

# Biomarkers of Endometrial Cancer

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**Abstract** EC is one of the commonest cancers worldwide, and its incidence is increasing particularly in the developed world. A patient usually presents with suspicious symptoms (typically PMB) and undergoes a range of investigations and treatment before a definitive diagnosis of EC is made (TVS, hysteroscopy and endometrial biopsy, CT/MRI, surgery for diagnosis, treatment and further staging).

Biomarkers have the potential to help screening, diagnosing and staging the disease and could complement conventional means. At the moment, biomarker utilisation and research are more relevant in facilitating staging of EC and thus guiding treatment and aiding prognosis. Biomarker utilisation in screening and diagnosis is much less developed.

**Keywords** Endometrial cancer • Biomarker • Screening • Diagnosis • Staging • Prognosis

## Introduction

### *Epidemiology*

According to the World Health Organisation, endometrial cancer (EC) is the seventh most common cancer among women worldwide [1]. The incidence varies among different regions, with ten times higher incidence in developed countries compared to developing or less developed countries [2]. In fact, EC occurs in 10–20 per 100,000 women annually, making it the commonest malignant tumour of the pelvis [2].

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The International Federation of Obstetrics and Gynaecology (FIGO) gives the following staging [3]:

- Stage I EC is confined to the corpus uteri:
  - IA confined to endometrium with no or less than half myometrium invaded
  - IB invasion equal to or more than half of myometrium
- Stage II involves the corpus with invasion into the cervical stroma but has not extended outside the uterus.
- Stage III has local or regional spread beyond the uterus:
  - Stage IIIA is invasion of serosa or adnexa or positive peritoneal cytology and possibly more than one of these.
  - Stage IIIB is vaginal or parametrial metastases.
  - Stage IIIC is metastases to pelvic (IIIC1) or para-aortic (IIIC2) lymph nodes or both.
- Stage IV is involvement of the bladder or bowel mucosa or distant metastasis:
  - Stage IVA is involvement of bowel or bladder mucosa.
  - Stage IVB is distant metastases including nodes in the abdomen or inguinal region.

Approximately 72% of EC cases are FIGO stage I at diagnosis, 12% are stage II, 13% are stage III, and 3% are stage IV [4, 5]. The overall survival of patients affected with EC is about 80% and this depends on the FIGO stage [3].

### **Aetiology and Risk Factors**

Despite significant research into the biochemical mechanisms and pathophysiology of EC, the precise aetiology is unknown [6]. EC rarely presents before the age of 40, and more than 80% of cases occur in postmenopausal women [7].

About 5–10% of the cases of EC have a hereditary basis, with hereditary non-polyposis colorectal cancer (HNPCC or Lynch syndrome) being the most common cause. In fact, women with HNPCC have a higher lifetime risk of developing EC than developing colorectal cancer (42% versus 30%) [8]. More than 90% of EC cases occur sporadically [2]. Risk factors include unopposed oestrogen stimulation of the endometrium such is the case in women suffering from polycystic ovary syndrome (PCOS), obesity, diabetes mellitus and oestrogen-secreting tumours [2, 9].

Nulliparity is also a risk factor for EC as well as the use of tamoxifen for the treatment for women with breast cancer [10, 11].

### **The Dualistic Model**

EC is commonly classified into two types. Type 1 tumours (about 80%) are endometrioid carcinomas arising in a background of hyperplasia in obese women [12]. These tumours are usually low grade, oestrogen related and follow a more

favourable course. In contrast, type 2 tumours (about 20%) are non-endometrioid (predominantly serous and clear cell) carcinomas arising in endometrial polyps or from precancerous lesions in the vicinity of an atrophic endometrium. These tumours are high grade, not oestrogen related, often invade the myometrium and (lymph) vascular spaces and have a high mortality rate [13]. At the time of operation, about one in every ten clinical stage I ECs has lymph node metastases, most commonly at pelvic lymph nodes, sometimes associated with para-aortic lymph node involvement [14]. The latter represent a more aggressive disease stage, and it is an independent predictor of poor outcome [15].

