

## CA125 and HE4 levels in a Czech Female Population Diagnosed with Endometrial Cancer in Preoperative Management

JIRI PRESL<sup>1</sup>, ZDENEK NOVOTNY<sup>1</sup>, ONDREJ TOPOLCAN<sup>2</sup>, PAVEL VLASAK<sup>1</sup>, RADEK KUCERA<sup>2</sup>,  
RADKA FUCHSOVA<sup>2</sup>, JINDRA VRZALOVA<sup>2</sup>, LUCIE BETINCOVA<sup>1</sup> and SARKA SVOBODOVA<sup>2,3</sup>

<sup>1</sup>Department of Gynecology and Obstetrics, Faculty Hospital in Pilsen, Pilsen, Czech Republic;

<sup>2</sup>Department of Nuclear Medicine, Laboratory of Immunoanalysis,  
Faculty Hospital in Pilsen, Pilsen, Czech Republic;

<sup>3</sup>Third Internal Medicine Department and First Medical Faculty, Charles University, Prague, Czech Republic

**Abstract.** *Aim: The aim of the present study was to compare the use of cancer antigen 125 (CA125) and human epididymis protein 4 (HE4) biomarkers in patients with endometrial cancer for preoperative management and to particularly focus on relationship between CA125 and HE4 and disease stage in predicting myometrial invasion or intrauterine tumor spread. Patients and Methods: Thirty-four patients diagnosed with endometrial cancer and 32 healthy controls were enrolled into the pilot study in the period between May 2012 and March 2013. Blood from all the females was collected and examined for CA125 and HE4. Based on standardized ultrasound examination, including gynecological examination, the clinical disease stage was determined. Results: We found a significant difference ( $p < 0.0001$ ) for means of serum levels of HE4: females with endometrial cancer, 75.5 pmol/l, versus healthy females, 40.0 pmol/l. A non-significant statistical difference was found for mean serum CA125 levels ( $p = 0.4442$ ): females with endometrial cancer 19.0 IU/l, versus healthy females, 15 IU/l. A significant correlation with histopathological disease stage was found for both biomarkers (Spearman correlation). Sensitivity and specificity, and the related cut-off for HE4 suggest that HE4 would be a more appropriate biomarker for differential diagnosis between benign and malignant states. Conclusion: Based on our pilot study, we found that parallel examination of HE4 and CA125 may support endometrial ultrasound finding verification prior to biopsy. This study is ongoing and we expect that results on a larger population may enable HE4 measurement to be implemented in routine practice.*

*Correspondence to:* Jiri Presl, MD, Department of Gynecology and Obstetrics, Faculty Hospital in Pilsen, Alej Svobody 80, Pilsen-Lochotin, CZ-304 60, Czech Republic. Tel: +420 377105240, e-mail: preslj@fnplzen.cz

**Key Words:** Endometrial cancer, tumor markers, CA125, HE4.

Endometrial cancer represents the most frequent gynecological malignancy in the Czech Republic. According to the latest data from the National Oncology Registry from 2010, the incidence rate is 34.86/100000 females (17), which means that 1,870 new cases occur every year. Mortality is around 5.48/100000 females (17), meaning 294 patients dying from endometrial cancer per year. The most frequent incidence appears in the fifth and sixth decade of the life. Only 20-25% patients will be diagnosed prior to the menopause.

No ideal serum biomarker is currently in use for the management of endometrial cancer. The aim of this study was to compare the potential use of cancer antigen 125 (CA125) and human epididymis protein 4 (HE4) as biomarkers for patients diagnosed with endometrial cancer for preoperative management and to particularly focus on the relationship between CA125 and HE4 and disease stage in order to predict myometrial invasion or intrauterine tumor spread.

### Patients and Methods

Thirty-four patients diagnosed with endometrial cancer and 32 healthy controls were enrolled into the pilot study in the period between May 2012 and March 2013.

The mean age of the healthy females was 58.5 (37.0-73.0) years and that of females with endometrial cancer was 65.0 (43.0-88.0) years. The age distribution is shown in Figure 1.

The group of patients with endometrial cancer included 18 patients with clinical stage IA, eight patients with clinical stage IB, six patients with clinical stage II and two patients with clinical stage IIIA disease.

Blood from all the females for CA125 and HE4 biomarker measurement was collected from the cubital vein in the morning between 7 and 10 a.m. using VACUETTE® collection system (Greiner Bio-One, Austria). Sera were separated by centrifugation at 1700 ×g for 10 min and all specimens were stored in aliquots, immediately deep-frozen and stored at -80°C until laboratory analysis, if not measured within 24 h after collection. Both biomarkers were measured in the Laboratory of Immunoanalysis at

