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HE4 as a biomarker for ovarian and endometrial cancer management

Jinping Li, MD, PhD,
Department of Biomedical Science, Mercer University School of Medicine at Savannah, 4700 Waters Avenue, Savannah, GA 31404, USA Tel.: +1 912 350 0411, Fax: +1 812 350 1281

Sean Dowdy, MD,
Department of Obstetrics and Gynecology, Mayo Clinic, Rochester, MN 55905, USA, Tel.: +1 507 266 7933, Fax: +1 507 266 9300

Tracy Tipton, BS,
Department of Biomedical Science, Mercer University School of Medicine at Savannah, 4700 Waters Avenue, Savannah, GA 31404, USA, Tel.: +1 912 350 0411, Fax: +1 812 350 1281

Karl Podratz, MD, PhD,
Department of Obstetrics and Gynecology, Mayo Clinic, Rochester, MN 55905, USA Tel.: +1 507 266 7932, Fax: +1 507 266 9300

Wei-Guo Lu, MD,
Women's Hospital, Zhejiang University School of Medicine, Xueshi Road Hangzhou 310006, PR China, Tel.: +86 571 8703 1130, Fax: +86 571 8706 1878

Xing Xie, MD, and
Women's Hospital, Zhejiang University School of Medicine, Xueshi Road Hangzhou 310006, PR China, Tel.: +86 571 8703 1130, Fax: +86 571 8706 1878

Shi-Wen Jiang, MD[†]
Department of Biomedical Science, Mercer, University School of Medicine at Savannah, 4700 Waters Avenue, Savannah, GA 31404, USA, Tel.: +1 912 350 0411, Fax: +1 812 350 1281

Jinping Li: li_s@mercer.edu; Sean Dowdy: dowdy.s@mayo.edu; Tracy Tipton: ttipton7@utmem.com; Karl Podratz: podratz.k@mayo.edu; Wei-Guo Lu: lbwg@zju.edu.cn; Xing Xie: xiex@mail.hz.zj.cn; Shi-Wen Jiang: Jiang_s@mercer.edu

Abstract

Ovarian and endometrial cancer will be diagnosed in over 63,000 women in 2009, resulting in 22,000 deaths in the USA. Histologic screening, such as pap smears for detection of cervical cancer, is not feasible for these diseases given difficulty with access to the tissue. Thus, a serum-screening test using a biomarker or panel of biomarkers would be useful to aid in cancer diagnosis, detection of recurrence and as a means to monitor response to therapy. In this review, we focus on the human epididymis protein (HE)4 gene, which appears to have potential as a biomarker for both of these diseases. The structure and methods of detection of HE4 are discussed. Preliminary data show that HE4 may have more potential than cancer antigen 125 in discriminating benign from

[†]Author for correspondence Department of Biomedical Science, Mercer University School of Medicine at Savannah, 4700 Waters Avenue, Savannah, GA 31404, USA, Tel.: +1 912 350 0411, Fax: +1 812 350 1281.

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cancerous ovarian masses, and has the strongest correlation with endometrial cancer of all markers tested to date. Utilizing risk stratification, a panel of biomarkers including HE4 may ultimately be useful for detecting ovarian and endometrial cancer at an early stage in patients at high risk.

Keywords

cancer antigen 125; cancer diagnosis and treatment; endometrial cancer; human epididymis protein 4; ovarian cancer

Ovarian and endometrial carcinomas (ECs) are the two most common malignancies of the female reproductive system. According to estimates by the American Cancer Society, in 2009 alone 21,550 women will be diagnosed with epithelial ovarian cancer (OC) and 14,600 deaths attributed to this disease, making it the leading cause of death by all gynecologic cancers. With 42,160 estimated cases leading to 7780 deaths, EC is the most prevalent gynecologic cancer and ranks second in the number of deaths. The high death rate of OC is mostly attributable to its late detection and the associated high tendency for diffuse metastasis and recurrence. For EC, the common presentation of vaginal bleeding and ready access to the endometrium for the purposes of biopsy often lead to earlier detection. Both malignancies are characterized by relatively high 5-year survival rates with stage I disease, but detection of either disease at an advanced stage translates into a poor prognosis. Early OC detection corresponds to a 92% 5-year survival rate, whereas the overall 5-year survival rate for OC is less than 50%. This is because only 19% of ovarian malignancies are diagnosed prior to extra-ovarian spread owing to the lack of obvious symptoms prior to progression. Likewise, the 5-year survival rate for EC diagnosed at local, regional and distant sites is 95, 67 and 23%, respectively [101]. There is clearly an urgent need for the development of early detection methods for these diseases. Novel biomarkers would also allow disease stratification, monitoring of response to therapy, and surveillance for post-surgical recurrence in women with a diagnosis of OC.