### **Clinical Presentation**

Typically, EC presents as postmenopausal bleeding (PMB). In premenopausal women, it presents as menorrhagia, intermenstrual or postcoital bleeding. EC is usually diagnosed early so women rarely present with systemic symptoms of malignancy like weight loss, tiredness or malaise.

### **Investigations**

#### Transvaginal Ultrasound

Transvaginal ultrasound (TVS) is an appropriate first-line procedure to identify which women with PMB are at higher risk of EC. The mean endometrial thickness in postmenopausal women is much thinner than in premenopausal women; therefore thickening of the endometrium may indicate the presence of pathology. In general, the thicker the endometrium, the higher the likelihood of important pathology, that is, EC. In the UK, the endometrial thickness threshold is 5 mm which provides adequate sensitivity without excessive false-positive rates in most women and a false-negative rate of 0.25–.50% [16]. European guidelines have a lower threshold (3–4 mm), but this leads to greater numbers of biopsies [17]. Some pathology may be missed; therefore, hysteroscopy and biopsy should be carried out in cases where endometrial thickness is below the threshold if there is a high clinical suspicion [17].

#### Hysteroscopy and Endometrial Biopsy

A definitive diagnosis in PMB is made by histology. Biopsy can be taken during hysteroscopy performed under local or general analgesia.

## Staging

Once histological diagnosis is established, CT of chest, abdomen and pelvis as well as MRI of pelvis should be performed to assess the extent of disease. Further staging is performed intraoperatively which includes exploration of the pelvis and abdomen with biopsy of any suspicious lesions, total abdominal or laparoscopic hysterectomy (TAH/TLH), bilateral salpingo-oophorectomy (BSO) and, where appropriate, complete pelvic and/or para-aortic lymphadenectomy [18].

## Treatment

This depends upon the stage [19].

- Stage I requires total abdominal hysterectomy with bilateral salpingo-oophorectomy. The role of lymphadenectomy is debated [18].
- In stage II there should be radical hysterectomy with systematic pelvic node clearance. Para-aortic lymphadenectomy may also be considered. Lymphadenectomy is important for staging and as a guide for adjuvant therapy.
- Stages III and IV are best treated with maximal de-bulking surgery. Although there is no conclusive evidence, a combination of surgery, radiation and chemotherapy (usually with doxorubicin).

## Molecular Biology and Genetics of EC

The endometrium undergoes structural modification in response to fluctuations of oestrogen and progesterone during the menstrual cycle. Long-lasting unopposed oestrogen exposure leads to endometrial hyperplasia, which increases the chance of development of type 1 EC. The molecular basis of this process is still not known, since the involvement of only a minority of factors is reproducible [20]. Aside from their morphologic and clinical features, type 1 and type 2 ECs are further distinguished by genetic alterations [21].

In general, the development of cancer is characterised by self-sufficiency in growth signals, insensitivity to growth inhibition, evasion of apoptosis, angiogenesis, invasion and metastasis [22]. Understanding pathogenesis at the molecular level is essential in identifying biomarkers for successful targeted therapies.

### *Type 1 (Endometrioid) EC*

The most frequent genetic alteration mainly affecting type 1 EC involves the PTEN gene—a tumour suppressor [23]. PTEN, located at chromosome 10q23, encodes a protein (phosphatase and tensin homolog, PTEN) with tyrosine kinase. PTEN has

been reported to be altered in up to 83% of type 1 EC and 55% of precancerous lesions [23]. PTEN inactivation is caused by mutations that lead to a loss of expression and, to a lesser extent, by a loss of heterozygosity. Thus, loss or altered PTEN expression results in aberrant cell growth and apoptotic escape. Loss of PTEN is furthermore probably an early event in endometrial carcinogenesis, as evidenced by its presence in precancerous lesions, and is likely initiated in response to known hormonal risk factors [23]. Its expression is highest in an oestrogen-rich environment. In contrast, progesterone promotes involution of PTEN-mutated endometrial cells. These observations are consistent with the well-documented clinical effects of progesterone-mediated suppression and resolution of invasive EC and its precursors [24]. PTEN mutation is well documented in endometrial hyperplasia with and without atypia [25].

