Review

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Unlocking the Potential of Liquid Biopsy: A Paradigm Shift in Endometrial Cancer Care

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Abstract

Endometrial cancer is one of the most prevalent gynecologic malignancies in developed countries, with its incidence steadily increasing each year. Early diagnosis is crucial for a favorable prognosis; however certain patients experience recurrence and distant metastasis after surgery, similar to advanced cancer patients, with limited treatment options. Therefore, effective strategies for early screening, diagnosis, predicting local recurrence, and guiding rapid treatment interventions are essential for improving survival rates and prognosis. Liquid biopsy, a method known for being non-invasive, safe, and effective, has attracted widespread attention for cancer diagnosis and treatment. Although its clinical application in endometrial cancer is less established than in other cancers, research on biomarkers using liquid biopsy in endometrial cancer patients is currently in progress. This review examines the latest advancements in non-invasive biomarkers identified through liquid biopsy and provides a comprehensive overview of their clinical applications in endometrial cancer. Additionally, it discusses the challenges and future prospects of liquid biopsy, offering valuable insights into the diagnosis and personalized treatment of endometrial cancer.

Keywords: liquid biopsy; endometrial cancer; biomarkers; non-invasive; personalized medicine; early diagnosis

1. Introduction

Endometrial cancer (EC), a malignant epithelial tumor of the uterus, ranks sixth among the most common cancers in women from developed countries [1]. Despite our expanding understanding about EC over time, both incidence and mortality rates continue to rise steadily [1, 2]. Prognosis in EC largely depends on the tumor stage at diagnosis. The 5-year survival rate for Stage I patients is approximately 92%, but declines significantly in more advanced stages, dropping to 74%, 48%, and 15% for Stages II, III and IV, respectively [3]. Current EC guidelines recommend surgery for early-stage, low-risk cases, and a combination of surgery and postoperative adjuvant therapy for high-risk or advanced cases [4]. Notably, since the International Federation of Gynecology and Obstetrics (FIGO) incorporated molecular typing into the EC staging system in August 2023 [5], molecular profiling has become an increasingly valuable tool for guiding treatment decisions. However, despite these advances, the prognosis for patients with advanced or recurrent EC remains poor [6]. This may be attributed to the limited sensitivity of current imaging techniques in detecting early metastases, while molecular subtyping does not aid in the early diagnosis. Within molecular subtype-guided therapy, patients with the copy-number low (NSMP) subtype, particularly those who are estrogen receptor-negative, tend to have a significantly worse prognosis [7, 8]. Notably, patients with P53-abnormal EC have the poorest outcomes; even without chemotherapy, approximately 40% of those

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with TP53-mutations remain disease-free for five years [9]. These challenges highlight the ongoing need for improved risk stratification and guidance in adjuvant therapy. Therefore, finding more reliable tools and sensitive biomarkers is crucial for early diagnosis and personalized treatment. Recently, liquid biopsy has gained significant attention as a promising tool for precision medicine, cancer diagnosis, and therapy [10]. This technique involves analyzing non-solid biological materials such as blood, urine, cervical fluid, uterine aspirate, and peritoneal lavage fluid. Compared to conventional tissue biopsies, liquid biopsies are non-invasive, repeatable, and allow for real-time monitoring of treatment response and disease progression. They also overcome challenges related to anatomical sampling, patient age, cost, reproducibility, and clinical complications. Furthermore, they offer a more comprehensive view of heterogeneous and multifocal metastatic tumors [11]. Although the use of liquid biopsy in EC is still developing compared to other malignancies, research in this area is expanding. It is expected to play an important role in early detection, risk assessment, treatment selection, and real-time disease monitoring in EC. While recent reviews have primarily focused on blood-based biomarkers in liquid biopsies for EC [12–14], this review takes a broader perspective. It examines studies that utilize a variety of biosources, including both blood-based and non-blood-based specimens from EC patients. Additionally, we explore the advantages, limitations, and future developments of liquid biopsy technologies, offering new insights and directions for the personalized diagnosis and management of EC.

2. Methods

For this review, we searched PubMed (MEDLINE) in February 2025 for full-text, English-language articles on liquid biopsy in endometrial cancer published between January 2019 and January 2025. The search used the keywords “liquid biopsy,” “endometrial cancer,” and “clinical relevance.” We screened 720 titles for relevance to liquid biopsy, ultimately selecting 82 articles focused on endometrial cancer for detailed analysis. Additionally, foundational studies published before 2019 were included if they were frequently cited in recent research, particularly those describing key classifications, methods, or biomarkers.

3. Biological Components of Liquid Biopsy

Liquid biopsy targets can be broadly categorized into two groups based on their biological nature. The first includes cell-free molecules such as proteins, lipids, carbohydrates, metal ions, nucleic acids, and small metabolites. The second consists of cellular or subcellular components, including extracellular vesicles, circulating mitochondria [12], circulating tumor cells, peripheral blood mononuclear cells (PBMCs), circulating cancer-associated fibroblasts (CAF) [13], and tumor-educated platelets (TEP) [14]. Detection methods vary depending on the specific target type in the sample.

3.1. Circulating Tumor Cells

Circulating tumor cells (CTCs), originating from primary solid tumors or metastatic sites, enter the peripheral circulation through processes such as cellular invasion, matrix degradation, and angiogenesis. Only a small subset of CTCs—those with stem cell-like properties or epithelial-mesenchymal transition (EMT) features—can survive and migrate. Most CTCs are quickly eliminated by the immune system or destroyed by shear forces [15]. CTCs have a very short half-life, ranging from 1 to 2.4 hours [16], and are extremely rare, with only 0 to 28 cells typically detected in 7.5 milliliters of blood [17]. Their high heterogeneity leads to variable surface biomarker expression [18], making detection and the development of standardized treatment guidelines challenging. CTC detection typically involves three main stages: enrichment, detection, and analysis. Enrichment methods use physical properties-such as density, size and electrical charge-or biological approaches based on specific binding to cell surface antigens, or a combination of both. Molecular detection methods for CTCs include: (i) Nucleic acid analysis: Techniques such as fluorescence in situ hybridization (FISH) [19], microarrays [20] and Polymerase Chain Reaction (PCR)-based techniques [21], and sequencing-based techniques [22] are employed to detect genomic DNA or RNA signatures from CTCs in various body fluids. While these methods offer high sensitivity, their accuracy can be

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affected by background substances, including non-specific DNA/RNA, PCR inhibitors, and cross-hybridization, potentially leading to false positives or reduced accuracy. (ii) Protein Analysis: This approach focuses on identifying and characterizing surface or intracellular proteins of CTCs using techniques such as microfluidic technology [23] and enzyme-linked immunospot (ELISPOT) [24, 25]. While this approach reduces extensive or invasive manipulation of the target cells, thereby minimizing potential cellular interference, it can be time-consuming. (iii) Cellular function analysis: Culturing CTCs in vitro allows for the study of their proliferation, transformation, and invasion capabilities. Although this method offers high specificity, it is prone to cultivation failure due to the low viability and heterogeneity of CTCs, as well as factors such as initial cell count, cancer type, and culture conditions. [26].

3.2. Circulating Tumor DNA and Cell-Free DNA

Extracellular DNA fragments known as cell-free DNA (cfDNA) are discharged into the bloodstream by a number of cellular processes, including necrosis, apoptosis, and secretion [27]. These fragments, which may be single- or double-stranded, gain stability in circulation by binding to cell membranes and extracellular proteins, protecting them from nuclease-mediated degradation and rapid clearance. Circulating tumor DNA (ctDNA), released by cancer cells, reflects the tumor genomes from various sites, including primary tumors, CTCs, and metastases. Unlike a single tissue biopsy, ctDNA captures the molecular heterogeneity of cancer. It contains key genetic alterations found in tumor tissues, such as chromosomal rearrangements, point mutations, copy number variations, epigenetic modifications, insertions, and deletions [28]. In cancer patients, ctDNA levels range from 0.01% and 10% [29]. It is typically shorter than cfDNA—about 134 to 144 base pairs—with a half-life of approximately 114 minutes, making it a valuable tool for real-time tumor monitoring and assessing treatment response [29, 30].

The concentration of ctDNA in plasma is relatively low, but due to less contamination from white blood cell DNA, it is typically the preferred choice in clinical tests [31]. PCR-based methods, including droplet digital PCR (ddPCR), digital PCR (dPCR), and quantitative PCR (qPCR), are commonly used for detecting cfDNA and ctDNA due to their cost-effectiveness and high sensitivity. However, their limited ability to detect multiple mutations has led to the growing use of Next-Generation Sequencing (NGS) techniques for more comprehensive analysis, such as CAncer Personalized Profiling by deep Sequencing (CAPP-Seq) [32].

