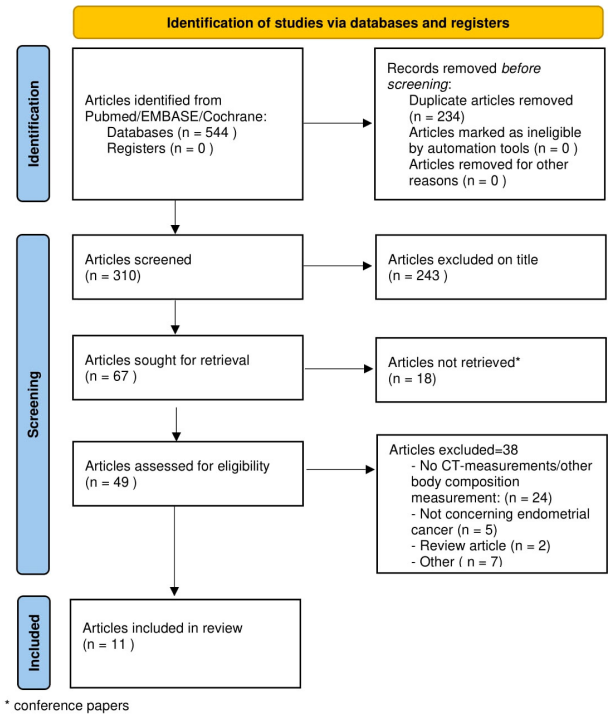

FIGURE 1
PRISMA flowchart of the selection of articles.

AXIS is more subjective in nature. To make the assessment more comparable, it was also converted to the previously mentioned scale. All scores were reviewed by two experts (AvdB and HW). Subdomains were scored separately and divided into three categories: good (if > 2/3 of the items were present and deemed acceptable), fair (if at least 1/2 of the items was present and deemed acceptable), or poor (if less than 1/2 of the items was present and deemed acceptable).

2.4 Outcome

We defined our primary outcome as the association of the type of AT distribution with patient characteristics and disease characteristics. The included patient characteristics consisted of BMI and sex steroid hormone levels; the disease characteristics were FIGO stage, histology, grade, myometrial invasion, tumor size, and

Risk of BIAS (ROB) of all cross-sectional studies.
Green = present, Yellow: partially present or unkown, Red = not present

D1 = Introduction
D2-11 = Methods
D12-16 = Results
D17-18 = Discussion
D19-20 = Other

FIGURE 2
Risk of bias table, AXIS.

Risk of BIAS (ROB) of all cohort studies.
Green = present, Yellow: partially present or unkown, Red = not present

D1-4 = Selection
D5 = Comparability
D6-8 = Outcome

FIGURE 3
Risk of bias table, NOS.

lymph node status. As a secondary outcome, we aimed to determine the relationship between AT distribution and patient prognosis defined as (disease-specific/overall) survival. Meta-analysis was not possible after consulting a statistician (predominantly) due to heterogeneity in the quantification in AT compartments measurement in the included studies.

3 Results

3.1 Data extraction and characteristics of eligible studies

The PRISMA flow chart is shown in Figure 1 and resulted in a total of 11 studies that fulfilled the inclusion criteria. Articles were published between 2011 and 2022. From these 11 articles, the following information was recorded: author, year of publication, journal, number of included patients, setting (university/teaching hospital/community hospital), EC subtype, FIGO stage, grade, mean age, mean BMI, AT measurements, level of imaging, and primary outcome and results (see Table 1). As shown in Table 1, the transverse CT plane of imaging that was used to measure the AT compartments was different between the studies that were included.

Five cohort and six cross-sectional studies were included. Seven studies were retrospective and four prospective. The number of participants in these studies ranged from 20 to 545. Ten studies used CT imaging, and one MRI to quantify visceral AT and SAT. Four studies included women from Asian ethnicity, six studies included women from European populations, and one study included South American women. All studies but one focused solely on EC, whereas this latter focused on gynecological cancers and did perform subanalyses for patients with EC. Three studies included ≥ 50% women with high-grade (grade III) EC. Four studies included > 50% low-grade (grade I/II) tumors, and, in the remaining four studies, the subdivision was not clear. Furthermore, the BMI distribution was not equal in all studies, and mean BMI ranged from 23.5 to 32.9 kg/m^2.

All included studies investigated AT compartments on CT scan; however, different terminologies were used to describe the same AT compartments (see Figure 4). To facilitate legibility for the reader, we added Figure 4.

There was considerable variation in the quality of the included studies. Four studies were scored as “poor” quality (26–29), three studies were scored as “fair” (14, 30, 31), and four studies were

TABLE 1 Study characteristics of included studies.

