

Human Epididymis Protein 4 (HE4)

Monitoring Patients With Epithelial Ovarian Carcinoma

Introduction

Human epididymis protein 4 (HE4), or WAP four-disulphide core domain protein 2 (WFDC2), was first identified in the epithelium of the distal epididymis and was originally predicted to be a protease inhibitor involved in sperm maturation.^{1,2} HE4 is the gene product of the *WFDC2* gene that is located on chromosome 20q12-13.1.³ The *WFDC2* gene is one of 14 homologous genes on this chromosome that encode proteins with WAP-type four-disulphide core (WFDC2) domains.³

HE4 belongs to the family of whey acidic four-disulfide core (WFDC2) proteins with suspected trypsin inhibitor properties^{3,4}; however, no biological function has so far been identified for HE4.^{5,6} The HE4 gene codes for a 13-kD protein, although in its mature glycosylated form the protein is approximately 20-25 kD and consists of a single peptide and two WFDC domains.⁶

Sensitivity and Specificity Comparable to CA125

HE4 has been reported to be expressed in a number of normal tissues, including epithelia of respiratory and reproductive tissues.^{3,4} Elevated levels were found in several tumor cell lines, including ovarian, lung, colon, and breast cancer.^{4,7} A number of independent microarray studies have shown that the *WFDC2* gene is overexpressed in patients with ovarian carcinoma relative to normal controls.⁷

In 2003, Hellström and colleagues showed that secreted HE4 was detected in high levels in the serum of ovarian

cancer patients.⁵ This group found that measurement of HE4 showed sensitivity and specificity comparable to that of CA125 for differentiating postmenopausal women with ovarian cancer from normal controls.⁵ They suggested that the HE4 assay may have an advantage over CA125 in that it is less frequently positive in patients with nonmalignant disease.⁵

Expression of HE4 in EOC

Drapkin and colleagues used immunohistochemical techniques to show that cortical inclusion cysts (CIC) lined by metaplastic Mullerian epithelium abundantly expresses HE4 relative to normal surface epithelium.³ Using tissue microarrays, they showed that HE4 expression was restricted to certain histologic subtypes of epithelial ovarian carcinomas (EOC).³

HE4 was expressed in 93% of serous and 100% of endometrioid EOC expressed HE4, whereas only 50% and 0% of clear-cell carcinomas and mucinous tumors, respectively, were found positive.³ This study also revealed that most nonovarian carcinomas do not express HE4, consistent with previous findings that HE4 protein expression is highly restricted in normal tissue of the reproductive tracts and respiratory epithelium.³

CA125 and HE4 in Combination

Moore and colleagues showed that HE4 had a slightly better sensitivity for detecting EOC than CA125.⁸ These researchers found that HE4 was particularly superior for detecting stage I disease, with no increase in sensitivity when combined with CA125 or any other marker.^{8,9} Overall, they found that combining CA125 and HE4

provided a more accurate predictor of malignancy than either alone.⁸

Montagnana and colleagues found that receiver operating characteristics curve analysis on healthy controls and patients with ovarian cancers revealed that HE4 had a significantly higher area under the curve than CA125 (0.99 vs 0.91), with a sensitivity and specificity of 98% and 100%, respectively.¹⁰

Mean HE4 levels were found to be significantly higher in patients with endometrial or ovarian cancer than in patients with ovarian endometriomas or other types of endometriosis.¹¹ These findings suggest that the HE4 test may be valuable in discriminating ovarian tumors from ovarian endometriotic cysts.¹¹

Shah and colleagues showed that the ability of serum HE4 levels to discriminate ovarian cancer cases from healthy and benign controls is not influenced by risk status.¹² Several other studies have indicated that including HE4 in a multivariate analysis of ovarian cancer risk served to improve the accuracy of screening and/or disease monitoring.^{9,13,14}

Monitoring Patients With Ovarian Cancer

The effectiveness of HE4 EIA as an aid in monitoring disease status in ovarian cancer patients was determined by assessing changes in HE4 levels in serial serum samples from 80 patients compared to changes in disease status.⁶

A study involving a total of 354 pairs of observations was undertaken with an average number of 4.4 observations per patient.⁶ A positive change in HE4 was defined as an increase in the value that was at least 25% greater than the previous value of the test. This level of change takes into account the variability of the assay and the biological variability. Sixty percent (or 76/126) of the patient samples with a positive change correlated with

disease progression, while 75% (or 171/228) of the patient serial samples with no significant change in HE4 value correlated with no progression. The total concordance was 70% (or 247/354). The data are presented in table 1.

Increase in HE4 concentration	Progression	No Progression	Total
>25%	76	57	133
≤25%	50	171	121
Total	126	228	354

Table 2 shows the resulting sensitivities and specificities of HE4 EIA compared to the disease status at various levels of HE4 EIA concentration. Sensitivity is represented as a concordance of HE4 EIA to progression of disease, and specificity is represented as a concordance of the HE4 EIA to the absence of disease progression.