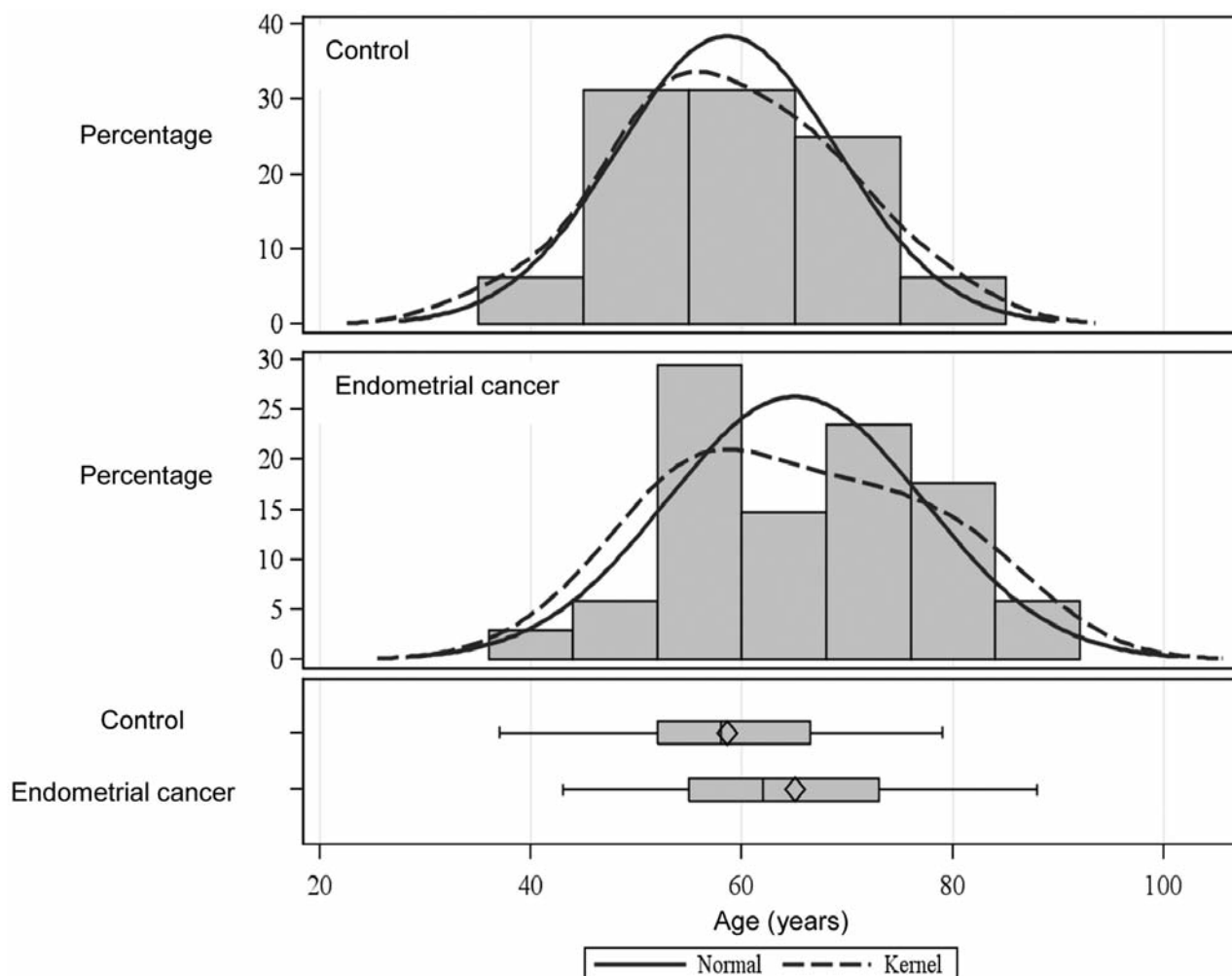


Figure 1. Age distribution of patients included in this study.

the Department of Nuclear Medicine in Pilsen, using commercially available chemiluminiscent kits using Architect 1000i instrument (Abbott, Chicago, IL, USA). Analytical sensitivity is set 1.0 kIU/l for CA125 and for HE4 limit of detection is 15 pmol/l and limit of quantification is 20 pmol/l.

Patients underwent expert standardized ultrasound examination and gynecological examination in order to determine the clinical stage of the disease prior to surgery.

In patients undergoing surgery, histological examination was performed perioperatively using frozen section method and based on these results, appropriate surgery to an extent reflecting the clinical stage of endometrial cancer was performed.

Statistical software SAS 9.3. (SAS Institute Inc., Cary, NC, USA) was used for all the statistical evaluations. Cut-off, sensitivity, specificity, positive and negative predictive value (PV+, PV-) and area under the receiver operating characteristic (ROC) curve (AUC) was established for both biomarkers. Wilcoxon's test was used for comparison between the two groups of patients (healthy and endometrial cancer).

Table I. Correlation of tumor markers levels with staging of endometrial cancer.