Author	Year	Journal	Included patients	Hospital of inclusion	Type of endometrial cancer	FIGO	Grade	Mean Age	Mean BMI	Adipose tissue measurements	Unit	Imaging	Aim	**Results**
Nakamura	2011	Oncology reports	122	University Hospital, Okayama	All	All	I - 50% II - 20.5% III - 16.4%	56.98	X	VFA, SFA, TFA *	cm^2	CT L4/L5	Determine fat accumulation in visceral and subcutaneous adipose tissue on CT. Study the relationship of these findings with clinical variables in the various histological types.	Patients with type I endometrial cancer have a statistically significant association with obesity-related biological parameters.
Donkers	2021	European journal of Obstetrics & Gynecology and Reproductive Biology	176	Royal Cornwell Hospital Trust, UK (academical hospital)	All	All	III - 100%	70.0	29.4	SAV, VAV, TAV *	cm^3	CT L5/S1	Investigate the relationship between body fat distribution, assessed by CT-scan, in relation to overall and disease-specific survival in high-grade (grade 3) endometrial cancer patients.	In non endometrioid endometrial cancer, high visceral fat percentage was an independent predictor of poor survival. Hypertension and diabetes mellitus were significantly associated with high BMI and high visceral fat percentage.
dePaula	2020	Nutrition	545	Leading cancer institute, Brazil	All	All	I - 16.1% II - 25.1% III - 58.8%	64.5	29.8	SATI, VATI, SMI *	cm^2/m^2	CT L3	Provide the percentiles of distribution of body composition parameters according to cancer staging and body mass index (BMI). Identify the contribution of age, BMI, and cancer staging in the variation of the different parameters of body composition.	BMI was associated with body fat parameters and low-radiodensity SM index. Cancer stage was associated with SM index, mean SMD, and high-radiodensity SM index.

(Continued)

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TABLE 1 Continued

Author	Year	Journal	Included patients	Hospital of inclusion	Type of endometrial cancer	FIGO	Grade	Mean Age	Mean BMI	Adipose tissue measurements	Unit	Imaging	Aim	**Results**
Ye	2016	BMC Cancer	200	Shanghai	All	I-III	I - 43.0% II - 42.5% III - 14.5%	54	24.7	VAT, SAT *	%	CT L4/L5	To assess the effect of visceral adiposity on clinical and pathological characteristics in patients with endometrial cancer.	Viscerally obese patients were more likely to be old and have positive lymph nodes as well as extrauterine disease.
Tangen	2019	Gynecologic Oncology	20	Haukeland University Hospital, Bergen	Endometrioid/ non-endometrioid	I/II	I/II - 50% III - 50%	X	25.2	VAV, SAV *	cm^3	CT L5/S1	Investigate the relation between level of steroids in blood and prognosis for endometrial cancer patients.	DHEA, DHEAS, progesterone, 21 OH progesterone and E1S were significantly increased in patients with long survival compared to patients with short survival. Estradiol levels were significantly positively correlated with visceral fat percentage.
Nattenmüller	2018	Oncotarget	54	University Hospital Heidelberg	X	All	X	X	28.4	TAT, VAT, SAT *	cm^2	CT L3/L4	Investigate the impact of body composition on overall survival (OS) in gynecological malignancies.	There was no statistically significant impact of any BC-parameters on OS.
Weelden	2019	BMC cancer	39	Radboudumc, Nijmegen (academical hostpital)	All	All	I - 10% II - 41% III - 48%	68.0	26.9	SAV, VAV, TAV *	cm^3	CT L5/S1	Explore the relation between BMI, visceral and subcutaneous fat volumes and sex steroids and lipids levels in endometrial cancer patients.	Serum estradiol is moderately correlated with BMI and VAV and strongly correlated with SAV. Other sex steroids and lipids have weak and moderate correlations with VAV or SAV

(Continued)

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TABLE 1 Continued

Author	Year	Journal	Included patients	Hospital of inclusion	Type of endometrial cancer	FIGO	Grade	Mean Age	Mean BMI	Adipose tissue measurements	Unit	Imaging	Aim	**Results**
Celik	2021	Obstetrics and Gynaecology Research	186	Istanbul University Institute of Oncology	Endometrioid/ non-endometrioid	All	I - 38.7% II/III - 61.3%	62.9	32.9	VAT, SAT *	cm^2	MRI umbilical	Explore the relationship between VAT/SAT and survival in endometrial cancer patients.	Visceral adipose tissue is a significant and reliable prognostic indicator for endometrial cancer prognosis.
Mauland	2017	oncotarget	227	Haukeland University Hospital, Bergen	Endometrioid/ non-endometrioid	All	I/II - 68% III - 32%	66.9	27.9	SAV, VAV, TAV *	ml, %	CT L5/S1	Explore CT-quantified abdominal fat volumes and fat distribution in relation to BMI, clinicpathological features and survival in endometrial cancer patients.	High VAV% independently predicts reduced survival in EC patients.
Cho	2020	biomedical	52	Soonchunhyang University College of Medicine, Seoul	All	All	X	X	X	VFA, SFA, TFA *	cm^2	CT L4/L5	Predict the effect of subcutaneous and visceral fat on endometrial cancer.	Unlike subcutaneous fat, visceral fat is more directly related to the development of endometrial cancer.
Wada	2022	International journal of clinical oncology	148	National Hospital Organization Kyoto Medical Center, Kyoto?	Endometrioid/ non-endometrioid	All	X	61.5	23.5	Visceral fat, Subcutaneous fat, V/S ratio	cm^2	CT umbilical	Investigate the association between prognostic factors of type 1 and 2 endometrial cancer and obesity parameters.	A V/S ratio > 0.5 is a possible factor for poor prognosis in type 1 endometrial cancer.