Compared with screening and personalized therapy, risk stratification and proper triaging could be achieved by using a biomarker with relaxed specificity. This could be used to refer patients to centers specializing in ovarian cancer treatment, given that these patients have been shown to have more favorable outcomes when treated at such centers [1]. Patients treated by gynecologic oncologists more often experience complete surgical staging, and OC patients treated in tertiary care centers under a multidisciplinary team of physicians experienced in the care of OC have fewer complications and longer survival rates [2–8]. Unfortunately, recent analysis of OC patient care suggests that fewer than 50% of OC patients in the USA are cared for by, or in cooperation with, a gynecologic oncologist [3,9]. Of course, this statistic is attributable to several factors other than inadequate triaging, such as patient inclination to be cared for by his/her primary physician, lack of health insurance, insufficient transportation, opposing fiscal incentives, and scarcity of specialized physicians relative to the number of OC cases [2]. Regardless, in a hypothetical scenario void of such hindrances, early triaging would still be unimaginable without proper risk-stratification tools.

Limited success was accomplished by the use of cancer antigen (CA)-125 for OC diagnosis and triaging given its low sensitivity for early-stage disease and limited specificity, particularly in younger patients. The identification of novel biomarkers for OC/EC detection is an expanding field yet, to this date, no biomarker panel for either malignancy offers sufficient sensitivity and specificity for clinical application in diagnosis, staging, or treatment monitoring. Recent studies indicate that human epididymis protein (HE)4 is highly expressed in OC and EC tissues, and increased serum levels of HE4 are detected in these

patients' serum. Serum expression of HE4 in non-gynecologic cancers has not been reported. Several studies indicate that in combination with CA-125, HE4, with its differentiation power for endometriosis, can be a promising marker for OC/EC diagnosis and/or stratification. Here we provide a comprehensive review on the HE4 structure, expression pattern and potential value as a biomarker for endometrial or ovarian carcinoma. While HE4 is our primary focus, progress in the development and assessment of other OC/EC biomarkers is also discussed.

Etiological & morphological similarity of ovarian & endometrial malignancies

Both ovarian and endometrial tissues develop from the Müllerian system [10–12] and are subject to tight control by reproductive hormones. As such, cancers arising from the two tissues share similarities in etiological factors, gene expression profiles, tumorigenic mechanisms, pathological changes and metastatic characteristics. For example, cyclin E and L1 adhesion molecule were independently found to be overexpressed in Müllerian-derived carcinomas [13,14]. Further, histological analysis of different cytological subtypes of EC and OC indicate a parallel spectrum of morphological changes [15,16]. Zorn and colleagues' analysis of gene expression profiles between subtypes of the two malignancies reveals a related expression profile in the clear-cell subtype across different organs. On the other hand, endometrioid and serous subtypes displayed expression profiles that were, for the most part, unique to their organ of origin. The authors were able to identify nine genes common to both diseases that were capable of distinguishing among the various histotypes [16]. Moreover, comparisons between uterine and ovarian carcinosarcomas, rare but characteristically aggressive gynecologic tumors, reveal no differences in patient demographics or overall survival for women presenting with these malignancies, perhaps lending justification for the use of similar therapeutic strategies in combating these diseases [17]. In addition, EC and OC share many of the same risk factors, including, nulliparity and frequency of ovulation [10,12]. The use of oral contraceptive hormones has been shown to reduce the risk of developing either condition [10]. In view of these parallels between EC and OC, it should be no surprise that a single marker or marker panel could be capable of recognizing both conditions. CA-125 has been found to be overexpressed in both OC and EC tissues, and increased CA-125 serum levels have been evaluated as a biomarker for the detection and monitoring of both malignancies. However, the unsatisfactory sensitivity and specificity of the CA-125 assay has prompted intensive efforts in the search for superior biomarkers [18–20].

Current biomarkers for ovarian & endometrial carcinomas

Cancer antigen 125 was first identified in 1981 by Bast *et al.* as a protein that is elevated in the serum of more than 80% of women diagnosed with epithelial ovarian cancer [20–22]. CA-125, a transmembrane protein with a large, glycosylated extracellular domain, resides on the luminal-cell interface and is actively secreted into the lumen where it is detected in the serum. Although it is currently the only serum tumor marker routinely used for detection of epithelial OC [19,20], CA-125 is expressed in a variety of coelomic-derived epithelial cells, including pleura, pericardium, peritoneum and Müllerian epithelia [21,23]. Its success as a molecular marker for OC detection stems from its low expression in normal ovarian epithelium coupled with a significant upregulation in serous and papillary tumors [21,24]. Moreover, the fact that CA-125 is luminaly secreted allows for relatively easy, noninvasive detection in a patient's serum.

In addition to its application for OC detection, several studies have suggested that CA-125 also has a promising potential as a prognostic marker. After total surgical removal of ovarian

tumors, the CA-125 half-life is approximately 6 days, and results from a number of longitudinal studies indicated that women with a CA-125 half-life of greater than 20 days have a high potential for recurrence and a poor prognosis. Moreover, the initial level of CA-125 prior to therapeutic intervention has been shown to have some prognostic significance [19,25–30]. In fact, evidence shows that the serum half-life of CA-125 is an independent prognostic indicator for survival, rate of progression, time to progression, and the potential for total remission. A relatively high false-positivity (up to 20%) associated with this method has limited its usefulness for the assessment of individual patients [19,25,31,32]. Nevertheless, it is suggested that this system could serve as an effective surrogate for evaluating new therapeutic agents in clinical trials, thus serving an obvious benefit to both patients and clinicians [19].

