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Current trends in identifying biomarkers in endometrial cancer

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ABSTRACT

Endometrial cancer (EC) is the sixth most common cancer in women and accounts for about 8.2 % of the worldwide incidence of cancer in women. Its incidence varies across regions and is rising as life expectancy increases. Although, there have been significant recent advances in our understanding of endometrial cancer. But there is paucity of a reliable screening test. When diagnosed at an early stage, EC is highly curable and has excellent overall 5 –year survival rates. Delayed diagnosis contributes to advanced stage at the time of presentation and therefore poor survival. Early diagnosis is key to improving survival. As of now, there are no validated biological makers for its early detection. The present article attempts to analyze the approaches which may contribute to find relevant biomarkers to be used as screening, diagnostic and prognostic value to patient with endometrial cancer.

Introduction

Endometrial cancer (EC) is the sixth most common cancer in women and accounts for about 8.2 % of the worldwide incidence of cancer in women. Its incidence varies across regions and is rising as life expectancy increases (1, 2). When diagnosed at an early stage, EC is highly curable and has excellent overall 5 –year survival rates (3). Delayed diagnosis contributes to advanced stage at presentation and poor survival.

Post menopausal women with vaginal bleeding undergo various tests to exclude EC, including transvaginal ultrasound scan (TVS), Outpatient hysteroscopy (OPH) and endometrial biopsy (EB) (4). While these procedures are expensive and difficult to perform, their diagnostic utility for EC is limited by poor specificity (TVS) and unacceptable levels of invasiveness and discomfort (OPH, EB) (3, 4). The measurement of endometrial thickness (ET) with TVS for instance , although minimally invasive and highly sensitive for EC detection ,is plagued by a markedly low specificity as multiple benign pathologies especially polyps, intracavitary fibroids or blood clot , artifacts create the appearance of a thickened endometrium .(3,4) Endometrial biopsy , the gold standard for diagnostic evaluation of women with suspected EC , can sometimes miss focal pathologies , especially when done blindly using office based sampling devices such as pipette. The procedure is not only painful in nulliparous women but has high risk of insertion failure (3, 5). Hysteroscopy with directed biopsy, on the other hand, has better diagnostic sensitivity but is expensive, has high failure rate in outpatient clinic and over 30 % women experience severe pain or vasovagal episode during its completion. There is theoretical risk of disseminating cancer cells into the peritoneum, rarely life-threatening complications

ensue, e.g. uterine perforation (3, 6).

The ideal EC detection tool should be simple, non invasive, have ability to reliably detect all EC at the earliest stage with few false positives and negatives. Such a tool may be used to screen high risk asymptomatic women, or those with Lynch syndrome, who have a high lifetime risk of developing EC. Early detection could enable conservative management options to young women desirous to complete childbearing and morbidity obese women in whom surgery is potentially hazardous (4). Diagnostic biomarkers that identify specific subtypes of EC, for example POLE, Ultramutated EC, would also provide prognostic and predictive information and could be used to monitor response to therapy and detect recurrent disease.

EC have traditionally been classified into two histological categories Type 1 and type 2 (Bokhmans dualistic model). (7) Type I tumors make up 80-90 % of endometrial cancers and are estrogen responsive, have a favorable prognosis, and may be preceded by a precancerous condition (atypical hyperplasia). Type II tumors, account for only 10-20 % of endometrial cancers and are usually estrogen independent, high grade and clinically aggressive. (7, 8)

Newer classification

A recent and pragmatic classification of EC based on a multiplatform (genomic and transcriptomic) analysis categories EC into 4 distinct molecular subtypes of prognostic relevance: [9,10]

- Polymerase epsilon (POLE)- Unmutated
- Microsatellite instability (MSI)- Hypermutated
- Copy number low - Microsatellite stable (MS)
- Copy number high – Serous type

Search for EC diagnostic markers

Human genome was decoded in 2001 and it opened avenues for information on complete human genome thereby enabling the discovery of biomarkers and new diagnostic tests [12-17]. The high throughput technologies like genomic, transcriptomic, proteomic, metabolomic and imaging analysis have enormous potential as large-scale biomarker discovery (14).

Nair et al [13] cfDNA fractions of uterine lavage samples and Lim et al [14] using the cervical swab samples [14], both using Next generation sequencing for biomarker detection in EC patients however found limited application due to high false positive rates. It also became evident that development of EC is solely not based on genomics only rather environmental factors play major role in disease causation [12]. There is lot of information at cellular, subcellular and intracellular levels that is not explained by genomics alone [15]. This led to integration of epigenomic transcriptomic, proteonomic and metabolomic data incorporation. The biomarker search at epigenetic based platforms had limitations as they were non quantitative or semi quantitative with poorly defined cut offs based on test and control genes and hence poor reproducibility in routine clinical setting [14].