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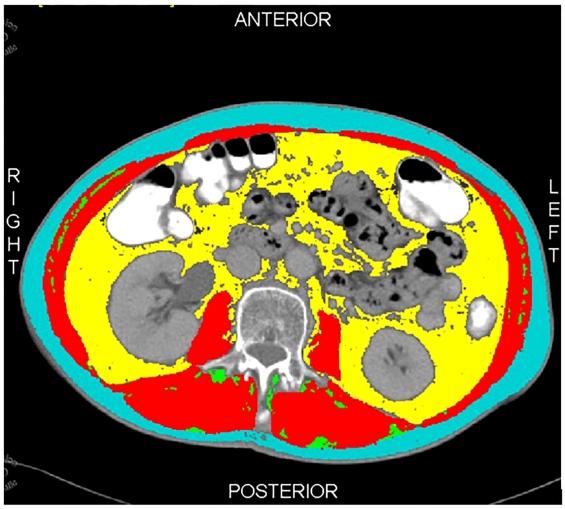

(Adipose) tissue compartment	Abrevations used	Color
Visceral AT	VAT, VAV	Yellow
Subcutaneous AT	SAV, SATI, SFA, SAT	Blue
Total AT	TAV, TFA, TAT	
Muscle	x	Red
Intra-muscular AT	x	Green

FIGURE 4
Explanation of AT distribution and different terminology.

scored as "good" quality (32–35). The reason for judging a study as "poor" was mostly due to lack of information in the methods and the results/outcome sections (see Figures 2, 3).

3.1.1 Relationship between AT distribution and patient characteristics

3.1.1.1 BMI

Five studies (n = 746) explored the correlation between BMI and CT scan–based AT distribution (28, 29, 34–36). All five studies found a significant positive correlation between AT distribution and BMI (Table 2) (28, 29, 34–36), indicating that patients with a higher BMI also demonstrated higher quantities of AT on their CT scan. This relationship was significant for all measured AT distribution parameters as applied in different studies, including visceral, subcutaneous, and total AT (TAT). The two studies that investigated the relation between BMI and V/S ratio and VAT% did not find a significant relation between these parameters (29, 35).

3.1.1.2 Sex steroid hormone levels

Two smaller studies (n = 20 and n = 39) in postmenopausal women compared sex steroid hormone levels in relation to AT distribution (14, 31). Tangen et al., in a highly selective cohort of women with poor and good prognosis, reported a positive correlation

TABLE 2 Relationship between adipose tissue (AT) distribution patient characteristics (BMI and sex steroid levels).

Relationship between AT distribution and BMI	Patients (n)	VFA/VAV	SFA/SAV	TFA/TAV	V/S ratio	VAT%
Cho	52	r^2 = 0.299 **p ≤ 0.0001**	r^2 = 0.528 **p ≤ 0.0001**	r^2 = 0.584 **p ≤ 0.0001**	x	x
Wada	145	R = 0.678 **p ≤ 0.01**	R = 0.872 **p ≤ 0.01**	R = 0.871 **p ≤ 0.01**	R = 0.05 p = 0.52	x
Ye	200	x	x	R = 0.667 **p ≤ 0.0001**	x	R = 0.743 p = 0.495
Nakamura	122	R = 0.743 **p ≤ 0.0001**	R = 0.895 **p ≤ 0.0001**	R = 0.907 **p ≤ 0.0001**	x	x
Mauland	227	r = 0.78 p ≤ 0.0001	r = 0.87 p ≤ 0.0001	r = 0.89 p ≤ 0.001	x	x
Relationship AT distribution and sex steroid levels	**Patiënts (n)**	**VAV**	**SAV**	**TAV**	**BMI**	**VAV%**
Tangen	20					
* E2		r = 0.42 p = 0.068	r = 0.005 p = 0.98	r = 0.24 p = 0.31	x	r = 0.47 p = 0.035
Weelden	39					
* E2		r = 0.58 **p ≤ 0.01**	r = 0.74 **p ≤ 0.01**	r = 0.74 **p ≤ 0.01**	r = 0.62 **p ≤ 0.01**	r = −0.06 NS
* A4		r = 0.29 NS	r = 0.43 **p ≤ 0.01**	r = 0.37 **p ≤ 0.05**	r = 0.26 NS	r = −0.17 NS
* DHEAS		r = 0.3 **p ≤ 0.05**	r = 0.3 **p ≤ 0.05**	r = 0.30 NS	r = 0.36 **p ≤ 0.05**	r = −0.10 NS

VFA, visceral fat area; VAV, visceral abdominal fat area; SFA, subcutaneous fat area; SAV, subcutaneous abdominal fat area; TFA, total fat area; TAV, total abdominal fat area; VAT%/VAV%, percentage of visceral adipose tissue; A4, androstenedione; DHEAS, dehydroepiandrosteronsulfate; x, outcome not reported; NS, not significant.
Bold values are statistical significant values.