The WHO and the Response Evaluation Criteria in Solid Tumor (RECIST) group have identified predictors to evaluate progression-free survival in clinical trials; however, the criteria neglects the use of CA-125 measurements in their definition of progression [21]. The Gynecologic Cancer Intergroup proposed a definition of disease progression that features both CA-125 concentrations and RECIST criteria [21,33]. For patients whose CA-125 levels have returned to normal following treatment, progression is defined as a twofold or more increase of CA-125 over the upper limit of normal on separate occasions at least 1 week apart. For those patients with elevated pretreatment CA-125 levels that fail to normalize, twofold or more increase of CA-125 over the nadir level is the criteria for progression. This definition was used retrospectively to compare the date of disease progression determined by CA-125 levels versus that determined by clinical and radiological criteria. Based on data from a trial of cisplatin and paclitaxel versus cisplatin and cyclophosphamide [34,35], the average time to determine progression was 54 days earlier when both criteria were employed, and the date of progression based on CA-125 measurement was up to 650 days earlier than the clinical diagnosis of progression [21,34,35].

Despite the many promising characteristics of CA-125 for OC diagnosis and monitoring, there are also several drawbacks. For example, in addition to marked overexpression in ovarian lesions, serum CA-125 is frequently elevated in association with irritation of the peritoneum and mesothelium caused by benign conditions such as menstruation, pregnancy and the postpartum state [21,36]. Likewise, elevated serum CA-125 concentrations are often seen in other diseases, such as liver disease (i.e., cirrhosis), congestive heart failure and primary liver cancer, especially if ascites is present. Furthermore, women with nonmalignant gynecological conditions, including benign ovarian cysts and endometriosis, tend to have serum CA-125 concentrations above the upper limit of normal, which is arbitrarily set at 35 U/ml. As a result, false-positives and low specificities are major concerns in regard to the use of CA-125 as an OC marker [19–21,37]. The low prevalence of OC (30–50 cases/100,000 women) further limits the achievable positive and negative predictive values of a population-wide, CA-125 screening test [38]. In fact, many specialists advise against CA-125 monitoring altogether because of the anxiety and stress it affords patients. Similarly, physicians struggle with the decision to start chemotherapy on asymptomatic patients with rising CA-125 levels. Consequently, several studies and clinical trials are currently underway to develop a more reliable detection system, many of which employ a panel of two or more complementary markers.

Considerably, fewer studies have been performed to evaluate the efficacy of CA-125 for EC detection and/or monitoring. This is partially due to the fact that timely EC detection (relative to OC) is often realized by virtue of early presentation of symptoms, such as vaginal bleeding. Nevertheless, EC's high incidence rate, together with the poor prognosis for advanced-stage patients justifies the need to identify diagnostic and prognostic biomarkers for EC. Although CA-125 is elevated in EC patients relative to healthy control

subjects, this upregulation is slightly below the clinically defined cut-off point for OC diagnosis [39,40]. Moreover, serum concentrations of CA-125 are elevated in only 10–20% of women with early-stage EC, and only 25% of asymptomatic patients with recurrences will present with elevated CA-125 levels [18,41,42]. Thus, CA-125's application for EC detection is essentially restricted to advanced-stage diagnosis. Yurkovetsky and colleagues highlighted this idea in a recent publication confirming the presence of considerably elevated serum cancer antigen levels (CA-125, CA15–3 and carcinoembryonic antigen) in stage III EC patients compared with women with stage I disease. The authors suggested that this may be caused by the ‘shedding’ of cancer antigens during EC progression and/or disease aggressiveness, thereby depositing increasing amounts of these proteins into the lymphatic system as the malignancy advances in stage. Conversely, that same tumor aggressiveness and potential for metastasis may actually drive the upregulation of cancer antigens [40]. Of course, these are not mutually exclusive mechanisms as both can be at play simultaneously. Regardless of the culprit, the fact remains that no accurate biomarkers for EC detection are currently available.

Recently, Yurkovetsky's group showed evidence of EC screening potential for prolactin, a single-chain peptide from the growth hormone family. The primary source of prolactin is the anterior pituitary gland, yet endometrial stroma also produce the protein during the secretory phase of the menstrual cycle. Studies have shown that prolactin's function is not limited to the regulation of breast development and lactation. Prolactin also acts as a cytokine with central roles in the immune and inflammatory processes [40,43,44]. Additionally, prolactin is able to serve as a paracrine/autocrine hormone, thereby influencing local angiogenic responses [40,45,46]. Given that blood vessel growth and remodeling is a key factor in cancer metastasis and lymphvascular invasion, prolactin could influence advancement of EC and other malignancies [40]. Accordingly, prolactin may not only serve as a marker for early detection of EC, but could also allow for reliable risk assessment for metastatic potential and/or probability for lymph node invasion. Early studies assessing prolactin's efficacy as an EC marker indicated its effectiveness in identifying recurrent diseases [40,47]. Recent data from Yurkovetsky and colleagues has also suggested that prolactin's diagnostic power in discriminating EC from healthy controls is superior to all other biomarkers examined to date. In their study involving 115 EC patients and 135 healthy control females, prolactin serum-marker assays were able to identify EC with a sensitivity of 98.3% and a specificity of 98%. No significant advantage was realized upon addition of other markers to the prolactin assay. Thus, prolactin measurement is sufficient to discriminate patients with cancer from healthy women. Since elevated levels of prolactin were detected in other cancers, including malignancies of the ovary, pancreas and lung, the authors concluded that the prolactin assay alone is not able to discern between the various cancer types and, consequently, cannot be used alone in the diagnosis of EC. The authors subsequently identified a panel of five markers, which included prolactin, eotaxin, growth hormone, E-selectin and thyroid-stimulating hormone. This combination has shown specificity for EC over OC and breast cancer, but it remains to be tested if it is effective in distinguishing EC from benign gynecologic diseases, such as endometriosis [40]. Future studies of prolactin's use as a biomarker for EC will need to address this issue prior to clinical trials.