Change in HE4 Concentration	Sensitivity	Specificity
10%	71%	62%
25%	60%	75%
50%	43%	88%
75%	38%	92%
100%	31%	95%

Conclusion

In postmenopausal women HE4 serum concentrations represent a valuable marker with which to distinguish epithelial ovarian carcinoma from benign ovarian disease.¹¹ In addition, it has been demonstrated that HE4 improves the effectiveness of CA125 when the two are used in combination.^{13,14} Moreover, with the addition of a symptom index¹³ and patient characteristics (eg, age at menarche, etc),¹⁴ the effectiveness may be enhanced even further.

Human Epididymis Protein 4 (HE4) 081700**Related Information** Cancer Antigen (CA) 125, Serum (002303)**Synonyms** HE4; WFDC2**Special Instructions** Values obtained with different assay methods should not be used interchangeably in serial testing. It is recommended that only one assay method be used consistently to monitor each patient's course of therapy. This procedure does not provide serial monitoring; it is intended for one-time use only. If serial monitoring is required, please order test 481700.**Specimen** Serum**Volume** 0.5 mL**Minimum Volume** 0.2 mL (**Note:** This volume does **not** allow for repeat testing.)**Container** Red-top tube or gel-barrier tube**Collection** If red-top tube is used, transfer separated serum to a plastic transport tube.**Storage Instructions** Refrigerate. Stable refrigerated or frozen for 72 hours.**Patient Preparation** No special patient preparations are required.**Causes for Rejection** Nonserum sample received**Reference Interval** See the table, **Distribution of HE4 Assay Values** below.**Use** The HE4 is an enzyme immunometric assay for the quantitative determination of HE4 in human serum. The assay is to be used as an aid in monitoring recurrence or progressive disease in patients with epithelial ovarian carcinoma. Serial testing for patient HE4 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer.**Limitations** HE4 levels tend to be higher in older women and in women who began menstruating at an older age, but these effects are small.¹⁴ Falsely elevated or depressed values of HE4 may occur in samples containing human antimouse antibodies.⁶ Levels of HE4 within the reference range do not preclude the presence of cancer, nor are elevated results an absolute indication of malignancy; thus, HE4 should not be used for cancer screening.⁶ HE4 should not be used for monitoring patients with mucinous or germ cell ovarian cancer. Results should be interpreted in conjunction with other clinical and laboratory findings.⁶**Methodology** Enzyme-linked immunosorbent assay (ELISA)**Ovarian Cancer Monitor 081610****Synonym** HE4 and CA125 Profile**Profile Includes** Human Epididymis Protein 4 (HE4); Cancer Antigen (CA) 125, Serum**Specimen** Serum**Volume** 1 mL**Minimum Volume** 0.8 mL (**Note:** This volume does **not** allow for repeat testing.)**Container** Red-top tube or gel-barrier tube**Collection** If red-top tube is used, transfer separated serum to a plastic transport tube.**Storage Instructions** Refrigerate.**Patient Preparation** No special patient preparations are required.**Causes for Rejection** Nonserum sample received**Reference Interval** See individual test descriptions.**Use** When used in combination, HE4 and CA125 provide a more accurate predictor of malignancy than either marker alone.⁸**Methodology** HE4: Enzyme-linked immunosorbent assay (ELISA); CA 125: Electrochemiluminescence immunoassay (ECLIA)For the most current information regarding test options, including specimen requirements and CPT codes, please consult the online Test Menu at www.LabCorp.com.

Distribution of HE4 Assay Values*					
	Number of subjects	0-150 pM	150.1-300 pM	300.1-500 pM	>500 pM
Apparently Healthy					
Females (Premenopausal)	76	72	3	0	1
Females (Postmenopausal)	103	97	5	0	1
Benign Conditions					
Pregnancy	22	21	1	0	0
Benign Gynecological Disease	347	324	18	1	4
Other Benign Disease	108	82	8	7	11
Hypertension/CHF	96	75	16	2	3
Cancer					
Ovarian Cancer	127	27	18	21	61
Breast Cancer	46	40	4	2	0
Lung Cancer	50	29	15	6	0
Endometrial Cancer	116	86	15	4	11
Gastrointestinal Cancer	56	47	8	0	1

*In this study 94.4% of the healthy female subjects had an HE4 assay value at or below 150 pMol.

References

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Abbreviations

CA125	Cancer antigen 125
CIC	Cortical inclusion cysts
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EOC	Epithelial ovarian carcinoma
HE4	Human epididymis protein 4
WAP	Whey acidic protein
WFDC2	WAP-type four-disulphide core



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