between VAT percentage (VAV%) and estradiol (E2) levels (r = 0.47, p = 0.035; Table 2). Notably, neither BMI, TAT volume (TAV), SAT volume (SAV), nor VAT volume (VAV) were found to be significantly correlated with E2 levels (31). In contrast, Weelden et al., in a cohort selected on the basis of availability of a broad hormone analysis and preoperative CT scan, found a positive correlation between E2 and SAV (r = 0.74, p < 0.01), TAV (r = 0.74, p< 0.01), BMI (r = 0.62, p < 0.01), and VAV (r = 0.58, p < 0.01) (see Table 3). Androstenedione (A4) was positively correlated with SAV (r = 0.43, p < 0.01) and TAV (r = 0.37, p < 0.05). Dehydroepiandrosteronesulfate (DHEAS) was positively correlated with BMI, VAV, and SAV (r = 0.36, r = 0.35 and 0.34, all p < 0.05) (14).

3.1.2 Relationship between AT distribution and disease characteristics

3.1.2.1 FIGO stage

The relation of AT fat distribution and FIGO stage was reported in three studies including a total of 948 patients (27, 33, 34). The largest study (n = 545) observed a lower mean SAT index (SATI) in patients with a higher FIGO stage (FIGO stage III/IV) (p = 0.034) (33). Whereas, two other studies (n = 403 in total) did not find any significant association between AT distribution and FIGO stage [low (I/II) vs. high (III/IV)] (27, 34). These two studies included quite different patient populations, with 38% endometrioid EC and 100% grade III tumors in the study by Donkers et al. and 82% endometrioid EC with only 32% grade III tumors in the study by Mauland et al. However, a combination of these study characteristics was quite similar to that in the first study by de Paula et al.

3.1.2.2 Histopathological characteristics

Two studies (n = 298) presented data on the relationship between AT distribution and histological subtype (27, 28). The first study, by Nakamura et al. (n = 122), that included predominantly grade I/II EC (>70%), observed that patients with endometrioid EC had a significant higher BMI (p = 0.006), increased subcutaneous fat area (SFA) (p = 0.005), and increased total fat area (TFA) (p = 0.006) when compared to patients with non-endometrioid subtypes (28). Donkers et al. (n = 176), who solely included grade III EC, however, did not find an association between any obesity parameters and endometrioid and non-endometrioid subtypes (27).

3.1.2.3 Lymph node status

The study from Ye and colleagues was the only study reporting specifically on histopathological features in relation to VAT%. The study mostly included low-grade EC and only 14.5% high-grade EC. Higher VAT% in this study was significantly associated with the presence of lymph node metastases (p = 0.042), unrelated to subtype. They did not find any statistically significant association between VAT% and histological subtype, grade, myometrial invasion depth, tumor size, or lympho-vascular invasion (35).

3.1.3 Relationship between AT distribution and patient prognosis

Five studies (n = 788), which were quite dissimilar in their patient cohorts, reported on survival parameters including overall survival (OS), progression-free survival (PFS), and disease-specific survival (DSS) (27, 29, 30, 32, 34). In two studies, the VAV% in relation to OS was evaluated (see Table 4). Mauland et al. (n = 227),

TABLE 3 Relationship between adipose tissue (AT) distribution and disease characteristics (FIGO stage, histology, and other histopathological features).

Relationship between AT distribution and higher FIGO stage	Patiënts (n)	SATI/SAV	VATI/VAV	TAV	VAV%	HRSMI	BMI
de Paula	545	**p = 0.034**	p = 0.085	x	x	**p = 0.044**	x
Mauland	227	p = 0.66	p = 0.79	p = 0.90	p = 0.21	x	x
Donkers	176	p = 0.17	p = 0.45	p = 0.17	p = 0.88	x	p = 0.036
Relationship between AT distribution and histology (Type I and II endometrial cancer)	**Patiënts (n)**	**VFA/VAV**	**SFA/SAV**	**TFA/TAV**	**VAV%**	**BMI**	
Nakamura*	122	p = 0.309	**p = 0.005**	**p = 0.006**	x	**p = 0.006**	
Donkers	176	p = 0.64	p = 0.28	p = 0.88	p = 0.97	p = 0.66	
Relationship between AT distribution (VAT%) and histopathological features **	**Patients (n)**	**Histology**	**Grade**	**Myometrial invasion depth**	**Tumor size**	**Positive lymph node status**	**LVSI**
Ye	122	p = 0.381	p = 0.069	p = 0.093	p = 0.791	**p = 0.042**	p = 0.582

SATI, subcutaneous adipose tissue index; SAV, subcutaneous abdominal fat volume; SFA, subcutaneous fat area; VATI, visceral adipose tissue index; VAV, visceral abdominal fat volume; VFA, visceral fat area; TAV, total abdominal fat volume; TFA, total fat area; VAV%, percentage of visceral fat volume; HRSMI, high-radiodensity skeletal muscle index; BMI, body mass index; LVSI, lympho-vascular invasion; x, outcome not included in article. *, significant in type II EC; **, (VAT % < 31.89% and VAT% ≥ 31.89%).
Bold values are statistical significant values.

