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# Evaluation of tumor marker HE4 assay on the Elecsys 2010 analyzer

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## ABSTRACT

**Introduction**: Whey-acidic protein human epididymis protein 4 (HE4) is a new promising biomarker for epithelial ovarian cancer. The measured HE4 values may depend on the testing procedure used. The aim of this study was to evaluate the

**Methods**: We evaluated a HE4 method on Elecsys 2010 analyzer. The method for quantitative determination of HE4 is direct, competitive chemiluminescent immunoassay. For quality control we use Elecsys PreciControl HE4 1 and 2. HE4 was measure on sera obtained from 56 women ( 20 healthy and 36 with epithelial ovarian cancer).

**Results**: The Roche HE4 assays showed a good linearity (r=0.99) and precision (intrassayed total CV<5%). The median HE4 serum concentrations was significantly higher among EOC patients than healthy females (p<0,05). Elevated levels HE4 were found in 78 % patients with epithelial ovarian cancer.

**Conclusions**: The presented results of the analytical evaluation methods for the determination of HE4 on the Elecsys 2010 analyzer showed an acceptable accuracy and precision.

**Keywords**: Human epididymis protein 4 (HE4), epithelial ovarian cancer, biomarkers

## INTRODUCTION

Epithelial ovarian cancer (EOC) is the fourth most common cause of cancer mortality in women and the leading cause of death from gynecological malignancies. The majority of women have advanced-stage disease at initial diagnosis and a 5-year survival of 10-30 %. The median survival after recurrence is only 2 years despite the advances in chemotherapy and secondary debulking surgery in selected patients (1).

The human epididymis protein 4 (HE4, also known as WFDC2) belongs to the family of whey acidic four-disulfide core (WFDC) proteins with suspected trypsin inhibitor properties. The corresponding gene codes for a 13 kD protein. In its mature glycosylated form the protein has a molecular weight of approximately 20-25 kD and consists of a single peptide chain containing two WFDC domains (2).

HE4 was first determined in the epithelium of the distal epididymis. It showed low expression in epithelia of respiratory and reproductive tissues including ovary, but high expression in ovarian cancer tis-

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sue. High secreted levels can also be found in the serum of ovarian cancer patients (3).

The measured HE4 value of a patients sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the HE4 assay method used. HE4 values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations (4,5).

The assay is to be used as aid in monitoring recurrence or progressive disease in patients with epithelial ovarian cancer. Serial testing for patient HE4 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer (6).

Moore and colleagues (7) have reported that the serum biomarker algorithm ROMA (Risk of Ovarian Malignancy Algorithm), which utilizes the dual marker combination of HE4 and CA125, along with menopause status, shows good performance for risk prediction of ovarian malignances (7).

## METHODS

Blood samples were collected from 36 female patients with epithelial ovarian cancer (mean age: 56 years, range 48-69 years). Control samples (N=20) were collected from healthy females. The blood was collected into the non-heparinized tubes and kept 30 min at room temperature, then centrifuged for 15 min at 3000 rpm. The sera were then stored at -20 °C until use.

The serum levels of HE4 were determined using a chemiluminescent enzyme immunoassay on the Elecsys 2010 Analyzer (Roche Diagnostics, Germany) according to manufacturer, s instructions. The HE4 assay is a two-step immunoassay for quantitative determination of HE4 antigen in human serum using CMIA technology. In the first step, sample and 2H5 anti-HE4 coated paramagnetic micropaticles are combined. HE4 antigen present in the sample binds to the anti-HE4 coated microparticles. After washing, 3D8 anti-HE4 acridinium-lebeled conjugate is added to create a reaction mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminiescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HE4 antigen in the sample and RLUs.

For quality control ElecsysPreciControl HE4 1 and 2 (Roche Diagnostics, Germany) were used.

Statistical analysis

The data were analyzed in SPSS version 18 (Chicago, IL, USA). Descriptive statistics and Pearson correlation were used. The difference between groups were considered significant if $p<0.05$.

## RESULTS

The results of the HE4 assay precision within-run and between-run analyses are showed in Table 1.

The coefficients of variation (CV%) values for the within-run precision were 6.9 – 8.2 %, with those for between-run ranging 7.9 – 9.8%. The results variation was greater at lower concentrations.

The HE4 assay accuracy results are presented in Table 2.