Epigenetic modifications, particularly ctDNA methylation patterns, have emerged as important biomarkers for cancer diagnosis and prognosis. Detection methods for ctDNA genomic regions, and genome-wide methods that enable comprehensive analysis [33]. PCR-based assays such as methylation-specific PCR (MSPCR) [34] and droplet digital MSPCR (ddMSPCR) [35], as well as target bisulfite sequencing, are examples of targeted approaches. Whole-genome bisulfite sequencing (WGBS) [36], TET-assisted pyridine borane sequencing (TAPS) [37], reduced representation bisulfite sequencing (RRBS) [38], and Infinium methylation arrays (HM450, HM850) [39] are examples of technologies used in genome-wide approaches, which include both site-specific and region-wide analysis. Advanced techniques, such as cfMeDIP-seq and nanopore sequencing, further enhance methylation profiling and are especially well-suited for analyzing the low-abundance, fragmented ctDNA found in liquid biopsies [33].

3.3. Circulating Tumor RNA and Cell-Free RNA

Cell-free RNA (cfRNA), including circulating tumor RNA (ctRNA) derived from cancer cells, consists of various types such as circular RNA (circRNA), microRNA (miRNA), and long non-coding RNA (lncRNA). cfRNA is released through passive mechanisms like normal cellular activity or cell death [40], as well as active secretion by cells. Although cfRNA has a plasma half-life of just 15 seconds, its stability is enhanced through interactions with proteins [41], proteolipid complexes, and extracellular vesicles [42]. Detection methods for cfRNAs and ctDNAs are similar and include quantitative reverse transcription PCR (qRT-PCR), reverse transcription PCR (RT-PCR), and RNA sequencing using NGS. Among these, qRT-PCR stands out for its high sensitivity, reproducibility, and accuracy in quantifying cfRNA [43].

 doi:10.20944/preprints202506.1856.v1

Individual cfRNA profiles vary significantly, and the lack of standardized clinical protocols often leads to false positive and false negative results. Consequently, researchers have tried to focus on RNA methylation. Common techniques for detecting RNA methylation include antibody-based immunoprecipitation combined with deep sequencing, mass spectrometry (MS), thin-layer chromatography, radioactive isotope incorporation, and bisulfite modification followed by sequencing [44]. Among these, methylated RNA immunoprecipitation sequencing (MeRIP-seq) remains a key method for identifying RNA methylation modifications [45].

3.4. Extracellular Vesicles

Extracellular vesicles (EVs), including exosomes, microvesicles and apoptotic bodies, are distinguished by their size, surface properties, biogenesis pathways, and molecular content. Exosomes, first discovered in the late 1960s [46], are the tiniest nanoscale EVs, typically ranging from 40 to 200 nm in diameter and having a density of 1.13 to 1.18 g/mL [47]. Produced by nearly all cell types under both healthy and diseased conditions, these vesicles facilitate the transfer of proteins, lipids, and nucleic acids, playing a key role in intercellular communication [47]. In cancer, EVs are involved in nearly every stage of disease progression, including the transformation of normal cells [48], tumor growth [49], angiogenesis [50], modulation of tumor microenvironment [51], invasion and metastasis [52], drug resistance [53], and EMT [54]. As such, EVs are considered promising candidates for cancer diagnosis, prognosis, and the development of therapeutic biomarkers.

Methods for EV enrichment and detection leverage their inherent properties, such as size, density, surface composition, and precipitation behavior. Currently, commonly used techniques include ultracentrifugation, ultrafiltration, precipitation, immunoaffinity capture, and lipid-based isolation [55]. Advanced approaches—such as microbeads, microfluidic chips, and thermal methods—are also being explored to enhance enrichment efficiency. Traditional detection methods like enzyme-linked immunosorbent assay (ELISA) [56] and Western blot analysis [57] remain reliable. However, emerging techniques, including colorimetry [58], fluorescence [59], flow cytometry [60] electrochemical analysis [61], electron microscopy [62], nanoparticle tracking analysis (NTA) [63], CRISPR/Cas-assisted methods [64], and single exosome detection [65], are enhancing the sensitivity and specificity of exosome research, particularly in the context of liquid biopsies.

3.5. Proteomics

Proteomics complements genomics, transcriptomics, and metabolomics by analyzing protein distribution, structure, interactions, and alterations within biological systems to offer a thorough understanding of biological processes [66]. Unlike the static nature of genomes, proteomes dynamically vary across different life stages and functional states. Early liquid biopsies efforts in cancer focused on identifying protein biomarkers in blood. Although over a hundred biomarkers, such as HE4 for ovarian cancer and SCC for cervical cancer, are used for treatment monitoring and recurrence assessment, their effectiveness in early detection is limited by insufficient specificity and sensitivity [67], underscoring the need for more advanced diagnostic approaches.

Technological advancements have revolutionized proteomics, shifting from traditional moderate-throughput methods such as ELISA and CLIA to high-throughput techniques like antibody/antigen arrays, proximity extension assays (PEA), reverse phase protein arrays (RPPA), and aptamer-based platforms [68]. MS now plays a central role, offering rapid protein sequencing, precise molecular weight determination, and quantitative detection of post-translational modifications [69]. In liquid biopsy analysis, MS is often integrated with liquid chromatography (LC), enzymatic digestion, and desalting, followed by electrospray ionization (ESI) and tandem MS scanning to enhance detection accuracy and sensitivity [70]. Recent advancements in MS instrumentation, including improved ion transmission efficiency and advanced noise-reduction algorithms, have significantly boosted single-cell and targeted proteomics.

While high-throughput methods support large-scale profiling, single-cell proteomics technologies address cellular heterogeneity. Techniques like mass cytometry for CTC immunophenotyping [71], microfluidics-based CTC isolation [72], and single-cell Western blotting [73] enable detailed protein expression analysis at the single-cell level, providing critical insights into

 doi:10.20944/preprints202506.1856.v1

cancer cell heterogeneity. However, antibody-based detection still faces limitations in specificity and throughput, necessitating further optimization to reduce cross-reactivity and enhance multiplexing capabilities.

3.6. Metabolomics

Metabolomics, systematically defined by Nicholson et al. in 1999 [74], focuses on the comprehensive analysis of low-molecular-weight metabolites (<1500 Da) using advanced spectroscopic, electrochemical, and computational techniques. Because metabolites rapidly respond to microenvironmental changes, they offer dynamic insights into physiological and pathological states, making metabolomics a highly sensitive approach for biomarker discovery [75]. As a non-invasive tool in liquid biopsy, metabolomics enables the identification of disease-associated biomarkers in biofluids; however, its clinical specificity still requires further validation to account for potential confounding factors. Metabolomics analyses typically follow two complementary strategies: non-targeted approaches, which explore global metabolite profiles for hypothesis generation, and targeted approaches, which quantitatively assess predefined metabolites in a hypothesis-driven manner [76]. Key analytical platforms for metabolomics include nuclear magnetic resonance (NMR) [77] and MS. NMR offers rapid, non-destructive analysis with high reproducibility; however, conventional ^{1}H NMR is limited in sensitivity and spectral resolution, especially for low-abundance metabolites or complex mixtures [77]. Emerging NMR technologies—such as two-dimensional spectroscopy [77] and cryogenic probe-assisted ^{13}C detection [78]—show promise in addressing these limitations.

MS-based technologies, including gas chromatography-MS (GC-MS) and liquid chromatography-MS (LC-MS), offer broader metabolite coverage and superior sensitivity [79]. LC-MS is ideal for non-volatile compounds, while GC-MS is suited for volatile metabolites. Recent advancements, such as ultra-performance LC-MS/MS (UPLC-MS/MS) [80] and nanoparticle-enhanced laser desorption/ionization MS (NPELDI-MS) [81], have further improved detection limits and ionization efficiency, enabling high-throughput metabolomics.

4. Application of Liquid Biopsy in EC

Liquid biopsies are increasingly recognized as valuable tools in EC management, with applications spanning early detection, prognosis, recurrence monitoring, and therapy guidance. In the following discussion, original research articles published on PubMed between January 2019 and January 2025, focusing on the use of liquid biopsy in EC, will be compiled and presented in a summary table along with relevant commentary. This analysis will be organized from two perspectives: studies based on blood-derived samples and those utilizing non-blood-derived specimens.

4.1. Blood-Based Liquid Biopsy in EC

Blood is the primary and most significant source for liquid biopsy. While tumor heterogeneity poses a major challenge in tissue-based sampling, liquid biopsy using blood allows for a more comprehensive and dynamic assessment of EC patients (Table 1).

Table 1. Application of liquid biopsy in blood samples.