TABLE 4 Relationship between adipose tissue (AT) distribution and survival.

Relationship between AT distribution and Survival	Patients (n)	Patient group	Fat distribution parameter	Outcome	p-value
Mauland	227	All patients	VAV% ≥ 37%	Reduced OS (#)	**0.005**
Donkers	176	All patients	VAV% > 34%	Reduced OS ($)	**0.006**
		Non-endometrioid		Reduced OS & DSS (#)	**0.026**
Celik	186	All patients	VAT index > 0.265	Reduced DSS ($)	**0.029**
Wada	145	Endometrioid	V/S ratio (> 0.5)	Reduced OS ($)	**0.005**
				Reduced PFS ($)	**0.008**
Nattenmuller	54	All patients	Any	No effect on OS ($)	NS

#, multivariable analyses; $, univariable analyses; VAV%, visceral fat percentage; VAT index, visceral adipose tissue index; V/S ratio, visceral/subcutaneous index; OS, overall survival; DSS, disease-specific survival; PFS, progression-free survival; NS, not significant.
Bold values are statistical significant values.

with 82% endometrioid EC and 32% grade III tumors in their cohort, found that a VAV% ≥ 37% was independently associated with a reduced OS (p = 0.005) (34). Donkers et al. (n = 176), including 38% endometrioid EC and 100% grade III tumors, observed a similar relationship, but with a different cutoff value (VAV% > 34%) and only in univariable analysis. However, in subgroup analysis within non-endometrioid patients in the Donkers study, this association remained significant in the multivariable analysis for OS (p = 0.006) and DSS (p = 0.026) (27).

A third study, by Celik and colleagues (n = 186), classified patients into a VAT index ≤ 0.265 and a VAT index > 0.265. This index could not be translated to a clinical percentage based on the study information (32). This study, including a somewhat higher risk population with 61% grade III tumors despite 71% endometrioid EC, found no significant difference in PFS (p = 0.186); however, DSS was more favorable in the lower VAT index group (p = 0.029) (32). Wada et al. (n = 145), including a cohort with a relatively lower mean BMI of 23.5 kg/m^2, explored the V/S ratio as a prognostic factor for PFS and OS in type I and II EC (29). The authors found that a V/S ratio > 0.5 was associated with a poor prognosis (OS and PFS) in univariable analyses including endometrioid (p = 0.0053 and p = 0.0080) but not in non-endometrioid EC (29). The remaining, smallest, study (n = 54) did not show a significant impact of AT distribution on OS (30). This study by Nattenmuller et al. also failed to provide any patients characteristics besides mean BMI.

4 Discussion

This review aimed to give an overview about the knowledge concerning AT distribution and EC. EC is considered to be affected by the obesity paradox, which presumes that, in contrast to an overall poorer prognosis due to obesity, obesity is associated with less aggressive biological subtypes of EC and, therefore, a better cancer specific prognosis may be found (9). However, this contrasts the observation that also the non-endometrioid or more aggressive subtypes show a rising incidence in obese women. As mentioned earlier, obesity is defined as a BMI above 30 kg/m^2 (3). This definition, however, does not differentiate between the amount of AT or muscle or cover the complexity of AT distribution in visceral and subcutaneous compartments. Therefore one possible explanation for the obesity paradox is that it considers obesity as one entity and disregards these distinct localizations, subcutaneously or viscerally, with most likely different metabolic activity and distinct effects on cancer development. Low-grade inflammation is associated with VAT rather than with the SAT, where there is high aromatase activity. To our knowledge, this may distinctly affect EC development and fuel the attention for AT distribution and the way that we portray obesity (15).

Overall, this review had a number of notable findings that we will discuss in details. First, there is a strong correlation between BMI and imaging-based AT distribution measures. Second, studies indicate a significant association between AT distribution and sex-steroid hormone levels. Third, there are indications that a relation between AT distribution and histopathological findings exists. This relation is not consistent in the included studies, which may, in part, be explained by inclusion bias, as studies varied widely in subtypes and grades included. Last, and maybe most importantly, in all studies reporting about patient prognosis, increased VAV is associated with a worse survival (OS, DSS, and PFS) (27, 29, 32, 34).

All included studies found a significant positive correlation between BMI and the amount of SAT VAT and TAT (28, 29, 34, 36). BMI is the easiest way of classifying obese patients, and, currently, CT scans are not routinely performed for AT distribution (only). A study by Kammerlander et al. reported that simple anthropometric measures of obesity such as waist circumference and BMI were accurate for assessing cardiovascular risk in men but not in women. In women, VAT measurement through CT scan allowed a more precise assessment of obesity-associated cardiometabolic and cardiovascular risk (21). This underscores that there is an additional and clinical value in supplementing routine BMI measurement with more sophisticated measurements of other obesity-linked variables, including AT distribution above all in women. A similar study has not been yet carried out in patients with cancer.