Besides CA-125 and prolactin, many potential markers have been investigated in the past decade. A comprehensive list of putative biomarkers for ovarian and ECs is provided in Table 1. Crucial information on their specificity and sensitivity is also compiled. Owing to their in-depth coverage within the body of this review, HE4 and CA-125 have been excluded from the table.

Discovery of HE4

HE4 (*WFDC2*) was first identified and characterized by Kirchhoff *et al.* upon differential cDNA screening of human epididymal tissue [48,49]. Subsequent studies revealed the expression of HE4 in a number of tissues outside of the male reproductive system. Using northern hybridization, Bingle *et al.* detected HE4 mRNA expression in lung, kidney and salivary gland [50]. Galgano *et al.* analyzed HE4 expression patterns in normal and malignant human tissues using a cDNA microarray. HE4 is expressed in relatively high levels in the trachea and salivary gland [51]. Applying quantitative realtime PCR, our laboratory has detected high levels of HE4 mRNA in the epididymis, trachea and lung, and intermediate levels in prostate, endometrium and breast. Little or no HE4 expression was detected in the colon, ovary, liver, placenta, peripheral blood cells and skeletal muscles [Jiang SW, Unpublished Data].

The deduced amino acid sequence of HE4 predicts a significantly large and odd-numbered cysteinyl content, suggesting that HE4 may participate in protein–protein interactions [48,49]. Further analysis showed that HE4 contains two whey acidic protein (WAP) domains. This conserved domain is common to whey proteins in the milk of several mammals and is characterized by a four-disulphide core arrangement of 50 amino acids, including eight cysteines. Generally, WAP domain-containing proteins are small, secretory molecules. Since the WAP domain proteins carry serine protease inhibitory activities and are secreted by pro-inflammatory cells, these proteins were thought to play a part in the natural defense against microorganisms [52]. Later *in vitro* studies using cloned WAP cDNA indicated varied functions that often include effects on cell growth and differentiation [50,53,54].

HE4 is one of several WAP proteins that are localized on human chromosome 20q12–13.1 [55]. Interestingly, results from several comparative genomic hybridization assays suggested that the 20q13 locus frequently exhibits chromosomal gains in various cancer types, including malignancies of the oral cavity, breast, ovary, colon, pancreas, stomach and uterus [56–59]. Indeed, this locus harbors several WAP proteins, including elafin and secretory leucocyte proteinase inhibitor (SLPI), that have been identified as candidate biomarkers for a number of cancers [60]. Although a significant proportion of those proteins containing WAP domains possess protease inhibitor function (e.g., SLPI and elafin), no such protease inhibitor activity has been assigned to HE4. While the high expression levels in epididymis suggest that HE4 may be involved in male fertility, the physiological role of HE4 has yet to be determined [61,62].

HE4 as a marker for ovarian cancer

Ovarian cancer is a relatively manageable malignancy when diagnosed at an early stage, but late-stage detection almost always translates into a poor prognosis. Researchers have been vigorously working on the identification of a more reliable biomarker to assist in early detection, as well as treatment- and general disease-monitoring. HE4 is among the most frequently upregulated genes in epithelial ovarian carcinomas based on gene expression profiles [63–69]. As described later, several groups have pioneered the task in determining HE4's efficacy as a molecular marker for OC.

In order to measure the serum concentration of HE4 and subsequently determine if it could be utilized as a molecular marker for OC, an HE4 detection method was required. Hellstrom and colleagues constructed a gene encoding HE4 fused with mouse or human Ig Fc domains. Mice were then immunized with the mouse-derived Ig Fc–HE4 fusion protein. The resultant hybridomas were screened against the human-derived Ig Fc–HE4 fusion protein, upon which two monoclonal antibodies were generated that recognized different HE4 epitopes.