Author and Year	Biomarkers	Detection Method	No. of participants (EC/control)	Clinical Significance/Findings	Accuracy	Ref.
CTCs						
Jiang et al., 2019	TOPO48 AAb, Survivin-expressing CCC	ELISA, RT-PCR–ELISA	80/ 80	Combination of TOPO48 AAb and survivin-expressing CCC improves early diagnosis (93.3% sensitivity) and prognostic	AUC:0.927 (0.871-0.984) for combined biomarkers; Sensitivity:74.5%	[160]

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				stratification (survival outcomes) in early-stage EC.	(TOPO48 AAb); Specificity: 100%(TOPO48 AAb)	
Herrero et al., 2021	ANXA2	qPCR and High-Throughput Screening	57 EC	ANXA2 expression in CTCs predicts EC recurrence and progression. Daunorubicin was identified as inhibiting ANXA2+ tumor cells.	N/A	[122]
Francini, et al., 2023	ER	CellSearch® System	10 stage I-II EC	CTCs were detected in ovarian vein samples (8/10 patients) during surgery, but not in peripheral blood samples. The potential prognostic value for recurrence risk requires validation in a larger cohort.	N/A	[102]
Law et al., 2023	Pan-CK, GATA3, HER2, HE4, CD13	V-BioChip Microfluidic Device	8 EC/9 other cancers	EC patients had preoperative expression of all four markers. CD13 was identified as an alternative prognostic marker for both cervical and CE.	N/A	[105]
cfDNA or ctDNA						
Bolivar et al., 2019	PTEN, KRAS, CTNNB1, PIK3CA	NGS	48 EC	Mutations in plasma were significantly associated with advanced stage, deep myometrial invasion, lymphatic/vascular invasion, and larger tumor size.	N/A	[82]
Benati et al., 2020	cfDNA、RTL	qRT-PCR	40/ 31	cfDNA RTL analysis may be a diagnostic tool for EC detection at an early stage, while its diagnostic performance seems unsatisfactory for cancer progression, staging, and grading.	AUC (95% CI): 0.87 (0.79-0.95); Sensitivity (95% CI):80.0% (64.35%–90.95%); Specificity (95% CI): 80.65% (62.53%–92.55%)	[84]
Gressel et al., 2020	Low molecular weight cfDNA	Fluorometric quantification	91/22	The concentration of LMW cfDNA was significantly higher in women with uterine cancer and associated with advanced stage, aggressive histology and worse OS.	N/A	[83]
Shintani et al., 2020	PIK3CA, KRAS	ddPCR	199 EC	ctDNA detection in pre-operative plasma was linked to advanced FIGO stage, aggressive histology,	N/A	[110]

 doi:10.20944/preprints202506.1856.v1

				LVSI, and shorter RFSand OS.		
Łukasiewicz et al., 2021	TEPs RNA, ctDNA	RNA-Seq and DNA Sequencing	53 EC, 38 benign gynecologic conditions, 204 healthy	ctDNA and TEPs presented the potential for EC diagnosis and tumor histology evaluation preoperatively.	TEPs AUC: 97.5% (vs. healthy), 84.1% (vs. benign); ctDNA AUC: 96% (tumor tissue); 69.8% (blood). CtDNA Sensitivity: 77.8%; CtDNA Specificity: 58%	[14]

Author and Year	Biomarkers	Detection Method	No. of participants (EC/control)	Clinical Significance/Findings	Accuracy	Ref.
Feng et al., 2021	PTEN, TP53, FAT4, ARID1A, ZFHX3, ATM, FBXW7	ddPCR	9 EC	Post-operative ctDN A detection predicted tumor relapse. DFS was shorter for ctDNA-positive cases.	AUC: N/A; Sensitivity:100%; Specificity:83.3	[103]
Grassi et al., 2021	Tumor-specific DNA junctions	qPCR	11 EC	Pre-surgical ctDNA was detected in 60% (6/10),and correlated with advanced stage and aggressive disease features. Post-surgical ctDNA detected in 27% (3/11), 2/3 experienced recurrence.	N/A	[111]
Beinse et al., 2022	ZSCAN12, OXT	Methylation-specific ddPCR	Retrospective: 108 tumor tissues; Prospective: 33 / 55	ZSCAN12 and OXT methylation in plasma offered high specificity and sensitivity for EC prediction.	AUC: 0.99; Sensitivity: 98%; Specificity: 97%	[86]
Kodada et al., 2023	DNMT3A, TET2, and others	NGS	21 EC	A poorer prognosis may be correlated with mutations related to ARCH (DNMT3A and TET2).	N/A	[85]
Ashley et al., 2023	129 genes with molecular barcoding	NGS	44 EC	Presence of ctDNA at baseline or post-surgery was significantly associated with reduced PFS. Correlation with disease stage, progression, and treatment response.	N/A	[112]
Recio et al., 2024	16 somatic single nucleotide variants (SNVs)	mPCR-NGS	101 stage I uterine malignancies (88% EC)	Post-surgical ctDNA detection is prognostic of poor RFSin patients with stage I EC.	N/A	[104]

 doi:10.20944/preprints202506.1856.v1

Blanc-Durand et al., 2024	TP53, DNMT3A, PIK3CA, PTEN, ERBB2, CTNNB1, PPP2R1A	NGS	61 EC	cfDNA sequencing in advanced EC provided 90% informative results and 87.5% accuracy in molecular subclassification.	N/A	[121]
Pamela et al., 2024	TP53, PIK3CA, PTEN, ARID1A, KRAS, CCNE1, ERBB2, FBXW7	Hybrid capture NGS for SNVs, indels, CNVs, fusions, MSI, bTMB	1,988 advanced / recurrent EC	TP53 mutations associated with worse OS.	N/A	[114]
Casas-Arozamena et al., 2024	PTEN, PIK3CA, TP53, ARID1A, KRAS, CTNNB1, PIK3R1, FBXW7, PPP2R1A, FGFR2	ddPCR, Targeted sequencing, Qubit fluorometry	198 EC	High pre-surgery cfDNA and detectable ctDNA correlate with poor DFS and DSS.	N/A	[115]

Author and Year	Biomarkers	Detection Method	No. of participants (EC/control)	Clinical Significance/Findings	Accuracy	Ref.
Jamieson et al., 2025	TP53, PIK3CA, PTEN, KRAS, CTNNB1, AKT1, BRAF, ERBB2	NGS	24 EC, 17 OC, 2 synchronous endometrial / ovarian carcinomas (SEOC), 1 endocervical adenocarcinoma	Preoperative ctDNA detection was associated with advanced stage, elevated CA125, and recurrence.	N/A	[113]
cfRNA or ctRNA						
Shan et al., 2020	lncRNA DLEU1	RT-qPCR	128 / 50 endometrial hyperplasia / 50 controls	Higher lncRNA DLEU1 levels were associated with advanced clinicopathological features and worse overall and DFS in EC patients.	AUC (95% CI): [EC vs. controls: 0.883 (0.826-0.926), EC vs. hyperplasia: 0.766 (0.697-0.826)]; Sensitivity: [EC vs. controls: 77.3%, EC vs. hyperplasia: 60.9%]; Specificity: [EC vs. controls: 92.0%, EC vs. hyperplasia: 90.0%]	[117]

 doi:10.20944/preprints202506.1856.v1

Fan et al., 2021	miR-20b-5p, miR- 143-3p, miR-195-5p, miR-204-5p, miR-423-3p, miR- 484	qRT-PCR	92 / 102	The 6-miRNA signature demonstrated very consistent diagnostic performance in three datasets across cohorts.	AUC: [Training: 0.748, Testing: 0.833, External Validation: 0.967]; Sensitivity: [Training: 78.4%, Testing: 77.1%, External Validation: 83.3%]; Specificity: [Training: 63.0%, Testing: 66.7%, External Validation100%]	[87]
Wu et al., 2022	miR-204-5p	RT-qPCR	52 / 60	Metastasis of lymph nodes was associated with down-regulation of serum miR-204-5p.	AUC (95% CI): 0.923 (0.847-1.000); Sensitivity: 87.2%; Specificity: 80%	[116]
Salim et al., 2022	miRNA133a-2, miRNA-21, miRNA-205	qRT-PCR	36 /15	These miRNAs could serve as potential prognostic biomarkers for endometrial carcinoma.	N/A	[118]
Kumari et al., 2023	miR-16, miR-99b, miR-20a, miR-145, miR-143, miR- 125a	qRT-PCR	10 /10	miR-16, miR-99b, miR-125a, and miR-145 could serve as diagnostic indicators for endometrioid EC.	AUC: 0.957 (miR-145); Sensitivity: 90% (miR-145);Specificity: 100% (miR-145)	[161]
Rostami et al., 2024	miR-155-5p, miR- 200b-3p, miR-589-5p, and others	Small RNA Sequencing	316 / 316	These RNAs hold potential as early biomarkers for EC, which could facilitate timely interventions. Relationships between EC and miRNAs were modified by body mass index, physical activity, and smoking status.	N/A	[88]