Studying the relation between AT distribution and sex-steroid hormone level is challenging because of the uncertain contribution of pre- and postmenopausal ovaries to the systemic sex-steroid

hormone levels. The retrospective nature of the included studies further complicates this. The two studies reporting on this outcome though included women with mean age of 66–68 and, therefore, presumably mostly postmenopausal women. Although sample size urgently needs to be enlarged, these studies demonstrate that AT distribution, specifically increased SAT and VAV%, is significantly associated with increased E2 levels. Future prospective larger studies are needed to confirm this relationship. We have recently set up the ENDOCRINE study, prospectively studying the effect of obesity, AT distribution, and oophorectomy on hormone levels in patients with EC and controls (37). This study may therefore be able to answer which AT compartment plays the most important role in E2 production and quantify how obesity and AT distribution contribute to differences in systemic sex-steroid hormone levels and resulting risk of EC.

The positive association between the higher amount of TAT and SAT and endometrioid type EC (28) fits with the classical etiological risk factors for endometrioid type EC (38). In the study by Nakamura, 70% of patients indeed suffered from low-grade endometrioid EC. This may therefore also support the lack of a similar association between AT distribution and subtype in the study by Donkers et al. (27), who only included high-grade EC, of which 60% of non-endometrioid subtype. The association between higher VAT% and a relative abundance of VAT with lymph node metastasis as reported by Ye et al. (35) may suggest a different and more aggressive tumor biology effect by VAT. Unfortunately, none of the other studies included lymph node metastasis as an outcome parameter. This more aggressive tumor biology might be in line with a study of Habanjar et al. They demonstrated that chronic low-grade inflammation resulted in a higher influx of macrophages in the tumor microenvironment, which stimulated angiogenesis, tumor cell motility, and infiltration. The macrophages also initiated the pre-metastatic site, promoting extravasation, survival, and sustained growth of tumor cells (39). Although speculative, as a higher amount of VAT results in a state of chronic low-grade inflammation, a higher incidence of lymph node metastasis may be expected (40).

Considering patient outcome, all studies reporting on this outcome demonstrated a worse prognosis, predominantly shown by a reduced OS and DSS, in patients with a higher VAV (27, 29, 32, 34). Relevant literature for comparison was mostly found in breast and colorectal cancer. A review in breast cancer by Picon-Ruiz et al. summarized that overall obesity was linked to both a shorter DSS and OS, both in pre- and postmenopausal women (41). Another breast cancer study focusing specifically on AT distribution found in their cohort a negative relation between the amount of SAT and OS but no relation between the amount of VAT and OS (42). This might be explained by the fact that patients with in the lowest VAT quartile were, on average, 12 years younger (48 years) compared with the patients in the highest quartile of VAT (60 years), affecting survival in itself. They also hypothesized that some parts of the abdominal SAT might have similar metabolic effects to VAT (41). However, this hypothesis has not been substantiated in other studies. A further study in (colo)rectal cancer in contrast showed a longer OS in patients with a higher SAT ratio but did not find VAT to be an independent prognostic factor (43). In a last study concerning colorectal patients, increased V/S ratio was significantly associated with a higher recurrence and shorter OS and DSS in patients with mid and low rectal cancer (22). These studies indicate that there is evidence on the role of AT distribution and survival in a number of cancer types. So far, there is evidence suggesting that AT distribution plays a role in the pathogenesis of several different cancer types. This evidence, however, is not conclusive yet and associations may be tumor specific.

There are a number of limitations that need to be addressed. First, studies used different measurements for displaying the AT distribution, like SAV, SAT, SATI, and SFA that are all used to display the amount of SAT. Using all these different terms makes the comparison and thus interpretation of these studies challenging (See Figure 4). Second, there is a plethora and heterogeneity in the quantification measures of the AT compartments in the included studies, precluding meta-analyses. For example, there is no agreement at what transverse CT-plane AT compartments are best measured. Because of the lack of a gold standard, all levels (L3 through S1) were accepted in this review but will need to be more standardized in future studies. In addition, this may have caused confounding in the results.

A broad search was performed to avoid missing any important studies in this research area. As a consequence, studies of moderate quality were also included, where varying degrees of selection bias were present, as documented in the risk of bias tables. This precluded strong conclusions.

To conclude, to our knowledge, this is the first review to summarize the evidence on the role of AT distribution on patient, disease characteristics, and prognosis in patients with EC. AT distribution may be the missing link between obesity and EC. There is strong evidence, already in these retrospective studies, that AT distribution affects patient prognosis in EC. Furthermore, correlations exist between AT distribution and patient and disease characteristics (including histology and lymph node status). Well-designed, prospective, and large-scale studies are essential to further understand and maybe find a way for more selective identification of women at risk of EC and even in therapeutic options for EC. Possible clinical applications might be improving the understanding of different drivers in the pathogenesis of EC and therefore develop a better tool in recognition of patients at risk and differentiate which patients would benefit from additional therapeutic options. Furthermore, specifying the role of obesity in the pathogenesis of EC supports educating the lay public in the importance of obesity prevention.