The monoclonal antibodies were then employed in the construction of a double-determinant ('sandwich') ELISA, which has been successfully used for the serum detection of HE4 [62,70,71]. However, large-scale screening assays for evaluating biomarkers with immunoassays face daunting challenges, such as quantity requirements for patient sample, cost of affinity reagents, and the amount of work required in preparing and employing those reagents. In light of these short-comings, Scholler *et al.* sought to develop a new type of antibody that might alleviate the challenges. The authors developed the 'biobodies' (Bbs) from diploid yeast transformed with recombinant plasmid DNA such that, upon secretion, the Bbs would be 'bio'-tinylated. As a consequence of this elegant *in vivo* biotinylation mechanism, chemical biotinylation is avoided and the detection role of recombinant antibodies through directed biotinylation is maintained. The researchers developed Bbs for HE4 and mesothelin, coupled them with polyclonal antibodies, and tested their accuracy in OC detection. They compared the results to those obtained using the standard CA-125 double-determinant 'sandwich' ELISA and demonstrated that detection using the Bbs produced similar accuracy as the previous assay, yet required considerably less serum and was significantly more cost effective. The authors found that HE4 and mesothelin are more effective in the detection of serous ovarian malignancies than those of endometrioid, clear cell or mucinous subtypes. Given that serous cell OC is the most common form of OC and the least likely to be diagnosed when restricted to the ovary, markers like HE4 and mesothelin bear strong implications for the improvement of OC management [72].

Several publications have demonstrated HE4's superiority over CA-125 as an OC biomarker. Specifically, HE4's ability to distinguish benign diseases from malignancies (i.e., its sensitivity) affords it with an advantage over CA-125 alone in OC detection [18,38,62,70,71]. Indeed, the use of CA-125 for detection of OC in premenopausal women is associated with a sensitivity and specificity so low that it is almost exclusively reserved for application in postmenopausal cases [18]. Moore *et al.* succeeded in validating HE4's complementary effect on CA-125's ability to detect OC upon assessing the efficacy of various, putative OC biomarkers, alone or in combination. Preoperative serum and urine samples collected from multiple institutes were screened to determine the levels of CA-125, soluble mesothelin-related peptide, HE4, CA72–4, activin, inhibin, osteopontin, EGF receptor, and human epidermal growth factor receptor 2 (*HER2* oncogene) in 259 patients with adnexal masses, of which 233 were eligible for analysis (67 invasive epithelial OCs and 166 benign ovarian neoplasms). As a single marker, HE4 had the highest sensitivity of 72.9% when specificity was set at 95%. The combination of CA-125 with HE4 achieved the highest sensitivity compared with all other single markers or dual-marker combinations, and the addition of other markers to the CA-125 plus HE4 panel only imposed a modest improvement in sensitivity for OC detection [18]. Havrilesky and colleagues obtained similar results in an independent study of another group of OC biomarkers, which included HE4, glycodelin, matrix metalloproteinase (MMP)7, SLPI, plasminogen activator, urokinase receptor (Plau-R), mucin (MUC)1, inhibin A, plasminogen activator inhibitor (PAI-1), and CA-125. The predictive value of single marker and multiple marker panel were assessed using two different cut-off points determined by receiver operating characteristic (ROC) curves, one being the best cut-off, as determined by the highest sensitivity plus specificity value, and the other being determined by using the upper limit of twice the standard deviation the study's reference cohort in accordance with the Clinical and Laboratory Standards Institute. The authors determined that HE4 displays the highest sensitivity among all other single markers in the detection of both early- (62.4–82.7%) and late-stage (74.6–92.5%) OC, regardless of which cut-off was used [38]. The researchers concurrently carried out a pilot study assessing the effectiveness of a particular biomarker panel (HE4 plus MMP7 plus glycodelin) for monitoring OC. This panel predicted recurrence prior to CA-125 elevation in 56% of cases.

Huhtinen and colleagues recently analyzed serum concentrations of HE4 and CA-125 in 225 women with OC, EC, endometriosis or healthy controls. The combination of HE4 plus CA-125 achieved a much improved sensitivity of 92.9% at 95% specificity compared with either HE4 (78.6% sensitivity) or CA-125 (78.6% sensitivity) alone. HE4 levels were elevated in both endometrial and ovarian malignancies, but not in endometriotic lesions. These results thus provide additional evidence for HE4's complementary association with CA-125 in the detection of OC. It is noteworthy that this study demonstrated unique abilities of HE4 and CA-125 in distinguishing patients with ovarian malignancy from those with benign ovarian or endometrial conditions. Increased CA-125 without HE4 elevation is specifically indicative of advanced endometriosis or ovarian endometrioma. On the other hand, elevated HE4 with normal CA-125 may suggest the presence of either ovarian or other types of cancers, including EC [73]. Dong *et al.* determined that HE4 has an advantage over CA-125 in ROC-area under the curve (AUC) and sensitivity with 100% specificity when ovarian cancer was compared with healthy controls or women with benign diseases. Conversely, the CA-125 assay had the advantage in ROC-AUC and sensitivity with 100% specificity when ovarian cancer was compared only with healthy controls [74]. This is attributable to the drastic difference (relative to HE4) in CA-125's expression in ovarian malignancies versus healthy women. Even in this situation, the HE4 plus CA-125 combination assay was still superior to CA-125 alone when OC was compared with different control groups [74].