EVs

Author and Year	Biomarkers	Detection Method	No. of participants (EC/control)	Clinical Significance/Findings	Accuracy	Ref.
Song et al., 2020	LGALS3BP	TMT Labelling, ELISA	87 EC / 12 AEH / 42 controls	Plasma exosomal LGALS3BP levels correlated with EC progression and poor prognosis.	AUC (95% CI): 0.7406 (0.6506–0.8305)	[120]
Zhou et al., 2021	miR-15a-5p, miR- 106b-5p, miR-107	ddPCR	115 / 87	Exosomal miR-15a-5p was highly predictive of the aggressiveness and p53 mutation status of EC	AUC: 0.813 (miR-15a-5p); 0.899 miR-15a-5p	[162]

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				tumours and markedly elevated in early-stage EC.	combined serum tumor markers (CEA and CA125)	
Sommella et al., 2022	APOA1, HBB, CA1, HBD, LPA, SAA4, PF4V1, APOE	LFQ-MS	36 / 36	Identified eight proteins significantly upregulated in serum exosomes, indicating potential as early- stage EC biomarkers.	AUC (95% CI): 0.98 (0.95-1) (Stage 1 EC); Sensitivity: 100% (Stage 1 EC); Specificity: 86.11% (Stage 1 EC)	[163]
Proteomics						
Tarney CM et al., 2019	CFB, TF, CAT, PSMB6, B2M, PCDH18	HPLC-MS/MS	112 / 112	Six proteins could distinguish EC cases from the control group, with strongest performance ≤ 2 years pre-diagnosis.	AUC (95% CI): 0.800.72–0.88; Sensitivity: 45.2% (cutoff: 0.5); Specificity: 96.4%(cutoff: 0.5)	[90]
Ura et al., 2021	CLU, SERPINC1, ITIH4, C1RL, APOC3, DSG1	2D-DIGE, WB, LC-MS/MS	15 / 15	Study identified 16 proteins with diagnostic potential for EC. Validation showed upregulation of CLU, ITIH4, SERPINC1, C1RL in EC serum and exosomes.	AUC: 0.9289; Sensitivity: 100%; Specificity: 86.67%	[89]
Ura et al., 2022	Gal-1, Gal-9, MMP7, FASLG, COL9A1	Proximity extension assay (PEA)	44 / 44	Combined proteins from the Immuno-oncology panel and the Target 96 Oncology III panel showed differential expression in early-stage Type I EC with high diagnostic accuracy	AUC (95% CI): 0.969 (0.939–0.999); Sensitivity: 97.67%; Specificity: 83.72%	[91]
Celsi et al., 2022	Suprabasin (SBSN) (isoforms 1 & 2)	2D-DIGE and MS, validated by WB	Proteomic: 10 /10, Validation: 30/30 (serum), 30/30 (tissue)	In serum or tissue, SBSN, particularly isoform 2, may be a novel biomarker for EC.	AUC: [Isoform 2 (serum): 0.75, (tissue): 0.79]	[99]
Mujammami et al., 2024	FABP-1, α-2 macroglobulin, ZAG, Ero1-α, haptoglobin, and others	2D-DIGE, MALDI-TOF- MS	8 diabetic EC / 8 non-diabetic EC	Downregulation of FABP-1 and haptoglobin, and upregulation of ERO1-α, α-2-macroglobulin, and ZAG in EC with diabetes indicated severe disease and poor prognosis.	N/A	[119]
Metabolomics						
Strand et al., 2019	183 metabolites	LC-MS	40 EC	Metabolite patterns were associated with survival. Methionine sulfoxide elevation was linked to poor prognosis.	AUC: [Model3: 0.965 (0.913–1)]	[106]

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Troisi et al., 2020	268 serum metabolites	GC-MS	Training: 120 (50 / 70), Validation: 1430	The EC screening of postmenopausal women using an ensemble EML algorithm achieved an accuracy rate of > 99%.	Sensitivity: 100%; Specificity: 99.86%	[100]

Author and Year	Biomarkers	Detection Method	No. of participants (EC/control)	Clinical Significance/Findings	Accuracy	Ref.
Forsse et al., 2020	17-OHP, 11-DOC, A4, E1, E2	LC-MS/MS	100 EC	Low levels of 17-OHP, 11-DOC, and A4 were associated with aggressive EC phenotypes and poor disease-specific survival.	N/A	[107]
Kozar et al., 2021	Ceramides, acylcarnitines, 1-methyladenosine	HPLC-TQ/MS	15 / 21	Combined panel identified as superior to individual biomarkers for early disease detection.	AUC (95% CI): 0.925 (0.905–0.945); Sensitivity: 94%; Specificity: 75%	[92]
Njoku et al., 2021	Phospholipids, sphingolipids	MS	67 / 69	Lipid metabolites effectively discriminated EC EC in women with BMI ≥ 30 kg/m2.	AUC: 0.95	[93]
Dossus et al., 2021	Amino acids, sphingolipids, carnitine	LC-MS/MS	853 / 853	Identified metabolites were associated with EC risk	N/A	[164]
Trabert et al., 2021	Pregnenolone, progesterone, 17-hydroxypregnenolone, and others	LC-MS/MS	EC: 65 / 345; OC: 67 / 413	17-hydroxypregnenolone was inversely associated with EC risk and positively associated with ovarian cancer risk.	N/A	[165]
Yan et al., 2022	6-keto-PGF1α, PA (37:4), LysoPC (20:1), PS (36:0)	UPLC-Q-TOF/MS	326 / 225	Specific biomarkers for endometrial polyps were identified to distinguish them from EC or hyperplasia.	AUC: [EP vs. EC: 0.915; EP vs. EH: 1.000]; Sensitivity: [EP vs. EC: 100%; EP vs. EH: 100%]; Specificity: [EP vs. EC: 72.41%; EP vs. EH: 100%]	[96]
Roškar et al., 2022	Leptin, IL-8, sTie-2, Follistatin,	Luminex xMAP™	91 / 111	Leptin was significantly higher in EC patients, especially in Type 1 EC.		[97]

 doi:10.20944/preprints202506.1856.v1

	Neuropilin-1, G-CSF	Multiplexing Technology		IL-8 levels were elevated in Type 2 EC, poorly differentiated G3 tumors and those with vascular invasion.	AUC: [Training: 0.94 Test: 0.81]	
Breeur et al., 2022	117 metabolites	LC-MS/MS, FIA-MS/MS	1706 EC	An inverse association between EC risk and a glycine/serine metabolite cluster was found.	N/A	[166]
Cheng et al., 2023	Ursodeoxycholic acid, PC (O-14:0_20:4), Cer (d18:1/18:0)	UHPLC-MS/MS	Discovery: 18 / 20, Validation: 20 EC / 20 atypical endometrial hyperplasia	Lipid biomarkers differentiated early-stage EC from healthy controls and AEH patients.	AUC: [Discovery: 0.903 Validation: 0.928]; Sensitivity: [Discovery: 83.3% Validation: 85%]; Specificit: [Discovery: 85% Validation: 85%]	[94]
Dahmani et al., 2023	11-oxygenated androgens (11KAST, 11OHAST, etc.)	LC-MS/MS	272 EC	Higher preoperative free 11KAST and postoperative 11OHAST levels were associated with increased risk of recurrence and poor DFS.	N/A	[108]
Hishinuma et al., 2023	LysoPC, TGs, amino acids	UHPLC-MS/MS	142 / 154	Histidine and tryptophan levels decreased with disease progression and recurrence risk.	AUC: [Top 5 metabolites: 0.997 (0.986-1)]	[109]

Author and Year	**Biomarkers**	**Detection Method**	**No. of participants (EC/control)**	**Clinical Significance/Findings**	**Accuracy**	**Ref.**
Benabdelkamel et al., 2024	338 metabolites	LC-HRMS	20 EC, 20hyperplasia, 19 controls	Plasma metabolic signatures distinguished EC and hyperplasia from healthy controls.	AUC: [15 metabolic variation: 0.821]	[95]
Multi-omics						
Hao et al., 2023	Metabolites and lncRNAs	LC-MS/MS, LncRNA sequencing	Endometrial dysplasia: 4, Stage I EC: 4, Stage III EC: 4, controls: 4	Metabolites and lncRNAs correlated with EC progression.	AUC: 2,3-Pyridinedicarboxylic acid: 0.69, hematommic acid,	[167]

 doi:10.20944/preprints202506.1856.v1

					ethyl ester: 0.69, maltitol: 0.69, 13 (S)-HODE: 0.88, D-mannitol:0.69	
Shen et al., 2024	Various metabolites and proteins	GWAS and Mendelian Randomization	121,885 participants (12,906 EC)	Key metabolites and proteins influenced EC subtypes.	N/A	[168]
Ding et al., 2024	CTCs, lncRNAs, and DNA methylation markers	Microfluidic CTC isolation, RT-qPCR, MSP/qMSP	71 / 14	Combined biomarkers improved diagnostic accuracy for EC compared to individual biomarkers alone.	AUC (95% CI): 0.94 (0.89–0.98); Sensitivity (95% CI): 89% (82–94%); Specificity (95% CI): 92% (85–96%)	[169]
Liu et al., 2024	CNV, FSD, NF	WGS	Training: 133 (66/67) Validation: 89 (44/45)	ML model was developed and maintained high performance in independent validation with stage I EC.	AUC: [Training: 0.991; Validation: 0.994]; Sensitivity: [Training: 98.5%; Validation: 97.8%]; Specificity: [Training: 95.5%; Validation: 95.5%]	[170]