Author contributions

AB - First authorship; HW: Equal contribution and last authorship; JP, AR, BW, LP, and RK: Equal contribution. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

1. International, W.C.R.F *Endometrial cancer statistics* (2022). Available at: https://www.wcrf.org/cancer-trends/endometrial-cancer-statistics/ (Accessed 2022 16-08).

2. Ali AT. Risk factors for endometrial cancer. *Ceska Gynekol* (2013) 78(5):448–59.

3. organization, W.-W.h. Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (Accessed 2023 17-01).

4. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature* (2006) 444(7121):875–80. doi: 10.1038/nature05487

5. Ford ES, Zhao G, Li C. Pre-diabetes and the risk for cardiovascular disease: a systematic review of the evidence. *J Am Coll Cardiol* (2010) 55(13):1310–7. doi: 10.1016/j.jacc.2009.10.060

6. Bhaskaran K, Douglas I, Forbes H, I, Leon DA, Smeeth L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5. *24 million UK adults Lancet* (2014) 384(9945):755–65. doi: 10.1016/S0140-6736(14)60892-8

7. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* (2008) 371(9612):569–78. doi: 10.1016/S0140-6736(08)60269-X

8. McCullough ML, Patel AV, Patel R, Rodriguez C, Spencer Feigelson H, Bandera EV, et al. Body mass and endometrial cancer risk by hormone replacement therapy and cancer subtype. *Cancer Epidemiol Biomarkers Prev* (2008) 17(1):73–9. doi: 10.1158/1055-9965.EPI-07-2567

9. Lennon H, Sperrin M, Badrick E, Renehan AG. The obesity paradox in cancer: a review. *Curr Oncol Rep* (2016) 18(9):56. doi: 10.1007/s11912-016-0539-4

10. De Pergola G, Silvestris F. Obesity as a major risk factor for cancer. *J Obes* (2013) 2013:291546. doi: 10.1155/2013/291546

11. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* (2004) 4(8):579–91. doi: 10.1038/nrc1408

12. Prentice AM, Jebb SA. Beyond body mass index. *Obes Rev* (2001) 2(3):141–7. doi: 10.1046/j.1467-789x.2001.00031.x

13. Blakemore J, Naftolin F. Aromatase: contributions to physiology and disease in women and men. *Physiol (Bethesda)* (2016) 31(4):258–69. doi: 10.1152/physiol.00054.2015

14. van Weelden WJ, Eldevik Fasmer K, Tangen IL, IntHout J, Abbink K, van Herwaarden AE, et al. Impact of body mass index and fat distribution on sex steroid levels in endometrial carcinoma: a retrospective study. *BMC Cancer* (2019) 19(1):547. doi: 10.1186/s12885-019-5770-6

15. Rakotoarivelo V, Lacraz G, Mayhue M, Brown C, Rottembourg D, Fradette J, et al. Inflammatory cytokine profiles in visceral and subcutaneous adipose tissues of obese patients undergoing bariatric surgery reveal lack of correlation with obesity or diabetes. *EBioMedicine* (2018) 30:237–47. doi: 10.1016/j.ebiom.2018.03.004

16. WHO

17. Duren DL, Sherwood RJ, Czerwinski SA, Lee M, Choh AC, Siervogel R, et al Body composition methods: comparisons and interpretation. *J Diabetes Sci Technol* (2008) 2(6):1139–46. doi: 10.1177/193229680800200623

18. Abraham TM, Pedley A, Massaro JM, Hoffmann U, Fox CS. Association between visceral and subcutaneous adipose depots and incident cardiovascular disease risk factors. *Circulation* (2015) 132(17):1639–47. doi: 10.1161/CIRCULATIONAHA.114.015000

19. Di Rollo DG, McGovern J, Morton C, Miller G, Dolan R, Morgan PG, et al. Relationship between BMI, CT-derived body composition and colorectal neoplasia in a bowel screening population. *Scott Med J* (2022) 67(3):93–102. doi: 10.1177/00369330221102237

20. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu C, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the framingham heart study. *Circulation* (2007) 116(1):39–48. doi: 10.1161/CIRCULATIONAHA.106.675355

21. Kammerlander AA, Lyass A, Mahoney TF, Massaro JM, Long MT, Vasan S, et al. Sex differences in the associations of visceral adipose tissue and cardiometabolic and cardiovascular disease risk: the framingham heart study. *J Am Heart Assoc* (2021) 10(11):e019968. doi: 10.1161/JAHA.120.019968

22. Lee KH, Kang BK, Ahn BK. Higher visceral fat area/subcutaneous fat area ratio measured by computed tomography is associated with recurrence and poor survival in patients with mid and low rectal cancers. *Int J Colorectal Dis* (2018) 33(9):1303–7. doi: 10.1007/s00384-018-3065-z

23. Page MJ, Mckenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *PloS Med* (2021) 18(3):e1003583. doi: 10.1136/bmj.n71

24. Downes MJ, Brennan ML, Williams HC, Dean RS. Development of a critical appraisal tool to assess the quality of cross-sectional studies (AXIS). *BMJ Open* (2016) 6 (12):e011458. doi: 10.1136/bmjopen-2016-011458