A noninvasive means of discriminating between malignant pelvic masses and benign lesions is important given that approximately 20% of women will develop an ovarian cyst or pelvic mass at some point in their lives. Without adequate means of discerning the malignant potential of these tumors, a considerable proportion of these women will have unnecessary surgery. Likewise, since CA-125 overexpression is undetectable in as many as 50% of early-stage ovarian malignancies, if such criterion is used to determine malignant status, many women who do need surgery may fail to receive adequate treatment in an acceptable time frame. A key concern that Moore and colleagues point to in a recent editorial is that no currently employed imaging technique or biomarker assay, by itself, is able to distinguish OC from benign ovarian diseases with an acceptable sensitivity and specificity [2]. MRI, PET and CT scans are useful for classifying women into low- to high-risk strata, but these examinations are far too costly to be employed for all women with an ovarian cyst or pelvic mass. Considering the radiation exposure and/or invasiveness, administration of these examinations to the asymptomatic population is difficult to justify. Since urine/serum-based assays are noninvasive and relatively inexpensive, it would be ideal to have a biomarker or multimarker panel with sufficient sensitivity and specificity to serve as an OC risk stratification tool. Sophisticated imaging techniques could then be employed in the moderate- to high-risk patients triaged for care in centers specializing in the treatment of ovarian carcinoma [2]. Recently, Fujirebio Diagnostics, Inc. developed such a tool that significantly improves differential diagnosis of pelvic masses. The kit uses an HE4 plus CA-125 combination assay, coupled with a risk of ovarian malignancy algorithm calculation, to categorize women presenting with a pelvic mass into groups based on malignant potential. The stratification kit correctly identified 91% of patients with epithelial ovarian cancer as high-risk for malignancy, while 75% of women with benign pelvic masses were appropriately assigned to the low-risk group [2,18,75]. This represents a significant improvement compared with the sensitivity and specificity achievable by the use of CA-125 alone. This commercially available kit has recently received regulatory clearance by the US FDA to be used for OC detection and monitoring in the USA [102].

Although HE4 has consistently shown promise as a complement to CA-125 and other biomarkers for OC detection/diagnosis, improvements need to be made before an effective population-wide screening test can be realized. Given the OC prevalence of only one in

2500 postmenopausal women in the USA, an acceptable screening assay would require a sensitivity of 75% and a specificity of around 99.7% to obtain the minimally tolerable positive predictive value of 10% for the detection of OC at all stages. No single marker to date has reached this benchmark, and even the best multi-marker panels have only been able to approach the threshold. It may be reasonable to employ a less than optimally specific biomarker panel in defined higher risk groups to increase the positive predictive value. Coupling a suboptimal marker to techniques, such as transvaginal sonography, although more invasive and expensive, should increase the overall positive predictive value [38].

HE4 application for endometrial cancer

Similar to OC, endometrial malignancies tend to carry favorable prognoses when early detection is realized. Diagnosis at a later stage, however, typically coincides with a poor prognosis and a relatively low 5-year survival rate. Fortunately, the presentation of signs and symptoms early on in the course of the malignancy, including postmenopausal bleeding, often precludes late diagnosis beyond the first stage. As a result, approximately 70% of ECs are diagnosed as stage I malignancies. Even so, the remaining 30%, along with certain high-risk groups, such as patients with human nonpolyposis colorectal cancer syndrome, phosphate and tensin homolog gene defects (Cowden syndrome), obesity, diabetes or breast cancer patients on tamoxifen, would certainly benefit from the advent of a reliable serum-marker panel to aid early diagnosis. Noninvasive means for detecting EC may improve detection, aid in the diagnosis of recurrence, and help monitor response to therapy. Some patients with stage I disease will carry intermediate- to high-risk factors such as depth of invasion and lymphovascular involvement, and may benefit from a reliable means of disease monitoring. Furthermore, an accurate biomarker for EC might provide preoperative prognostic value to help guide the extent of surgical staging, thus contributing to the goal of improving overall patient care [76]. Presently, recurrent EC is detected through presentation of clinical symptoms and imaging techniques, which generally only leads to advanced-stage detection. The use of CA-125 assays for recurrent EC detection is largely limited to later-stage detection since only 10–20% of women with stage I EC and 25% of those with asymptomatic recurrences will have elevated serum CA-125 levels [41,42,76–81]. Taken together, these data suggest that a more reliable EC biomarker is needed.