The application of high throughput technology in study of RNA is known as transcriptomics. It allows the characterization of genetic expression at m RNA level [15]. However, EC transcriptome in cancer genome could not provide information on differential expression in EC verses non-EC endometrial tissue [16]. It is still expected that as transcriptomics reflect dynamic cell state, they have great potential to yield useful biomarkers [17].

Proteomics

Nowadays protein identification with altered expression in cancers is done by employing advanced techniques like two-dimensional gel electrophoresis and mass spectrometry-based analysis [18]. Whereas 2D gel electrophoresis has low throughput [19] others like Enzyme linked immunosorbent assay (ELISA) and Immunohistochemistry (IHC) could easily be employed in resource limited settings as well [20].

The choice of biological specimens' chosen for protenomic analysis is dictated by ease of availability and invasiveness of procedure to obtain sample. Hysterectomy, endometrial biopsy specimens and uterine lavage samples are viable source of cancer derived proteins but their retrieval is not easy. Blood is most accessible and minimally invasive fluid for examination but its use is limited by low quantity of tumor related signals in circulation in early stage of disease process [21, 22].

An effective sample preparation is essential for successful biomarker discovery [23]. Tissue based proteomic studies could be done by using laser capture micro dissection (LCM) but there is no standard technique for sample preparation from blood and it depletes high abundant proteins [17]. Although the acceptability of blood (serum/ plasma) as potential source of biomarkers for EC is high but the real challenges comes from the matrix and therefore have poor concordance with time derived proteins.

Blood as source of biomarkers

Peripheral blood has circulating tumor cells (CTC), circulating tumor DNA (ct DNA), proteins and extracellular vesicles all having potential to serve as biomarkers. Table-1 shows the various blood-based biomarkers that are under research. Unfortunately, currently there is insufficient data to support any of the blood-based biomarkers for EC diagnosis. There have been inconsistent study findings, poor accuracy and lack of validation of blood-based markers rather they have been used for prognostification. The most promising blood base markers include HE4 (WFDC2) and CA 125 (MUC 16) which are also found in tissue based EC samples and have been validated in literature studies [25,26] .The sensitivity and specificity of HE4 in EC specimen was 0.65 and 0.9 in study by Li and colleagues [26]. The results using CA 125 are unsatisfactory [25]. To improve diagnostic accuracy of HE4 and CA125 some researchers have suggested to add body mass index (BMI) { Knifie et al [27]} and Prolactin {Yurkovetsky [28]}. Prolactin has been found to increase in ovarian, lung and pancreatic carcinoma limiting its utility as EC marker [28].

Table -1 Blood markers for EC

Promising		
Marker	**Technique**	**Nature**
Prolactin	Bead based immunoassay	Secretion by stromal cells
Human Epididymis Protein 4(HE4)	Enzyme immunoassay	Whey acidic protein family
CA125, CA72.4, CA 15.3	ELISA	Mucin family glycoprotein
Human chitinase 3 like protein 1 (YKL-40)	ELISA	Glycoprotein chitinase family
Adiponectin	ELISA	Adipokine metabolic, immune function

Limited use biomarkers	
Cyclophilin A	**TNF Receptor 1A**
Sperm associated antigen	Colony stimulating factor CSF1
Growth related oncogen ALPHA (cxcl1)	Alpha fetoprotein
Growth differentiation factor 15 (GDF -15)	Alpha beta glycoprotein
FAS	Complement (C3, C4, C4B)
Leucine rich glycoprotein (LRG1)	Clustering (CLU)
Transthyretin	Antithrombin III
inter alpha trypsin inhibitor family heavy chain related peptide	Alpha 1 antitrypsin
Cleavage high molecular kininogen	Visfalin (NAMPT)
ICAM	TSH, ACTH, FSH
Interleukin 31,33, 2R	

Inconsistent biomarkers
Matrix metalloprotein MMP 2,7,9
Serum Amyloid A (SAA)
apolipoproteins (A-IV0 C1
Dickkopf related protein 3 precursor
VEGF

Proteomic analysis of tissue samples

The tissue biomarkers have been broadly categorized as – chaperones / heat shock proteins (HSP 10, 27, 70, 71) , enzymes (pyruvate kinase (PK) , phosphoglycerate kinase (PGK -1) , phosphoglycerate mutase 2 (PGAM 2) , alpha enolase (ENO -1) , enzyme inhibitors (alpha -1- antitrypsin precursors (SERPINA 1) , calcium binding proteins calgranulin (S- 100A8/9) , calgizzarin (S-100A11) , calyphosine (CAPS), fatty acid binding protein (epidermal fatty acid protein (FABP5) and calyphosine protein among others. Molecular chaperons are implicated in tumor cell proliferations and differentiation but their non specificity is a major hurdle [29, 30, and 31. In combination with other proteins, however they are likely to be strong candidate for EC detection and warrant further exploration.

