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# HE4 Related Publications



**1** Hellström I, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. *Cancer Res.* 2003; 63: 1999-2003.

Hellström et al investigated the WFDC2 gene which is present in epithelial cells of human epididymis and is an epididymis-specific, fertility related protein, also known as HE4. Two monoclonal antibodies (Mabs) were derived, 2H5 and 3D8 that recognize different HE4 epitopes. Monoclonal antibodies 2H5 and 3D8 were used to develop an ELISA assay to measure HE4 in postmenopausal women. The HE4 ELISA was run on 37 ovarian cancer patients, 65 healthy controls and 19 patients with benign ovarian disease; in addition the levels of CA125 were measured in all patients as well for comparison. Neither marker was able to identify all cases of ovarian cancer with HE4 missing 7 cases and CA125 missing 8. Analysis of the data indicates that HE4 and CA125 have comparable sensitivity and specificities; however HE4 is a better marker in the detection of early cases of ovarian cancer when compared with benign ovarian disease.

	Sensitivity at 96 % Specificity	
	HE4	CA125
Early stage vs Normal	86%	71%
Late stage vs Normal	80%	80%
All cases vs Benign	67%	40%

The authors conclude that based on their analysis HE4 has a better ability to discriminate cancer from benign disease based on it's specificity in this patient population.

**2** Moore R, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. *Gynecol Oncol.* 2008; 108: 402-408.

Moore and colleagues investigated a series of 9 novel biomarkers alone and in combination in patients with ovarian cancer. The objective of this study was to find biomarkers that were complementary to CA125 for use in a multiple marker panel in the triage of patients with a pelvic mass to appropriate centers for surgery. 233 patients all of which underwent pelvic mass surgery were evaluated with the following markers: CA125, HE4, Soluble Mesothelin Related Peptide (SMRP, MESOMARK™), CA72-4, activin A, inhibin, osteopontin, epidermal growth factor (EGFR), and serum HER-2/neu. 67 patients were diagnosed with invasive

epithelial ovarian cancer and 166 with a benign condition of the ovary. Only SMRP and HE4 resulted in sensitivity higher than that of CA125 at 95% specificity (43.3% sensitivity). The combination of HE4 and CA125 resulted in the highest sensitivity over any other single marker or dual combination. See the chart below for representative sensitivity and specificity values from the study.

Biomarker	Sensitivity at 95 % Specificity
CA125	43.3%
HE4	72.9%
SMRP	53.7%
CA125+HE4	76.4%
CA125+SMRP	56.8%
CA125+HE4+SMRP	74.7%

No other single marker or combination of markers significantly increased the sensitivity more than CA125 and HE4. In a comparison of Stage I cancer to benign disease, HE4 alone had the highest sensitivity at 45.9% at 95% specificity. This study has demonstrated that HE4 alone and in combination with CA125 has a greater sensitivity at a set specificity. In addition, HE4 was shown to complement the CA125 test in patients with both ovarian cancer and benign conditions. The authors conclude that this combination of markers may be useful in the triage of women as a part of an algorithm to ensure they are treated at the appropriate center for care.

**3** Moore R, et al. Utility of a Novel Serum Tumor biomarker HE4 in Patients with Endometrioid Adenocarcinoma of the Uterus. *Gyn. Onc.* 2008; 110(2): 196-201.

There is a need for sensitive tumor markers as an aid in screening women at high risk for endometrial cancer as well as those who are at risk for recurrence of disease following diagnosis. Researchers investigated 4 biomarkers (CA125, HE4, CA72-4, SMRP) alone and in combination in patients with endometrioid tumors to evaluate the ability of biomarkers to identify women with cancer. 171 patients with endometrial cancer and 156 healthy subjects were obtained for the analysis of tumor markers. Of the patients with endometrial cancer 122 had stage I disease, 17 had stage II, 26 with stage III and 6 with stage IV. Of the markers that were evaluated CA125 and HE4 were significantly elevated compared to the healthy controls in all stages and stage I disease, neither CA72-4 nor SMRP were statistically elevated compared to the control group.



This study illustrated the performance of HE4 in endometrial cancer as the best in all stages of disease when compared to CA125, CA72-4 and SMRP. A combination of CA125 and HE4 raised the sensitivity compared to CA125 alone and HE4 in patients with Stage II-IV disease. HE4 alone or in combination with CA125 appears to be an excellent marker and may be an effective tool for the early detection of recurrence or monitoring the response to therapy.