Only a handful of research groups have begun investigating HE4's efficacy as a serum marker for endometrial malignancies. Data from these studies indicate a promising value for HE4 as a component of the biomarker panel in EC detection. Moore and colleagues measured preoperative serum samples from patients with endometrioid adenocarcinoma [76], along with healthy postmenopausal women as controls, for levels of HE4, soluble mesothelin-related peptide, CA72-4 and CA-125. The results suggested that, as a single marker for EC, HE4 was the most accurate of the group regardless of stage. The single marker ROC-AUC values for HE4 were higher than all other markers investigated for stage I, stages II–IV and all stages combined (ROC-AUC: 76.7, 83.6 and 78.7%, respectively). The sensitivity of the HE4 assay was also highest of all other single markers regardless of stage (sensitivity at 90% specificity for stage I, stages II–IV and all stages combined: 48.4, 71.4 and 55.0%, respectively). The addition of CA-125 to the HE4 assay considerably increased the sensitivity compared with that achievable by CA-125 alone (50.1 vs 24.6% at 95% specificity, respectively). The dual-marker combination assay of CA-125 plus HE4 was also advantageous according to ROC-AUC analysis compared with HE4 alone for stages II–IV (ROC-AUC: 86.6 vs 83.6%, respectively), but had no such advantage over HE4 alone in the analysis of stage I malignancies (ROC-AUC values for HE4 alone were identical to those for CA-125 plus HE4; 76.7%) or when all stages were combined (ROC-AUC for HE4 alone vs HE4 plus CA-125: 78.7 and 79.4%, respectively). These data suggest that HE4 is the most accurate and sensitive EC marker identified to date, and as such will likely prove

beneficial alone or as a component of a biomarker panel for the detection of EC. Moreover, CA-125 is frequently used for monitoring response to treatment in EC patients, and HE4's considerable advantage over the others (including CA-125) in identifying stage I endometrioid adenocarcinomas indicates its potential prognostic value in the earlier detection of EC recurrence.

In their analysis of serum HE4 and CA-125 levels in women with EC, OC, endometriosis and disease-free controls, Huhtinen *et al.* also reveal data suggestive of a role of HE4 in both OC and EC detection. HE4 mean serum levels were considerably elevated in both ovarian (1125.4 pM; $p < 0.001$) and endometrial (99.2 pM; $p < 0.001$) malignancies, but not in ovarian endometriotic cysts (46.0 pM) or other forms of endometriosis (40.5 pM), compared with the healthy control group. Serum CA-125 levels, on the other hand, were significantly elevated in OC (1117.1 U/ml; $p < 0.001$), aggressive endometriotic lesions with deep myometrial/peritoneal invasion (40.8 U/ml), and ovarian endometriotic cysts (44.3 U/ml), but not in the EC group (22.0 U/ml; $p = 0.029$), compared with healthy controls (8.9 U/ml) [73]. This study further corroborates HE4's superiority over CA-125 in discriminating between malignancies and benign diseases.

Expert commentary

New technologies for diagnosis of EC and OC in early stages are urgently needed. This need is particularly apparent for women with ovarian cancer as the majority are diagnosed in advanced stages with low rates of cure. Many approaches have been tested, so far without success. Most recently, Petricoin *et al.* reported on the use of proteomic spectra generated by mass spectroscopy that completely discriminated serum from patients with and without ovarian cancer. However, that technique was ultimately discredited and now, 7 years later, we are still without an effective screen for ovarian cancer. Until now, CA-125 has been the most effective serum marker for ovarian cancer. In this review we discuss findings showing that HE4 may correlate even better than CA-125 with the presence of ovarian and endometrial cancer. Ultimately, a panel of markers, including CA-125 and HE4 may prove clinically useful. The most difficult challenge will be to identify the most effective markers to be used in that panel. This challenge is even more problematic with ovarian cancer, as the heterogeneity of gene expression between different tumors appears to be much more pronounced than in cancers of other organ systems.

Five-year view

Although an inexpensive, sensitive and specific serum test would be the most attractive approach to screen women for ovarian and endometrial cancer, fundamental limitations of this method must be recognized. Measurement of CA-125 is very useful in the clinic setting for detecting recurrence in patients who have a known diagnosis of ovarian cancer in the past, and who displayed elevated CA-125 levels at diagnosis. In fact, over 95% of CA-125 elevations in this patient population are due to recurrent cancer. This is because of the high incidence of recurrence in this population. On the other hand, CA-125 is a very poor test to screen for ovarian cancer in the general population because of the low incidence. A screening test for ovarian cancer must have a very high specificity, over 99.9%, in order to be useful clinically and avoid unnecessary surgery in large numbers of patients. One of the strengths of HE4 is that it appears to correctly identify benign lesions in comparison to CA-125. But owing to the extreme heterogeneity of genetic abnormalities observed in ovarian cancer, such a screening test would require the use of multiple markers, of which CA-125 and HE4 may be a component. To achieve high specificity other modalities are likely to be required to be used in combination with serum markers, such as ultrasound and high-resolution MRI. Furthermore, patients at risk must be identified to improve the utility

of such a test. For example, morbidly obese women have a risk of endometrial cancer that is ten times the risk of women of normal weight. Thus, a screen for endometrial cancer is likely to be most useful and most cost effective in obese women. Future efforts to diagnose ovarian and endometrial cancer in early stages will be dependent not only on improving screening methods, but also on continued epidemiologic investigation. The promise of effective, innovative and safe treatments for advanced endometrial and ovarian cancer has been slow to be realized over the past decade. With continued persistence and cooperation between disciplines, early diagnosis in targeted populations at high risk may become a reality and significantly reduce the impact of these diseases in women.