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 (31-37) PTEN mutations are related to early stage, low rate of p53 over expression and longer survival in women with EC .(38) Loss of PTEN function does 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 . (39)

MSI (Microsatellite instability) which is the hallmark of defects in DNA mismatch repair genes occurs in 11-45 % of type 1 EC (39, 40, and 41). Whereas MSI is an independent predictor of a favorable outcome in colorectal cancer (42), conflicting data emerge from the literature as far as the prognostic relevance of MSI in the Type 1 EC is concerned. Alterations in Beta catenin expression has been reported both in type 1 EC and atypical hyperplasia and therefore appears to represent an early event in endometrial carcinogenesis. Saegusa et al 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 . (43)

K- ras mutations which are most commonly seen in type 1 EC has been associated with lymph node metastasis and poor survival. (43, 44, 45) For example, Mizuuchi et al investigated 49 cases and concluded that the presence of K-ras mutations was an independent predictor of unfavorable clinical outcome (p=0.034) after adjusting for tumor stage, depth of myometrial invasion and patient age

Vascular endothelial growth factor (VEGF)is an important endothelial cell mitogen that acts through specific receptors, namely flt-1 and flk -1/KDR (46). In EC, an increase in VEGF expression has been associated with advanced tumor stage, high tumor grade, deep myometrial invasion, lymphovascular space involvement and lymph node metastases. (47, 48, 49)

The proportion of aneuploid tumors among EC ranges from 16 to 28 % and significantly correlates with old age at diagnosis, type 2 histology, high tumor grade and lymph node involvement. In most studies patients with aneuploid tumors have significantly poorer survival at multivariate analysis, after adjusting for the common clinical –pathological variables (51, 52, 53). In fact, some authors have suggested including DNA ploidy among criteria for the selection of high-risk patients who might benefit from adjuvant treatment.

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 tumors. The presence of steroid receptors correlates with low grade, type 2 histology as well as favorable outcome in many studies (21, 27). Hormone receptor status in curettage and hysterectomy specimens has been reported to be highly correlated with favorable prognosis and with good to very good reproducibility for pathological staining assessment. On the contrary, loss of estrogen and progesterone receptors in curettage specimens has been significantly associated with aggressive phenotypes and poor survival in patients with EC (27). Table-2 shows the various tissue-based biomarkers that are under research.

Table 2 Tissue based biomarkers for EC

Consistent	
Chaperonin 10 9CPN)	
Piruvate Kinas (PK)	
Limited evidence	
Cyclophilin A	Alpha enolase
Caligizzarin	Superoxide dismutase
Epidermal fatty acid binding protein	Fibrinogen beta chain
Calgrunulin A	Anterio gradient protein
HSP 27, HSP 47	Macrophage migratory inhibitor
Proinhibin	Alpha 1 antitrypsin
Transgelin	Protein deglycase
Phosphoglycerate kinase	Annexin 1-2
Creatinekinas B	Peroxidases 1-4
Glutathione synthetase	Costar family
Serotransferrin receptor	Desmin

Urine sample

Urine is cheap, easily accessible, easy to collect and in study by Mu et al [54] found up regulation of Zinc alpha 2 glycoprotein, alpha 1 acid glycoprotein and CD 59 in EC patients compared to normal healthy subjects. The urine-based biomarkers rely on renal excretion of these markers or urinary contamination by uterine biomarkers. This poses major problems and makes them unreliable as many of them occur in urine as uterine shedding biomarker rather than true urinary excreted ones [55].

Ideal biomarker

The majorities of biomarkers are at discovery phase and fail to pass validation studies. Subject selection bias [56] is a problem with validation of these markers. Racial differences, age related normal control (healthy) subjects and postmenopausal woman selection create discriminatory results and skewed findings. A small sized study leads to erroneous conclusions. Many a times spurious signals [57] are introduced as a result of specimen collection at different time points, storage conditions, all these mandate standard operating procedures for all steps involving sample collection, processing and interpretation.

Conclusion

Biomarkers have the potential to help screening, diagnosing and staging the disease and could complement conventional means. At the moment, biomarkers utilization and research are more relevant in facilitating staging of EC and thus guiding treatment and aiding prognosis. Biomarker utilization 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.

Recommendations

Therefore, further new studies should be undertaken so that we are able to work out the best possible biomarker for EC. The following parameters may be included for a better outcome

- Sufficiently sized, population
- Test criteria applied for new surgical staging procedures with lymphadectomy should be better standardized
- Reproducibility
- Practical application in routine clinical settings

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