4.1.1. Early Diagnosis

The utility of cfDNA and cfRNA in the diagnosis of EC has been demonstrated [82]. Compared to cfRNA, cfDNA is generally preferred by researchers due to its greater stability and the more advanced state of detection technologies. Researchers have investigated cfDNA from various perspectives. For instance, in terms of concentration, Gressel et al. [83] found that the median concentration of low-molecular-weight (LMW) cfDNA was significantly higher in EC patients compared to healthy controls. In contrast, Benati et al. [84] examined relative telomere length (RTL) in cfDNA and found EC patients had markedly shorter RTL than healthy individuals with promising early diagnostic accuracy (AUC = 0.87). More commonly, studies focus on gene mutations [14, 85] or DNA methylation [86], utilizing either PCR- based or sequencing technologies. For example, Beinse et al. [86] identified hypermethylation of the ZSCAN12 and OXT genes in the ctDNA of EC patients. Using ddPCR, they achieved high diagnostic sensitivity and specificity (both 97%), successfully detecting ctDNA in 14 of 31 plasma samples collected before surgery or chemotherapy, including cases from both early and advanced stages. These findings highlight the potential of ctDNA methylation analysis as a non-invasive and personalized tool for monitoring and managing EC.

Similarly, cfRNA is being explored as a potential diagnostic biomarker. Fan et al. [87] identified six miRNAs that were overexpressed in the serum of EC patients. The diagnostic performance of this six-miRNA signature yielded AUCs of 0.748, 0.833, and 0.967 in training, testing, and external validation cohorts, respectively. Moreover, the expression levels of miR-143-3p and miR-195-5p in tissues, as well as miR-20b-5p in serum exosomes, were consistent with their serum levels, further supporting their diagnostic relevance. In addition to verifying the potential of miRNAs as early biomarkers, Rostami et al. found that the association between EC and miRNA expression is modulated by factors such as body mass index, physical activity, and adherence to a Western diet [88].

An increasing number of studies have demonstrated that the plasma protein profiles or metabolomic features can aid in the early diagnosis of EC [89-97]. However, the specificity of these diagnostic approaches remains limited, underscoring the need to combine multiple biomarkers-such as cfDNA, cfRNA, proteins, and metabolites-to enhance accuracy [95, 98]. With the advancement of

 doi:10.20944/preprints202506.1856.v1

artificial intelligence (AI), machine learning (ML) has been increasingly applied for biomarker screening and model development to enhance diagnostic performance diagnostic performance [99-101]. For instance, Troisi et al. applied an ensemble machine learning (EML) algorithm to screen and detect EC in postmenopausal women, achieving an accuracy rate of 99% [100]. Despite these advances, interpreting the results from such "black box" models remains a key challenge for researchers moving forward. In terms of sample collection site, Francini et al. [102] offered a novel perspective. In their preliminary study on CTC detection during early-stage EC surgery, 80% of patients had detectable CTCs in the ovarian vein, whereas none were found in peripheral blood samples. This suggests that ovarian vein sampling may offer greater sensitivity for CTC detection. In contrast, Kodada et al. [85] identified DNMT3A and TET2 mutations in ctDNA from peripheral plasma that were absent in tumor tissue, indicating challenges in distinguishing tumor-specific mutations from age-related clonal hematopoiesis (ARCH). Their findings suggest that background noise in EC diagnostics might be reduced by analyzing ctDNA from non-blood specimens such as uterine lavage fluid.

4.1.2. Recurrence Monitoring

Surgical removal of the tumor remains the primary approach in EC treatment, often followed by personalized adjuvant therapies based on postoperative assessment. Recurrence monitoring typically relies on radiographic imaging and serum tumor markers. However, these conventional methods often lack the sensitivity to detect minimal residual disease (MRD) or micrometastases after surgery. As a result, there is a critical need for more sensitive and specific biomarkers to enable early detection of recurrence and metastasis, which could significantly improve patient outcomes.

Emerging evidence suggests that ctDNA is a more accurate biomarker for monitoring EC recurrence. Feng et al. [103] used ddPCR to track common tumor-specific mutations, including PTEN, FAT4, ARID1A, and TP53, in the plasma of EC patients, achieving 100% sensitivity and 83.3% specificity. Their findings highlight ctDNA's superior predictive value over traditional markers like CA125 and HE4. Recio et al. [104] further confirmed this through longitudinal ctDNA monitoring post-surgery. They demonstrated that patients with positive ctDNA at both the initial time point and longitudinally had significantly worse recurrence-free survival (RFS) (HR = 6.2; $p = 0.0006$ and HR = 15.5; $p < 0.0001$, respectively), with recurrence rates of 58% and 52%, compared to 6% and 0% in ctDNA-negative individuals. This suggests that postoperative ctDNA detection is a strong predictor of outcomes and a key risk factor for recurrence. Similar conclusions were drawn by Grassi et al. [103]. Likewise, Law et al. [105] used microfluidic technology to investigate CTC-related markers in gynecologic malignancies. Although the study encompassed various cancer types, the findings in EC were particularly notable. Markers such as PanCK, GATA3, HER2, and HE4 were consistently detected in preoperative samples. During follow-up, the reappearance of these markers was strongly associated with disease recurrence in EC patients, often preceding clinical symptoms. This suggests these molecular markers could serve as early indicators of relapse, offering a critical window for timely intervention.

4.1.3. Prognostic Prediction

Prognostic biomarkers help identify patients with aggressive tumors and offer valuable insights into long-term outcomes, independent of treatment strategies. Their main purpose is to predict prognosis and guide treatment intensity to improve survival in EC patients. With advancements in MS technology, many researchers have applied non-targeted metabolomics to identify prognostic metabolites in EC [106-109]. However, the predictive value of these metabolites is evident only when assessed in combination, as no highly specific individual markers have been identified. With growing insights into genomics, attention has increasingly shifted toward cfDNA and cfRNA. Studies show that cfDNA is associated with tumor size, disease stage and classification, invasive characteristics, cancer progression, lymphovascular invasion [82, 85, 110-115], and overall survival (OS), supporting its potential as a prognostic marker. Similarly, cfRNA holds promise. [116-118]. For example, Wu et al. [116] found that reduced serum miR-204-5p levels correlate with lymph node metastases, while

 doi:10.20944/preprints202506.1856.v1

Shan et al. [117] proposed serum lncRNA DLEU1 as a prognostic biomarker linked to adverse clinical features and poor survival outcomes in EC.

In addition to free protein biomarkers in the blood [99, 119], exosomal proteins are also being investigatied. Song et al. [120] examined exosomal LGALS3BP as a potential biomarker for EC and found it significantly elevated in plasma exosomes from EC patients. Higher LGALS3BP levels were associated with increased cell proliferation, migration, angiogenesis, and poor prognosis. These findings highlight the potential of non-invasive markers from various sources, but further validation is needed to confirm their prognostic value and clinical utility in guiding treatment for EC.

4.1.4. Treatment Guidance

Modern treatment options such as molecular targeted therapy and immunotherapy have improved survival in patients with advanced or metastatic EC. However, systemic anti-cancer treatments face challenges like primary resistance, lack of initial response, and acquired resistance. Additionally, tumor molecular profiles often change during therapy, necessitating continuous monitoring to evaluate treatment response and predict resistance. Blanc-Durand et al. [121] demonstrated that cfDNA profiling in advanced EC provided 89% molecular information and 87.5% concordance with tissue biopsies. This method guided targeted therapy in 16% of patients, yielding a median PFS of 7.7 months and a 56% response rate. These findings highlight the potential of cfDNA analysis to enhance personalized treatment strategies for advanced EC.