25. Wells GA. *The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses*. Canada (2014).

26. Cho JH, Kim JS, Park S, Kim WY. Value of computed tomography-defined visceral fat area as a risk factor for endometrial cancer. *Clin Exp Obstetrics Gynecology* (2021) 48(2):359–64. doi: 10.26717/BJSTR.2020.28.004609

27. Donkers H, Fasmer KE, Mcgrane J, Pijnenborg JMA, Bekkers R, Haldorsen IS, et al. Obesity and visceral fat: survival impact in high-grade endometrial cancer. *Eur J Obstet Gynecol Reprod Biol* (2021) 256:425–32. doi: 10.1016/j.ejogrb.2020.11.050

28. Nakamura K, Hongo A, Kodama J, Hiramatsu Y. Fat accumulation in adipose tissues as a risk factor for the development of endometrial cancer. *Oncol Rep* (2011) 26 (1):65–71. doi: 10.3892/or.2011.1259

29. Wada M, Yamaguchi K, Yamakage H, Inoue T, Kusakabe T, Abiko K, et al. Visceral-to-subcutaneous fat ratio is a possible prognostic factor for type 1 endometrial cancer. *Int J Clin Oncol* (2022) 27(2):434–40. doi: 10.1007/s10147-021-02060-1

30. Nattenmuller J, Rom J, Buckner T, Arvin J, Bau B, Sohn C, et al. Visceral abdominal fat measured by computer tomography as a prognostic factor for gynecological malignancies? *Oncotarget* (2018) 9(23):16330–42. doi: 10.18632/oncotarget.24667

31. Tangen IL, Fasmer KE, Konings GF, Jochems A, Delvoux B, Xanthoulea S, et al. Blood steroids are associated with prognosis and fat distribution in endometrial cancer. *Gynecologic Oncol* (2019) 152(1):46–52. doi: 10.1016/j.ygyno.2018.10.024

32. Celik E, Yirgin IK, Celik HG, Engin G, Sozen H, Ak N, et al. Does visceral adiposity have an effect on the survival outcomes of the patients with endometrial cancer? *J Obstet Gynaecol Res* (2021) 47(2):560–9. doi: 10.1111/jog.14560

33. de Paula NS, Chaves GV. Percentiles for body composition parameters based on computed tomography in patients with endometrial cancer. *Nutrition* (2020) 79-80:110873. doi: 10.1016/j.nut.2020.110873

34. Mauland KK, Eng Ø, Ytre-Hauge S, Tangen IL, Berg A, Salvesen HB, et al. High visceral fat percentage is associated with poor outcome in endometrial cancer. *Oncotarget* (2017) 8(62):105184–95. doi: 10.18632/oncotarget.21917

35. Ye S, Wen H, Jiang Z, Wu X. The effect of visceral obesity on clinicopathological features in patients with endometrial cancer: a retrospective analysis of 200 Chinese patients. *BMC Cancer* (2016) 16:209. doi: 10.1186/s12885-016-2230-4

36. Cho J, Kim JS, Park S, Kim WW. Value of computed tomography-defined visceral fat area as a risk factor for endometrial cancer. *Biomedical* (2020) 28(1). doi: 10.26717/BJSTR.2020.28.004609

37. van den Bosch AAS, Pijnenborg JMA, Romano A, Haldorsen IS, Werner HMJ, et al. The role of fat distribution and inflammation in the origin of endometrial cancer, study protocol of the ENDOCRINE study. *PloS One* (2022) 17(10):e0276516. doi: 10.1371/journal.pone.0276516

38. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol* (1983) 15(1):10–7. doi: 10.1016/0090-8258(83)90111-7

39. Habanjar O, Diab-Assaf M, Caldefie-Chezet F, Delort L. The impact of obesity, adipose tissue, and tumor microenvironment on macrophage polarization and metastasis. *Biol (Basel)* (2022) 11(2). doi: 10.3390/biology11020339

40. Cancello R, Clement K. Is obesity an inflammatory illness? role of low-grade inflammation and macrophage infiltration in human white adipose tissue. *BJOG* (2006) 113(10):1141–7. doi: 10.1111/j.1471-0528.2006.01004.x

41. Picon-Ruiz M, Morata-Tarifa C, Valle-Goffin JJ, Friedman ER, Slingerland JM, et al. Obesity and adverse breast cancer risk and outcome: mechanistic insights and strategies for intervention. *CA Cancer J Clin* (2017) 67(5):378–97. doi: 10.3322/caac.21405

42. Bradshaw PT, Cespedes Feliciano EM, Prado CM, Alexeeff S, Albers KB, Chen WY, et al. Adipose tissue distribution and survival among women with nonmetastatic breast cancer. *Obes (Silver Spring)* (2019) 27(6):997–1004. doi: 10.1002/oby.22458

43. Karacelik T, Kaya B, Korkmaz M, Karaağaç M, Araz M, Eryilmaz MK, et al. Prognostic significance of adipose tissue distribution and metabolic activity in PET/CT in patients with metastatic colorectal cancer. *J Gastrointest Cancer* (2022). doi: 10.1007/s12029-022-00819-x

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