Mutations in PIK3CA may contribute to the alteration of the phosphatidylinositol 3-kinase (PI3K)/AKT signalling pathway mainly seen in type 1 EC [26]. PIK3CA gene mutations occur in 24–39% of the cases of type 1 EC and frequently coexist with PTEN mutations [27]. PIK3CA mutations have been associated with adverse prognostic factors such as high-grade and myometrial invasion [27].

The accumulation of sequence changes in DNA segments, which occurs because of inactivation of intranuclear proteins constituting the mismatch repair system, is known as microsatellite instability (MSI) [27]. MSI has been demonstrated in 20% of sporadic type 1 EC [26]. Microsatellites are short segments of repetitive DNA bases that are scattered throughout the genome. Inactivation of MutL protein homolog 1 (MLH1), a component of the mismatch repair system, is a common event in type 1 EC. This alteration occurs through hypermethylation of CpG islands in the gene promoter, a process known as epigenetic silencing [27]. MSI and abnormal methylation of MLH1 are early events in endometrial carcinogenesis and have also been described in precancerous lesions [28].

Other genetic alterations in type 1 EC include mutations of K-ras and beta-catenin genes [26].

## *Type 2 (Serous and Clear Cell) EC*

The most common genetic alteration in serous EC is in p53, the tumour suppressor gene. This occurs in up to 90% of serous EC [29]. The p53 gene is located on chromosome 17 and is important in preventing the propagation of cells with damaged DNA. The exact mechanism behind the cause of this mutation is still unclear. It is postulated that mutation in one allele occurs early during the development of serous carcinoma, and loss of the second normal allele occurs late in the progression to carcinoma [29].

Other frequent genetic alterations in type 2 ECs are inactivation of p16 and overexpression of HER-2/neu [30]. P16 inactivation was found in 45% of serous carcinomas and some clear cell cancers. The p16 tumour suppressor gene is located on chromosome 9p21 and encodes for a cell cycle regulatory protein. Thus, inactivation of p16 leads to uncontrolled cell growth [30].

## Diagnostic/Screening Markers

EC is detected after pathology assessment of uterine aspirates, hysteroscopy-guided biopsies and curettage. Although these methods are considered the gold standard for screening, they still have some limitations and drawbacks [31]. First, they may cause significant discomfort. Second, as tools for diagnosis, they have only a moderate ability to predict the final pathology, and third, they require a trained pathologist for interpretation.

A study by Colas et al. compared gene expression screening on 52 carcinomas and 10 normal tissues to identify potential biomarkers [32]. These were further validated in an independent series of 19 tissue samples by RTqPCR and on 50 carcinoma and non-carcinoma uterine aspirates [32]. A panel of potential genes differentially expressed was identified (ACAA1, AP1M2, CGN, DDR1, EPS8L2, FASTKD1, GMIP, IKBKE, P2RX4, P4HB, PHKG2, PPFIBP2, PPP1R16A, RASSF7, RNF183, SIRT6, TJP3, EFEMP2, SOCS2 and DCN) which correlated to their expression in the corresponding primary endometrial tumours [32]. The authors proposed that such a minimally invasive and highly sensitive and specific method for the identification of EC which has the potential to increase patient comfortability as alternative methods of diagnosis is based in more invasive techniques [32]. It could also provide a molecular tool for supporting pathologist decision and hence help gynaecologists to reduce the number of unnecessary hysteroscopies. Furthermore, among the potential clinical applications for these newly discovered molecular biomarkers could be a screening programme within high-risk populations designed to improve the early detection of EC [32]. Large validation studies need to be conducted first before such results are translated in clinical practice.

DNA methylation is notable because of its early occurrence in carcinogenesis, stability and detectability using highly sensitive and specific assays [33]. Based on the hypothesis that candidate DNA methylation markers demonstrate low values in benign tissues, large differences between carcinomas and benign tissues and highly statistically significant differences by disease status, Wentzensen et al. were able to identify an eight-marker panel obtained from endometrial brushings with substantial discrimination (ADCYAP1, ASCL2, CDH13, HS3ST2, HTR1B, MME, NPY, SOX1) [33]. These findings provide a proof of principle that it may be possible to develop diagnostic molecular testing as an adjunct to the classification of endometrial biopsies or brushings performed to assess suspicious vaginal bleeding [33]. What is more, this test could enable triage patients with carcinoma, while reducing overtreatment of innocuous lesions [33]. This could be particularly important among women with limited health care access as rapid identification of carcinomas may increase chances of cure and reduce the need for more aggressive treatment secondary to disease progression, whereas ruling out high-risk lesions could allow many women to safely opt for conservative management [34]. Again, validation of this biomarker panel in large prospective studies is imperative before the results can be applied in practice.