The statistically significant correlation between labeled and measured HE4 values was obtained ($r=0.99$), presented by the following equation: $y= -1.20+0.99x$, where y represents the measured HE4 levels, and x labeled HE4 levels. An intercept (-1.20) presents the systematic errors of the method, which was not statistically significant ($p>0.05$).

TABLE 1. Within-run and between-run precision of HE4 assay

| Sample | Sample value (n) | Mean value (pmol/L) | Sd (pmol/L) | CV(%) |
|---|---|---|---|---|
| Within-run | | | | |
| PreciControl HE4 1 | 20 | 45.5 | 3.7 | 8.2 |
| PreciControl HE4 2 | 20 | 341.5 | 23.5 | 6.9 |
| Between-run | | | | |
| PreciControl HE4 1 | 20 | 42.4 | 4.1 | 9.8 |
| PreciControl HE4 2 | 20 | 347.7 | 29.2 | 7.9 |

TABLE 2. Accuracy of HE4 assay on Elecsys 2010 analyzer.

| Calibrators (pmol/L) | 20.0 | 50.0 | 100.0 | 500.0 | 1000.0 |
|---|---|---|---|---|---|
| Measured values* (pmol/L) | 29.7 | 49.2 | 98.4 | 497.1 | 992.1 |

*Mean of two measurements of calibrators

TABLE 3. HE4 measurements

| Group | N | Median (pmol/L) | Range (pmol/L) |
|---|---|---|---|
| EOC | 36 | 139.6 | 34.2 – 1383.0 |
| Healthy controls | 20 | 32.1 | 11.2 – 72.3 |

EOC, epithelial ovarian cancer

Serum samples were collected from 36 ovarian cancer patients (EOC). Results of HE4 measurements in different groups are shown in Table 3.

The median serum HE4 concentrations were significantly higher among all ovarian cancer patients compared with group of healthy subjects.

Patients with ovarian carcinoma showed significantly higher HE4 levels of 139.6 (range: 34.2 – 1383.0) pmol/L compared to healthy controls levels of 32.1 (range: 11.2 – 72.3) pmol/L. In the present study, significantly elevated HE4 concentrations were found in 28 of 36 cases of ovarian cancer, and cut off levels of 74 pmol/L gave the sensitivity rate of 78%.

## DISCUSSION

Human Epididymis Protein 4 (HE4) is a 25 KD single peptide chain with a low expression in epithelia of repertory and reproductive tissues, but highly expressed in ovarian cancer tissue. HE4 is a novel serological marker used especially for ovarian cancer diagnosis because of its high sensitivity (8). The percentage of increase in HE4 values has been used as an aid in monitoring recurrence or progressive disease in patients with invasive epithelial ovarian cancer. There is no clinically accepted cut-off for use in monitoring cancer progression in epithelial ovarian cancer (9). Currently, several biomarker panels are being evaluated in increase the sensitivity and specificity of ovarian cancer diagnosis. The combination of CA125 and HE4 has been evaluated to improve ovarian cancer diagnosis. The data suggests that by combining these markers the predictive accuracy in ovarian malignancy is better than by applying any of the markers alone (10). As a single tumor marker, HE4 had the highest sensitivity for detecting ovarian cancer, especially in stage I disease.

The analysis of HE4 by competitive chemiluminescent immunoassay on Elecsys 2010 analyser is rapid and easy to perform. The linearity range of assay is from 20 to 1000 pmol/L, which is acceptable for routine clinical use. The CV for precision in this assay is not greater than 10% at the lowest measurable concentration. The obtained CV% values for precision were for the within-run precision were 6.9 – 8.2 %, with those for between-run ranging 7.9 – 9.8%. The results variation was greater at lower concentrations.

The median concentrations in women test group were 139.6 (range 34,2 – 1383,0 ) pmol/L. Reference interval for HE4 ranges between 0 to 74. The median serum HE4 concentrations were significantly higher among all ovarian cancer patients compared with group of healthy subjects. Elevated levels HE4 were found in 78 % patients with epithelial ovarian cancer.

## CONCLUSION

Early detection is a critical focus of ovarian cancer research because of its potential to reduce suffering and morbidity. HE4 assay values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and reagent specificity. The results reported by the physician must include the identity of the HE4 assay used. The presented results of the analytical evaluation methods for the determination of HE4 on Elecsys 2010 analyzer showed an acceptable accuracy and precision.

## COMPETING INTERESTS

Authors have no conflict of interest to report.

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