In CTCs from high-risk EC patients, Herrero et al. [122] identified overexpression of Annexin A2 (ANXA2), which was associated with reduced OS and PFS. High-throughput screening identified daunorubicin as a potential therapeutic agent that inhibits ANXA2-driven metastasis by reducing the invasiveness of ANXA2-overexpressing cells. For non-endometrioid EC subtypes, Shen et al. used multi-omics analysis to identify proteins such as IL32 and GRB7, which are involved in key oncogenic pathways like MAPK signaling and cytokine-cytokine receptor interactions. These findings not only deepen our understanding of EC pathogenesis but also provide potential targets for molecularly tailored therapies.

4.2. Non-Blood-Based Liquid Biopsy in EC

Non-blood-based liquid biopsies offer a promising alternative to traditional blood sampling in EC. Blood-based biomarker detection can be challenging, particularly in early-stage tumors, due to the low abundance of circulating signals [123]. Alternatively, the close anatomical connection between the uterine cavity, lower reproductive tract, and urinary system presents new opportunities for biomarker discovery in EC [124]. Examples of these findings are detailed in Tables 2 and 3.

Table 2. Application of liquid biopsy in urine samples.

Author and Year	Category of Liquid Biopsy	Biomarkers	Detection Method	No. of participants (EC/control)	Clinical Significance/Findings	Accuracy	Ref.
Kacírová et al., 2019	Proteomics	CDH1, VTN, HSPG2	Nano HPLC-ESI-MS/MS	5 / 7	Down-regulation of key proteins suggested potential urinary biomarkers for early detection of EC.	N/A	[171]
Ritter et al., 2020	miRNA	miR-3973; -4426; -5089-5p and -6841	RT-qPCR	10 / 30	These biomarkers served as promising candidates for urine-based liquid biopsies in detecting EC.	N/A	[127]

 doi:10.20944/preprints202506.1856.v1

Costas et al., 2023	cfDNA	47-gene panel (POLE, TP53)	NGS	19 / 20	Evaluating urine for somatic mutations offered a non-invasive, accurate approach for detecting EC and molecular classification.	AUC: 0.99; Sensitivity (95% CI): 100.0% (82.4%-100.0%); Specificity (95% CI): 95.0% (75.1%-99.9%)	[126]
Njoku et al., 2023	Proteomics	SPRR1B, CRNN, CALML3, TXN, FABP5, C1RL, MMP9, ECM1, S100A7, CFI	SWATH-MS with ML	50 / 54	Discriminated EC patients from symptomatic controls suggested its potential as a non-invasive diagnostic tool.	AUC (95% CI): 0.92 (0.86–0.97); Sensitivity: 83.7%;Specificity: 83.9%	[128]
Chen et al., 2023	Metabolomics	Baicalin, 5beta-1,3,7(11)-Eudesmatrien-8-one, Indolylacryloylglycine, Edulitine, Physapubenolide	UPLC-MS	42 EC (22 PT / 20 CR)	The predictive biomarkers presented great potential diagnostic value in fertility-sparing treatments for EC patients.	AUC: [Training: 0.982, Validation: 0.851]; Sensitivity: [Training: 97.5%, Validation: 86.4%]; Specificity: [Training: 96.7%, Validation: 90.0%]	[129]
Chen et al., 2024	Metabolomics	ADP-mannose, docosatrienoic acid, hippuric acid	UPLC-MS	146 / 59	Combined urine-serum metabolomics effectively distinguished EC from controls, high-risk from low-risk EC, and type I vs II EC.	AUC: [Training: 0.953; Validation: 0.972]; Sensitivity: [Training: 0.857; Validation: 0.846]; Specificity: [Training: 0.876; Validation: 0.974]	[130]
Fu et al., 2024	Metabolomics and Transcriptomics	10 metabolites (histamine, 1-methylhistamine, methylimidazole acetaldehyde, etc.)	LC-MS	110 / 110	The combination of these biomarkers demonstrated enhanced diagnostic accuracy	AUC: Combined:0.90; Sensitivity: Combined:	[131]

 doi:10.20944/preprints202506.1856.v1

		and 3 hub genes (RRM2, TYMS, TK1)			compared to individual markers.	>0.85; Specificity: Combined: >0.85	

Table 3. Application of liquid biopsy in other samples.

Author and Year	Category of Liquid Biopsy	Biomarkers	Detection Method	No. of participants (EC/control)	Clinical Significance/Findings	Accuracy	Ref.
Uterine lavage fluid/ Uterine aspirates							
Casas-Arozamena et al., 2020	cfDNA, CTCs	PTEN, PIK3CA, TP53, CTNNB1, KRAS, etc.	NGS, ddPCR, CellSearch system	60 EC	Genetic alterations were detected in 93% of EC through UAs. ctDNA was associated with high-risk tumors and disease progression.	N/A	[134]
Casas-Arozamena et al., 2023	cfDNA	BAT26, BAT25, NR24, NR21, Mono27	ddPCR	90 EC	A high concordance (96.67%) between MSI determinations in cfDNA and the standard of care was confirmed.	N/A	[135]
Yang et al., 2023	cfRNA	miR-146a-5p, miR-183-5p, miR-429	Real-time PCR	42 / 40	miR-146a-5p, miR-183-5p, miR-429 were significantly upregulated in EC.	AUC: miR-183-5p: 0.675, miR-429: 0.709, miR-146a-5p: 0.685	[136]
Cervicovaginal fluid / Cervicovaginal lavage							
Cheng et al., 2019	Metabonomi cs	Phosphocholine, Malate, Asparagine	NMR Spectroscopy	21 / 33	Metabolomic biomarkers in CVF for non-invasive detection of EC were identified and validated using ML algorithms.	AUC: [Training: 0.88-0.92; Test: 0.75-0.80]; Sensitivity (95% CI): Forests: 0.75 (0.19–0.99); Specificity (95% CI): Forests: 0.80 (0.28–1.00)	[148]
O'Flynn et al., 2021	Cytology	Malignant endometrial cells	Cytological analysis	103 / 113	Vaginal cytology demonstrated higher sensitivity (90.2%) compared to urine cytology (72.0%) but lower specificity.	Sensitivity: [Vaginal: 90.2%, urine:72.0%, combined: 91.7%]; Specificity: [Vaginal: 88.7%,	[138]

 doi:10.20944/preprints202506.1856.v1

						urine: 94.9%, combined:88.8 %]	
Łaniewski et al., 2022	Proteomics	72 proteins (TIM-3, VEGF, TGF-α, IL-10, CA19–9, CA125, etc.)	Multiplex Immunoas says	66 / 126	Identified lavage proteins could discriminate EC from benign conditions.	AUC (95% CI): Combined: 0.91 (0.78-0.97) Sensitivity: 86.1% (combined); Specificity: 87.9% (combined)	[144]
Yi et al., 2022	Metabolomi cs & Proteomics	Amino acid and nucleotide metabolism biomarkers	LC-MS/MS	44 / 43	Urine/intrauterine brushing metabolites correlate with tissue pathways (amino acid/nucleotide metabolism).	AUC: 0.808 (urine) 0.847 (intrauterine brushing); Sensitivity: Urine: 74.7% (top 5 metabolites)	[150]
Pelegrina et al., 2023	Somatic mutations	47 genes panel (POLE, TP53, PTEN, etc.)	NGS	139 / 107	POLE mutations indicated excellent prognosis, TP53 mutations were associated with significant DFS differences among molecular subtypes.	AUC: 0.83 (self-collected); Sensitivity: 73% (clinician and self-collected); Specificity: [80% (clinician-collected), 90% (self-collected)]	[139]
Evans et al., 2023	DNA methylation	ZSCAN12, GYPC	WID-qEC	12 / 375	WID-qEC test demonstrated superior diagnostic accuracy compared to transvaginal ultrasound in detecting uterine cancers.	AUC (95% CI): 0.943 (0.847–1.000); Sensitivity (95% CI):90.9% (62.3–98.4); Specificity (95% CI): 92.1% (88.9–94.4)	[140]