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Key Issues

- When diagnosed at an early stage, ovarian cancer and endometrial carcinoma patients tend to have good prognoses. Late-stage detection of either malignancy, however, typically translates into a poor prognosis. An accurate biomarker for early detection of these diseases is, thus, urgently needed.
- Cancer antigen (CA)-125 is elevated in ovarian and endometrial cancers as well as in benign conditions such as endometriosis. The diagnostic value of CA-125 is compromised by its high false-positive rate. New biomarkers with improved specificity are required.
- Multiple putative markers for ovarian and endometrial cancer been discovered. Only a handful have been tested in a sufficient number of patients. One protein, the human epididymis protein (HE4), has shown a strong potential for clinical application.
- HE4 is a small circulating peptide and a sensitive detection kit has been developed.
- Combining the use of HE4 with CA-125 has improved diagnostic specificity by excluding some benign conditions.
- HE4 can be used for stratification of patients with a pelvic mass. Proper triage of patients using HE4 may control costs associated with evaluation of pelvic masses.
- In the future, HE4 may be a component of a serum screening test for ovarian or endometrial cancer.

Table 1

Putative biomarkers for ovarian and endometrial carcinomas.

Name	Marker type	Malignancy	Site	Sample size	Application/indication	Sensitivity/specificity	Additional remarks	Ref.
Cathepsin B	Protein	EC	Paraffin-embedded tissue	64 EC tissue; 12 normal	Prognosis/tumor aggressiveness and survival prediction	N/A	Cathepsin-B levels determined by immunostaining	[82]
IL-6	Protein	EC	Serum	20 healthy women; 19 benign disease; 19 EC; 13USPC	Diagnosis/differentiating marker for USPC	N/A	IL-6 slightly elevated in EC; significantly elevated in USPC	[83]
YKL-40	Protein	EOC	Serum	19 borderline tumors; 76 EOC	Treatment monitoring/predictor of survival and chemoresistance to second-line treatment	N/A	Elevated in 65% stage I and II EOC;74-91% stage III and IV	[84-86]
Glycan markers	Oligosaccharide	EOC	Serum	48 EOC; 24 healthy controls	Diagnosis/potential screening tool	91.6/95.8%	N/A	[87]
ERCC5	Gene	EOC	N/A	LOH analyzed in 52 samples; mRNA measured in 90 tumors	Treatment monitoring/indicator of resistance to platinum chemotherapy	N/A	ERCC5 codes for XPG protein, a key factor in the nucleotide excision repair pathway	[88]
c0* and rc1‡ of CA-125, KLK5 and KLK7	Protein panel	EOC	Serum	98 EOC (14 Stage I, 5 II, 73 III and 6 IV)	Treatment monitoring/indicator of response to chemotherapy	AUC = 0.82	N/A	[89]
c0* of KLK7, KL10, B7-H4 and spondin2	Protein panel	EOC	Serum	98 EOC (14 Stage I, 5 II, 73 III and 6 IV)	Post-surgical monitoring/predictor of short-term (1-year) survival	AUC = 0.89	N/A	[89]
Leptin, prolactin, osteopontin, IGF II, macrophage inhibitory factor and CA-125	Protein panel	EOC	Serum	156 EOC; 362 healthy controls	Diagnosis/potential screening tool	84-98/95%	Three different platforms tested, two sample cohorts employed	[90]
PRL	Protein	EC	Serum	115 EC; 135 healthy controls	Diagnosis/potential screening tool	98.3/98.0%	Stage l-lll patients investigated	[91]
hK6	Protein	EC	Serum	22 healthy women; 20 benign disease; 20 EC; 17USPC	Diagnosis/differentiating marker for USPC	N/A	hK6 serum levels comparable in benign, healthy and EC, but elevated in USPC	[92]

Name	Marker type	Malignancy	Site	Sample size	Application/indication	Sensitivity/specificity	Additional remarks	Ref.
UPAR	Protein	EC	Paraffin-embedded tissue	58 subjects with EC	Prognosis/marker for aggressive EC	N/A	UPAR levels determined by immunostaining	[93]
Bcl-2	Protein	EOC	Urine	77 healthy women; 161 benign disease; 150EOC	Diagnosis/distinguishing EOC from healthy subjects and benign gynecologic disease	N/A	Bcl-2 levels examined by ELISA in subject urine samples	[94]
CK-20	mRNA	EC	EC tissue	20 EC; 5 healthy controls	Prognosis/detection of micrometastasis in lymph nodes	100/76%	CK-20 expression levels determined by RT-PCR	[95]

*c0 refers to the marker level prior to treatment; c1 is the level after the first treatment.

‡rc1 is the log(c0/c1), used for comparing pre- and post-treatment levels.

AUC: Area under the curve; Bcl-2: B-cell lymphoma 2; CK: Cytokeratin; EC: Endometrial carcinoma; EOC: Epithelial ovarian cancer; ERCC5: Excision repair cross-complementing rodent repair deficiency, complementation group 5; hK6: Human kallikrein-6; LOH: Loss of heterozygosity; N/A: Not applicable; PRL: Prolactin; RT: Real time; UPAR: Urokinase plasminogen activator receptor; USPC: Uterine serous papillary carcinoma; XPG: Xeroderma pigmentosum, complementation group G; YKL-40: Chitinase-3-like-1.