Higher serum CA 125 levels correlate with the extrauterine disease and advanced cases and are used as a marker to evaluate prognosis and recurrence in EC (see section below on Prognosis and Staging). However, a CA 125 level greater than 35 U/mL is not useful in diagnosing early stages of EC [35]. Moore et al. have proved that serum HE4 is elevated in all stages of EC and is more sensitive in early-stage cancer compared to CA 125 [36]. Although there is sufficient evidence in regard to the accuracy of HE4 for the diagnosis of EC, there is currently not enough data to estimate its value in clinical practice [35]. Such quantification warrants further large-scale studies. Finally, there is evidence that patients with EC have significantly different expression patterns of several serum biomarkers as compared to healthy controls with a high sensitivity (98.3%) and specificity (98.0%) [37].

In conclusion, the role of biomarkers in the screening and diagnosis of EC is still in its infancy, and further studies are needed to validate these promising findings before they are translated to clinical practice.

## Prognostic/Staging Biomarkers

### *Tissue Biomarkers*

Expression of p53 protein and/or p53 gene mutations have been detected in 7–43% of EC and have been associated with advanced stage, high grade, deep myometrial invasion, type 2 histology, lymph node metastasis and, ultimately, lower survival compared with EC patients without p53 alterations [38–43].

PTEN mutations are related to early stage, low rate of p53 overexpression and longer survival in women with EC [44]. On the other hand, Steinbakk et al. failed to evidence any prognostic relevance for PTEN status in curettages from patients with FIGO stages I–II type 1 EC [43]. Therefore, loss of PTEN function did not appear to impact on survival of patients with early disease, but it was associated with a better clinical outcome in those with advanced or recurrent disease [45].

MSI, which is the hallmark of defects in DNA mismatch repair genes, occurs in 11–45% of type 1 EC [45–47]. Whereas MSI is an independent predictor of a favourable outcome in colorectal cancer [48], conflicting data emerge from the literature as far as the prognostic relevance of MSI in type 1 EC is concerned [49].

Alterations in  $\beta$ -catenin expression have been reported both in type 1 EC and atypical hyperplasia and therefore appear to represent an early event in endometrial carcinogenesis [26]. Saegusa et al., who assessed 199 cases of type 1 EC, found a significant association between  $\beta$ -catenin mutations and low-grade histological malignancy ( $p = 0.048$ ), as well as between  $\beta$ -catenin mutations and lack of lymph node involvement [50].

K-ras mutations which are most commonly seen in type 1 EC have been associated with lymph node metastasis and poor survival [26, 51, 52]. For example, Mizuuchi et al. investigated 49 cases and concluded that the presence of K-ras

mutations was an independent predictor of unfavourable clinical outcome ( $p = 0.034$ ) after adjusting for tumour stage, depth of myometrial invasion and patient age [52].

Vascular endothelial growth factor (VEGF) is an important endothelial cell mitogen that acts through specific receptors, namely, flt-1 and flk-1/KDR [53]. In EC, an increase in VEGF expression has been often associated with advanced tumour stage [54], high tumour grade [55], deep myometrial invasion [56], lymphovascular space involvement [54] and lymph node metastases [54].

The proportion of aneuploid tumours among EC ranges from 16 to 28% and significantly correlates with old age at diagnosis, type 2 histology, high tumour grade and lymph node involvement [57–60]. In most studies patients with aneuploid tumours have significantly poorer survival at multivariate analysis, after adjusting for the common clinical-pathological variables [57, 59, 60]. In fact, some authors have suggested including DNA ploidy among criteria for the selection of high-risk patients who might benefit from adjuvant treatment [58, 59].