**Sensitivities for individual and combined tumor marker assays at 90% Specificity**

Marker combination	All stages	Stage I	Stage II-IV
CA125	34.6%	30.0%	46.7%
HE4	55.0%	48.4%	71.4%
CA72-4	10.2%	7.6%	17.6%
SMRP	11.3%	14.4%	16.6%
CA125+HE4	57.3%	51.6%	73.0%

**4 Moore R, et al. A Novel Multiple Marker Bioassay Utilizing HE4 and CA125 for the Prediction of Ovarian Cancer in Patients with a Pelvic Mass. *Gyn Onc.* 2009; 112(1): 40-46.**

This publication reports on a prospective, multi-center trial to validate a predictive model based on the combination of CA125 + HE4 to assess the risk for Epithelial Ovarian Cancer (EOC) in women with a pelvic mass. Patients were enrolled if they were scheduled to undergo surgery for the removal of an ovarian mass; those diagnosed with ovarian cancer were staged according to protocol. In all, 12 sites enrolled a total of 531 evaluable patients. 352 women were diagnosed with benign disease and 179 women were diagnosed with a malignancy. Each patient was classified into low or high risk of malignancy using their menopausal status, CA125 value and HE4 value in a predictive probability algorithm. High risk patients were classified based on a predictive probability of >13.1% for premenopausal women and >27.7% for postmenopausal women. The median serum levels for CA125 in patients with benign disease were 20.5 IU/mL and in cancer cases was 210.9 IU/mL. Median serum levels for HE4 in benign cases was 58.6 pM and in cancer cases was 274.4 pM. The results of the analysis of this patient group using the combination of HE4 and CA125 are listed below.

**Distribution of patients into low and high risk groups: Benign disease vs EOC and Low Malignant Potential tumors**

Menopausal	Sensitivity	Specificity	NPV
<b>Status</b>			
Combined	88.7%	74.7%	93.9%
Premenopausal	76.5%	74.8%	95.0%
Postmenopausal	92.3%	74.7%	92.6%

*This study demonstrates the benefit of CA125 in combination with HE4 in the risk of malignancy algorithm to identify women with a pelvic mass who are better treated by an ovarian cancer specialist in a surgical center.*

**5 Huhtinen K, et al. Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. *British J of Cancer.* 2009; Published Online doi: 10.1038/sj.bjc.6605011; 1-5.**

This study investigated the concentration of HE4 and CA125 in the serum of patients diagnosed with endometriosis, ovarian cysts, endometrial cancer and ovarian cancer as well as healthy controls. There were a total of 225 patients tested: 66 normals, 129 endometriosis, 16 endometrial cancer, and 14 with ovarian cancer. Mean HE4 concentrations were below the normal value (70pM) for all patients with endometriosis and controls, and was highly elevated in patients with both ovarian and endometrial cancer. CA125 showed a similar elevation in patients with ovarian cancer; however it was also elevated in patients with ovarian cysts and endometriosis. The combination of HE4 + CA125 was shown to accurately differentiate healthy patients and patients with endometriosis from those diagnosed with ovarian cancer.



**Accuracy and Sensitivity at 95% Specificity  
for ovarian cancer and ovarian endometriosis**

Tumor Marker	Accuracy (%)	Sensitivity (%)
<b>Ovarian Cancer vs Ovarian Endo</b>		
CA125+HE4	94.0	78.6
CA125	92.8	64.3
HE4	91.6	71.4
<b>Ovarian Cancer vs Control</b>		
CA125+HE4	96.3	92.9
CA125	96.3	78.6
HE4	93.8	78.6

Huhtinen, et al concludes that HE4 is a valuable marker and in combination with CA125 provides a more accurate tool for differential diagnosis of ovarian cancer from an ovarian cyst than either marker alone.

**6 Wang S, et al. The Application of HE4 in Diagnosis of Gynecological Pelvic Malignant Tumor. *Clin Oncol Cancer Res.* 2009; 6: 72-74.**

Wang et al investigated the HE4 test in the differential diagnosis of pelvic mass in a Chinese population of women. A total of 86 patients diagnosed with a pelvic mass were included in the study, 56 diagnosed with benign lesions and 30 with malignant lesions. These patients were evaluated against a normal control group of 46 healthy women. The range of serum HE4 values is illustrated in the chart below.