Author and Year	Category of Liquid Biopsy	Biomarkers	Detection Method	No. of participants (EC/control)	Clinical Significance/Findings	Accuracy	Ref.
Martinez-Garcia et al., 2023	Proteomics	SERPINH1, VIM, TAGLN, PPIA, CSE1L, CTNNB1	MS	22 / 19	6 protein biomarkers in cervical fluids were identified to distinguish women with abnormal uterine bleeding who are EC and those who are non-EC.	AUC: [UF: > 0.71, LDHA, ENO1, PKM: > 0.9; M1: up to 0.83 (SERPINH1); M3: up to 0.84	[145]

 doi:10.20944/preprints202506.1856.v1

						(TAGLN)]; Sensitivity: [M1: up to 83%; M3: up to 89%]; Specificity: [M1: up to 81%; M3: up to 78%]	
Illah et al., 2024	DNA methylation	ZSCAN12, GYPC	WID-qEC	28 / 74	The WID-qEC test reliably detected uterine cancers (endometrial and cervical) across sampling devices and collection methods (gyn. vs. patient self-sampling).	AUC (95% CI): 0.96 (0.91–1.00); Sensitivity: 92.9% (gyn)、75.0% (self); Specificity: 98.6% (gyn)、100.0% (self)	[141]
Zhao et al., 2024	DNA methylation	CDO1m, CELF4m	qMSP	21 / 275	Dual-gene methylation showed high sensitivity (85.7%) and specificity (87.6%) for EC screening.	AUC (95% CI): 0.867 (0.788–0.946) for dual methylation; Sensitivity (95% CI): 85.7% (0.707–1.000); Specificity: 87.6% (0.837–0.915)	[142]
Cai et al., 2024	DNA methylation	CDO1, CELF4	qPCR	40 / 98	Combined test specificity (95.9%) outperformed transvaginal ultrasound (ET) and CA125 and detected all Type II EC cases.	AUC (95% CI): 0.917 (0.853–0.91) for combined test; Sensitivity (95% CI): 87.5% (73.2–95.8); Specificity: 95.9% (89.9–98.9)	[143]
Njoku et al., 2024	Proteomics	HPT, LG3BP, FGA, LY6D, IGHM	SWATH-MS	53 / 65	Cervico-vaginal fluid protein signatures showed superior accuracy over plasma in detecting stage I EC and advanced tumors, effectively	AUC (95% CI): [Cervico-vaginal: 0.95 (0.91–0.98), Plasma: 0.87 (0.81–0.93)]; Sensitivity: [Cervico-vaginal: 91%	[146]

 doi:10.20944/preprints202506.1856.v1

						(83%–98%), Plasma: 75% (64%–86%)]; Specificity: [Cervico-vaginal: 86% (78%–95%), Plasma: 84% (75%–93%)]	
Harris et al., 2024	Proteomics	Angiopoietin-2, Endoglin, FAP, MIA, VEGF-A	Multiplex immunoassays	66 EC / 108 benign	5 key biomarkers significantly elevated in EC. Multivariate model showed prognostic value for tumor grade, size, invasion, and MMR status.	AUC: 0.918; Sensitivity: 87.8%; Specificity: 90.7%	[147]
Lorentzen et al., 2024	Metabolomics	Lipids, amino acids, and other metabolites	UPLC-MS	66 / 108	Metabolic dysregulation linked to tumor characteristics (size, myometrial invasion); improved noninvasive detection and risk stratification; multivariate models achieved high diagnostic accuracy.	AUC: 0.800-0.951 (25-feature model); Sensitivity: 78.6% (for EC); Specificity: 83.3% for EC, 79.6% for benign	[149]
Tampons							
Bakkum-Gamez et al., 2023	DNA methylation	28 Methylated DNA markers	qMSP	100 / 92	The sensitivity to detect EC was high even when vaginal fluid samples were collected before endometrial sampling.	AUC (95% CI): 0.91 (0.85–0.97); Sensitivity (95% CI):82% (70%–91%); Specificity (95% CI): 96% (87%–99%)	[172]
Cervical scrapings and Vaginal swabs							
Kim et al., 2022	Genomic DNA	100 EC-related genes	NGS	39 / 11	Cervical swab-based gDNA genomic data demonstrated enhanced detection ability and enabled patient classification.	Sensitivity: 67%; Specificity: 100%	[157]

Author and Year	Category of Liquid Biopsy	Biomarkers	Detection Method	No. of participants (EC/control)	Clinical Significance/Findings	Accuracy	Ref.

 doi:10.20944/preprints202506.1856.v1

Wen et al.,2022	DNA methylation	BHLHE22, CDO1	MPap	494 EC	MPap test showed high sensitivity and specificity for EC detection.	AUC (95% CI): [Stage 1: 0.91 (0.87–0.94), Stage 2: 0.90 (0.84–0.95)]; Sensitivity (95% CI): [Stage 1: 92.9% (80.5–98.5%), Stage 2: 92.5% (82.9–100.0%)]; Specificity (95% CI): [Stage 1: 71.5% (64.8–77.5%), Stage 2: 73.8% (67.6–79.4%)]	[153]
Herzog et al., 2022	DNA methylation	GYPC, ZSCAN12	qPCR	562 (various groups)	The WID-qEC test offered a non-invasive EC screening and triage with high sensitivity and specificity.	AUC: 0.94 (Barcelona); Sensitivity: [97.2% (FORECEE), 90.1% (Barcelona), 100% (PMB Cohort)]; Specificity: [75.8% (FORECEE), 86.7% (Barcelona), 89.1% (PMB Cohort)]	[154]
Wever et al., 2023	DNA methylation	ADCYAP1, BHLHE22, CDH13, CDO1, GALR1, GHSR, HAND2, SST, ZIC1	qMSP	103 / 317	DNA methylation marker analysis in urine, cervicovaginal self-samples, and clinician-taken cervical scrapes achieved high diagnostic accuracy for EC detection.	AUC: [Urine: 0.95, Self-samples: 0.94, Scrapes: 0.97]; Sensitivity:[Urine: 90%, Self-samples: 89%, Scrapes: 93%]; Specificity: [Urine: 90%, Self-samples: 92%, Scrapes: 90%]	[155]
Wang et al., 2024	DNA methylation	RASSF1A, HIST1H4F	qPCR	19 / 75	Methylation levels of RASSF1A/HIST1H4F increased with endometrial lesion severity.	AUC: RASSF1A: 0.938; HIST1H4F: 0.951	[156]
Other samples							
Mayo-de- las-	cfDNA	KRAS, PIK3CA	NGS, qPCR	50 / 7	KRAS/PIK3CA mutations were detected in 47.4% of	N/A	[158]

 doi:10.20944/preprints202506.1856.v1

Casas et al., 2020					peritoneal lavages and correlated with tumor tissue.		
Ayyagari et al., 2023	Metabolomics & Proteomics	SOAT1, CE	ELISA, colorimetric assay, RT-qPCR, IHC	32 / 16	SOAT1 and CE may be associated with malignancy, aggressiveness, and poor prognosis.	AUC: Peritoneal fluid SOAT1: 0.767; Sensitivity: 80%; Specificity: 67%	[159]

4.2.1. Urine Samples

Urine contains diverse components, including malignant cells, tumor-derived nucleic acids, peptides/proteins, endogenous metabolites, and secretory organelles [125]. Costas et al. [126] evaluated the utility of somatic mutation analysis in urine for non-invasive EC detection and molecular classification. Using NGS, they achieved a 100% mutation detection rate in EC cases, showing high concordance between urine and tumor samples, particularly when applying the Proactive Molecular Risk Classifier for EC (ProMisE) algorithm. These results suggest that urine-derived cfDNA, such as transrenal ctDNA (TR-ctDNA), may serve as a reliable biomarker for early EC diagnosis and prognosis. Similarly, Ritter et al. [127] identified miRNAs, such as miR-10b-5p and miR-205-5p in urine, with miR-10b-5p demonstrating diagnostic potential in EC patients. While additional validation is required, these studies highlight the promise of urine-based miRNA profiling for non-invasive screening.

Beyond nucleic acid biomarkers, urine proteins and metabolites have also been investigated. Unlike Ritter et al. [127], who relied on case-control studies to detect protein concentration differences, Njoku et al. [128] applied machine learning to develop a diagnostic model using 10 urinary markers, achieving an accuracy of 0.92. Similarly, instead of analyzing urine metabolites alone, Chen et al. [129], [130] combined serum and urine data, yielding an AUC of 0.922, demonstrating a valuable model-building approach for EC. Furthermore, Fu et al. [131] integrated metabolomics with transcriptomics, identifying differential metabolites and hub genes in urine associated with EC. This multi-omics strategy suggests that combining urine-based biomarkers with transcriptomic profiles could improve early EC detection.

As a distinct and promising sample source for liquid biopsy, urine offers a non-invasive, easily accessible, and disease-specific tool for EC diagnosis and management—potentially overcoming several limitations of blood-based sampling.

 doi:10.20944/preprints202506.1856.v1

4.2.2. Uterine Lavage Fluid and Uterine Aspirates

Uterine lavage fluid or uterine aspirates (UAs) represent a promising source for liquid biopsy due to their direct contact with tumors. Since Maritschnegg et al. [132] first detected shed EC cells in uterine lavage fluid, subsequent studies have further explored the diagnostic potential of these samples [133]. Casas-Arozamena et al. [134] provided the first comprehensive characterization of UAs, ctDNA, and CTCs. Their NGS analysis revealed genetic mutations in 93% of tumor samples, predominantly in genes such as PTEN, PIK3CA, and TP53. Notably, CTCs and ctDNA were found in 38.9% and 41.2% of cases, respectively, particularly among patients with high-risk tumor, suggesting their value as biomarkers for aggressive disease. Furthermore, they also demonstrated strong concordance between MSI results from UAs and cfDNA samples and those from traditional tissue, highlighting UAs as a viable tool for personalized monitoring and management [135].