Both HER2 overexpression and amplification have been linked to poor prognosis and survival in EC [61–63]. Following the successful development of targeted therapy against HER2 in breast cancer, reports on HER2 overexpression have sparked considerable interest for a potential novel HER2-based therapy in EC. Trastuzumab (Herceptin, Genentech, San Francisco, California), a humanised monoclonal immunoglobulin (Ig) G1 antibody against HER2/neu, is now Food and Drug Administration (FDA) approved in the treatment of HER2-overexpressing breast cancer and HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma [61]. In vitro studies have demonstrated that trastuzumab results in antibody-dependent cellular cytotoxicity in the range of 25–60% against HER2-overexpressing uterine serous carcinoma which can be augmented by both IL-2 and simultaneous administration of the heterodimerization inhibitor pertuzumab (Omnitarg, Genentech) [61].

Hormone receptor status has consistently been shown to be a relevant prognostic marker that could also influence the choice of treatment for metastatic disease, due to higher response rates reported for hormone-receptor-positive tumours [64]. The presence of steroid receptors correlates with low-grade, type 2 histology as well as favourable outcome in many studies [65, 66]. Hormone receptor status in curettage and hysterectomy specimens has been reported to be highly correlated with favourable prognosis and with good to very good reproducibility for pathological staining assessment [65]. On the contrary, loss of oestrogen and progesterone receptors in curettage specimens has been significantly associated with aggressive phenotypes and poor survival in patients with EC [66].

### *Serum Biomarkers*

Elevated serum CA 125 levels (>35 U/mL) have been found in 11–34% of patients with EC [61–64]. Preoperative serum CA 125 concentrations correlate with stage [67–71], depth of myometrial invasion [65–67], tumour grade [69, 71, 72], cervical invasion [73], peritoneal cytology [71, 73] and lymph node status [67, 71, 73]. A



preoperative test that could accurately recognise nodal disease would prevent both overtreatment (i.e. unnecessary pelvic and para-aortic lymphadenectomy) and undertreatment (i.e. withholding complete lymphadenectomy or adjuvant postoperative treatment to patients with lymph node metastases). However, the optimal cutoff value has not been determined yet, and correlations between the levels of CA 125 in serum and lymph node metastases remain inconsistent, warranting further research in this field [74].

Several studies have investigated whether serum CA 125 assay may provide additional information for the identification of those patients with high risk of sub-clinical extrauterine spread who need a lymphadenectomy [67, 71, 73]. Scambia et al. found CA 125 levels >65 U/mL in 22% of patients with negative lymph nodes compared to 58% of cases with histologically proven positive nodes ( $p = 0.022$ ) [67]. Sood et al. observed that preoperative serum CA 125 > 65 U/mL was the strongest predictor of extrauterine disease with a risk ratio of 6.5 (95% CI = 2.5–17.1) [71]. Other authors confirmed that serum CA 125 level was an independent risk factor for lymph node involvement [73, 74].

Serum CA 153 levels are elevated in 24–32% of patients with EC and correlate with tumour stage [67, 69]. Scambia et al. detected CA 153 levels >30 U/mL in 47% of patients with stage III disease compared with 18% of those with stage I–II disease ( $p = 0.01$ ) and found a significant relationship between serum CA 153 positivity (>30 and >50 U/mL) and shorter survival ( $p = 0.0004$  and  $p = 0.00025$ , respectively) [67].

## Conclusions and Future Approaches

Biomarkers have the potential to help screening, diagnosing and staging the disease and could complement conventional means. At the moment, biomarker utilisation and research are more relevant in facilitating staging of EC and thus guiding treatment and aiding prognosis. Biomarker utilisation in screening and diagnosis is much less developed.

There are important limitations that need to be overcome in the future to allow adequate implementation of new biomarkers to guide clinical care in EC. Suggestions for future research include [64]:

1. Sufficiently sized, population-based biomarker studies linked to state-of-the-art clinically and histopathologically annotated patient series.
2. The test criteria applied for new surgical staging procedures by lymphadenectomy should be better standardised, and figures for reproducibility, sensitivity and negative predictive value for the procedure should be established.
3. Introduction of new imaging methods and biomarkers for test development needs to meet strict standards for reproducibility and test quality before incorporation into stratification schemes that define target populations.
4. Studies of new potential markers need to be done in a prospective multicentre setting to document their performance in a routine clinical setting.

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