Group	Average level of serum HE4
Normal Control	34.1 pM
Benign Lesions	39.1 pM
Malignant Lesions	248.7 pM

The optimal cut-off in this patient population was 51.6 pM resulting in a sensitivity and specificity for HE4 of 86.7% and 98.0% respectively. Although this was a small sample size the data supports that of other published studies. Further investigation in a large scale study is required in China to verify HE4 as a marker for early diagnosis.

**7 Shah CA, et al. Influence of Ovarian Cancer Risk Status on the Diagnostic Performance of the Serum Biomarkers Mesothelin, HE4 and CA125. *Cancer Epidemiol Biomarkers Prev.* 2009; 18(5): 1365-1372.**

Researchers investigated the ability of three biomarkers, CA125, HE4 and Mesothelin to discriminate ovarian cancer from healthy controls in order to determine the potential value of these markers in screening women at high-risk for ovarian cancer. A total of 711 patients were evaluated; 444 healthy controls, 124 benign controls and 143 cases of invasive ovarian cancer. All participants completed a questionnaire that was used to classify them into a high or average risk group. In the patient population both CA125 and HE4 performed equally and better than Mesothelin. Sensitivity and specificity values for the different markers and risk groups are summarized below. All markers performed better when compared with the Healthy controls and HE4 performed the best when compared with the benign controls. Shah et al, conclude that further studies should be carried out to evaluate the use of these markers in the early detection of women at high risk for ovarian cancer.

**Diagnostic accuracy of biomarkers  
for average and high-risk women**

	Sensitivity at 95 % Specificity	
	Cases vs Healthy	Cases vs Benign
<b>CA125</b>		
Average Risk	79.4%	58.8%
High Risk	82.9%	63.4%
<b>HE4</b>		
Average Risk	80.4%	61.8%
High Risk	87.8%	75.6%
<b>Mesothelin</b>		
Average Risk	53.9%	43.1%
High Risk	39.0%	34.2%



**8** Havrilesky, et al. Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence. *Gynecol Oncol.* 2008; 110: 374-382.

**Sensitivity and Specificity of each individual marker**

Marker	Sensitivity Early stage	Sensitivity Late stage	Specificity
HE4	82.7%	92.5%	86.3%
CA125	45.9%	58.5%	98.2%
MMP7	45.9%	62.7%	89.4%
Glycodelin	63.2%	64.2%	79.0%
Plau-R	69.9%	74.6%	75.8%

The authors used two algorithms to identify early ovarian cancer. The two-step test used HE4 as the first step to stratify patients for further testing. If step one was elevated, this initiated step 2 which included testing with CA125, Glycodelin, and Plau R. This step was considered positive if any of the 3 markers were elevated. The sensitivity for early stage disease in the 2-step method was 76.7% at 97.2% specificity; in late stage disease the sensitivity and specificity were 84.6% and 97.2%. The one-step test used a combination of all the biomarkers, resulting in a sensitivity and specificity of 80.5% and 96.5% in early stage and 89.2% and 97.2% in late stage disease using the best cut-off. The authors concluded that with their method and combination of markers that they were able to approach the level of sensitivity and specificity needed for an effective screening test for the general population.

**Additional References**

- Hellström I, KE Hellström. SMRP and HE4 as biomarkers for ovarian carcinoma when used alone and in combination with CA125 and/or each other. *Adv Exp Med Biol.* 2008; 622: 15-21.
- Dong L, et al. [The values of serum human epididymus secretory protein 4 and CA(125) assay in the diagnosis of ovarian malignancy.] *Zhonghua Fu Chan Ke Za Zhi.* 2008; 43(12): 931-6. [Article in Chinese]
- Lowe KA, et al. Effects of personal characteristics on serum CA125, mesothelin, and HE4 levels in healthy postmenopausal women at high-risk for ovarian cancer. *Cancer Epidemiol Biomarkers Prev.* 2008; 17(9): 2480-7.
- Drapkin, et al. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Res.* 2005; 65: 2162-2169.
- Bingle L, V Singleton, CD Bingle. The putative ovarian tumour marker gene HE4 (WFDC2), is expressed in normal tissues and undergoes complex alternative splicing to yield multiple protein isoforms. *Oncogene.* 2002; 21(17): 2768-73.
- Gagnon A, B. Ye. Discovery and application of protein biomarkers for ovarian cancer. *Curr Opin Obstet Gynecol.* 2008; 20: 9-13