Further supporting the utility of endometrial fluid analysis, Yang et al. [136] used real-time PCR to analyze specific miRNAs —miR-429, miR-146a-5p, and miR-183-5p—in endometrial fluid, underscoring their diagnostic potential for EC. This miRNA profiling offers a less invasive alternative to traditional diagnostic procedures, potentially improving early detection and intervention. However, uterine lavage collection can cause notable patient discomfort and requires specialized equipment and trained personnel, limiting its routine clinical use [137].

4.2.3. Cervicovaginal Fluid and Cervicovaginal Lavage Fluid

Cervicovaginal fluid, which contains shed tumor cells originating from the lower reproductive tract, serves as an additional effective screening tool for minimally invasive sample collection compared to uterine lavage fluid, which has more limitations in its application. In recent years, researchers have carried out extensive studies on cervicovaginal fluid or cervicovaginal lavage fluid based on cytological analysis [138], somatic mutations [139], DNA methylation [140-143], proteomics [144-147] , metabolomics [148, 149], and multiomics [150] approaches.

In contrast to the limited specificity of traditional cytology tests [138], growing attention has turned to DNA methylation as a more accurate diagnostic approach. Evans et al. [140] assessed the methylation status of ZSCAN12 and GYPC in cervicovaginal samples using the WID-qEC test. Compared to conventional ultrasound, WID-qEC demonstrated superior performance, achieving 92.1% specificity, 90.9% sensitivity, and an AUC of 94.3%. These results were further validated by Illah et al. [141], confirming WID-qEC as a highly sensitive and specific diagnostic method. Collectively, these studies suggest that cervicovaginal lavage offers a practical, minimally invasive alternative to traditional diagnostic procedures.

Beyond non-targeted approaches that screen proteins or metabolites for diagnostic [144-146, 148, 150] or stratification models [147, 149]. Pelegrina et al. [139] made a significant advancement by applying NGS to assess somatic mutations in cervicovaginal samples for non-invasive EC detection and molecular classification. The ClassEC test identified mutations in 73% of EC cases, with 80% specificity in clinician-collected samples and 90% in self-collected ones. Importantly, the test stratified EC into four molecular subtypes with distinct prognoses: POLE mutations were linked to favorable outcomes, while TP53 mutations predicted poor prognosis. This integration of molecular profiling with non-invasive sampling offers a promising alternative to traditional invasive diagnostics and represents a major step forward in personalized treatment for EC.

 doi:10.20944/preprints202506.1856.v1

4.2.4. Tampons

Tampons, as widely accepted and non-invasive intravaginal hygiene products, present a promising method for EC detection. Fiegl et al. [151] demonstrated that DNA methylation analysis of tampon-collected samples could distinguish EC from benign conditions with 100% sensitivity and 97.2% specificity in women aged 50–75, excluding CIN III and cervical cancer. Similarly, Bakkum-Gamez et al. [152] used tampons to collect vaginal pool samples and identified hypermethylation in nine genes in EC patients, achieving an AUC of 0.88, 76% sensitivity, and 96% specificity. This approach not only allows for convenient self-collection, improving patient compliance, but also enables repeated sampling for long-term monitoring in high-risk populations.

4.2.5. Cervical Scrapings and Vaginal Swabs

In addition to tampons, vaginal swabs and cervical scrapings are valuable sources for molecular DNA testing in EC. These low-cost, minimally invasive methods can be easily incorporated into routine outpatient visits. Multiple studies have demonstrated high diagnostic sensitivity and specificity in detecting tumor driver gene methylation through vaginal swabs [153-156]. Notably, Herzog et al. [154] evaluated methylation of the GYPC and ZSCAN12 gene regions in cervical, vaginal, and self-collected swab samples from patients with EC symptoms, reporting EC detection sensitivities of 100%, 90.1%, and 97.2%, respectively. This highlights the potential of self-sampling to support early detection while reducing the need for in-person visits. Interestingly, cervical lavage fluid also revealed abnormal methylation in these genes, validating the reliability of vaginal swabs and cervical smears. Furthermore, Kim et al. [157] successfully detected key gene mutations—such as PTEN, PIK3CA, TP53, and ARID1A—from genomic DNA in cervical smear samples with 100% specificity, aiding the optimization of ProMisE-based molecular classification for EC.

However, future research should prioritize pre-diagnostic sampling. Since most existing studies have focused on already-diagnosed individuals, earlier sampling could better reflect real-world diagnostic scenarios and reduce bias from tumor cell shedding during clinical procedures.

4.2.6. Peritoneal Surgical Lavage Fluid and Peritoneal Fluid

Peritoneal surgical lavage fluid and peritoneal fluid have emerged as promising sources for detecting mutations and other genetic alterations associated with EC, offering diagnostic and prognostic value among various biopsy fluids. To validate the utility of peritoneal lavage fluid, Mayo-de-las-Casas et al. [158] used a highly sensitive qPCR method and found that, in EC cases with known hotspot mutations, cfDNA from peritoneal lavage had a significantly higher detection rate (47%) compared to plasma (10.5%). This indicates that peritoneal lavage may better reflect the tumor mutational landscape, particularly in early-stage disease. Similarly, Ayyagari et al. (159) evaluated sterol-O-acyl transferase 1 (SOAT1) and cholesterol ester (CE) levels in plasma, peritoneal fluid, and endometrial tissue from EC patients and controls. Elevated levels were observed in tumor tissues and peritoneal fluid from EC patients, while plasma levels were comparable between groups. The strong correlation between SOAT1, CE, and poor overall survival suggests these markers are linked to tumor aggressiveness and unfavorable prognosis. Thus, SOAT1 and CE may serve as prognostic biomarkers and potential therapeutic targets, with peritoneal fluid offering a more informative medium than blood for detection.

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5. Future Directions and Prospects

Liquid biopsy is poised to become an essential component of EC management in the near future. Techniques involving cervicovaginal fluids, uterine aspirates, and circulating biomarkers—combined with genomic, proteomic, metabolomic, and multi-omics analyses—offer transformative potential for early detection and personalized treatment. These technologies promise to improve diagnostic accuracy, reduce reliance on invasive procedures, and enable more targeted therapeutic strategies. Early detection through such methods could significantly enhance patient outcomes by allowing timely, individualized interventions. Integrating multi-omics approaches offers a comprehensive view of EC, uncovering potential therapeutic targets and providing deeper insights into tumor behavior, treatment response, and resistance mechanisms. Successfully translating these innovations into clinical practice will require close interdisciplinary collaboration among gynecologists, oncologists, geneticists, data scientists, and bioinformaticians. Such collaboration is key to developing integrated diagnostic platforms that improve diagnostic precision and enable personalized treatment strategies tailored to each patient's molecular and clinical profile. Recent advancements in imaging, histopathology, and molecular diagnostics emphasize the importance of an integrated approach that combines various testing methods to enhance cancer diagnosis and treatment. AI technology, among the fastest-growing fields, holds limitless potential for integrating and optimizing these diverse diagnostic modalities, particularly in liquid biopsy [173]. Furthermore, as we advance in this field, it is imperative to carefully manage ethical considerations regarding patient data privacy, and the potential of misinterpretation of genetic information. Ensuring the accessibility of these technologies in high- and low-resource settings is essential to broaden their impact and address disparities in cancer care [174]. The clinical application of liquid biopsy necessitates large-scale validation before it can be adopted as routine practice. Although studies in other cancers have shown promising results [175-178], EC presents unique challenges that require dedicated clinical trials. Large-scale validation is crucial for transitioning liquid biopsy into routine clinical use. We advocate for EC-specific trials to confirm the clinical utility of these innovations and establish new standards that improve prognosis and quality of life for EC patients.

6. Conclusions

Liquid biopsy is a minimally invasive and effective tool for cancer management, enabling real-time molecular profiling of tumors and capturing their dynamic complexity. Its ability to allow repeat sampling makes it especially valuable for monitoring tumor progression, particularly when traditional biopsies are not feasible. While liquid biopsy has demonstrated clinical utility in other cancers and is already integrated into practice, its application in EC is only now gaining broader recognition.

To fully integrate liquid biopsy into standard EC care, several challenges must be addressed, including standardization, development of external quality control programs tailored to specific biomarkers, and accreditation of laboratories conducting these analyses. Additionally, a robust regulatory framework is needed to guide clinical use, address ethical concerns, and ensure responsible implementation. Expanded research and large-scale clinical trials are crucial for validating its effectiveness and refining its role in patient care. Taken together, these efforts are essential to unlock the full potential of liquid biopsy, paving the way for more personalized, precise, and effective treatment strategies in EC.

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