Parameter	Spearman correlation coefficient (r)	p-Value
CA125	0.6120	0.0002
HE4	0.5047	0.0027

## Results

Box-plots in Figures 2 and 3 show a highly significant difference ( $p < 0.0001$ ) between median serum HE4 levels: 75.5 pmol/l in females with endometrial cancer *versus* 40.0 pmol/l in healthy females. Comparison of medians for

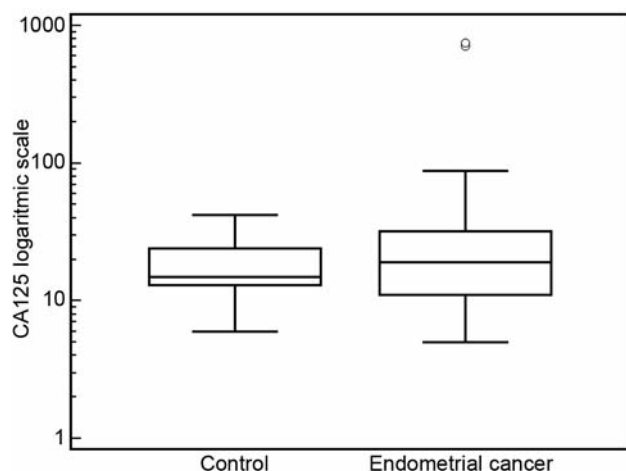


Figure 2. Box and whisker plot for CA125 (central boxes represents the values from the lower to upper quartile, 25-75% the middle line represent medians, and line extends from the min to max value excluding far out values).

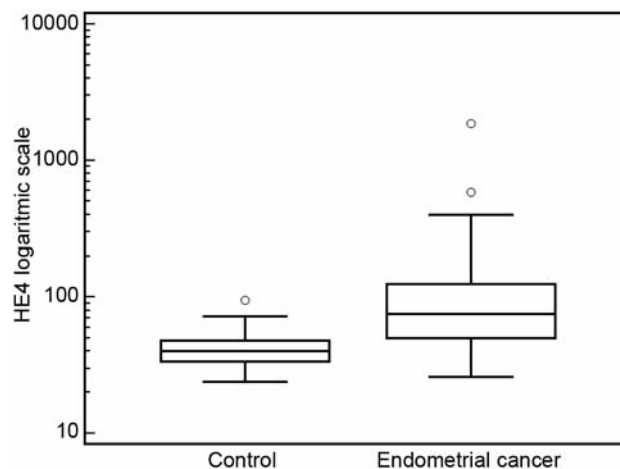


Figure 3. Box and whisker plot for HE4 (central boxes represents the values from the lower to upper quartile, 25-75% the middle line represent medians, and line extends from the min to max value excluding far out values).

Table II. Sensitivity, specificity and related cut-offs for both biomarkers.

Marker	AUC	Cut-off (kIU/l, pmol/l)	Specificity	Sensitivity	PV+	PV–
CA125	0.5699	32.00	93.75	26.47	81.82	54.55
		31.00	87.50	32.35	73.33	54.90
		42.00	96.88	20.59	87.50	53.45
HE4	0.8056	90.00	96.88	41.18	93.33	60.78
		62.00	90.63	55.88	86.36	65.91
		73.00	96.88	52.94	94.74	65.96
		49.00	78.13	79.41	79.41	78.13

AUC: area under the curve; PV+: positive predictive value; PV–: negative predictive value; CA125: cancer antigen 125; HE4: human epididymis protein 4.

CA125 showed no significant difference ( $p=0.4442$ ): 19 KIU/l females with endometrial cancer *versus* 15 KIU/l healthy females. Both biomarkers significantly correlated with histopathological staging of the disease (Spearman) as shown in the Table I.

Sensitivity and specificity and the related cut-offs for both biomarkers are shown in the Table II. These data suggest that HE4 would be a more appropriate biomarker for differential diagnosis between benign and malign endometrial findings.

## Discussion

Endometrial cancer represents the most frequent gynecological malignancy in the Czech Republic (17). The majority of patients are diagnosed and treated in early stages due to early clinical symptoms, namely bleeding. Oncogynecology teams must stratify patients to low- or high-

risk cancer based on the histological diagnosis to allow for proper operative and postoperative management. This stratification is at present based only on clinical symptoms, gynecological examination and imaging techniques, particularly ultrasound examination. No ideal serum biomarker has been defined, neither for potential endometrial cancer screening, for therapy response monitoring, for follow-up nor for prediction of intrauterine or extrauterine spread for better surgery design and extent.

Myometrial invasion, including tumor grading, represents the most important decision factor within clinical stage I disease for the extent of surgery for pelvic and para-aortic lymphadenectomy. When considering other very frequent comorbidities and factors, such as arterial hypertension, diabetes mellitus, age, overweight, such extensive surgery may be very risky and this has often been discussed among the experts at international meetings (1, 16).

CA125 has a very low sensitivity and specificity as a biomarker in such cases (7, 9, 20, 21). It is claimed that only 10 to 20% patients in the early stages and 25% of asymptomatic patients with disease recurrence have elevated serum CA125 levels (5, 10, 14). Only a significantly elevated serum level of CA125 may signal extrauterine tumor spread (3). When compared to ovarian cancer patients, CA125 levels closely correlate with disease regression or progression (8). Postoperative serum CA125 increase has 84-94% sensitivity for prediction of cancer relapse (18, 19). Based on these facts, it is evident that new biomarkers for patients with endometrial cancer are needed.

Many recent publications demonstrate that serum biomarker HE4, which has led to significantly improved diagnosis of ovarian cancer (15), is also elevated in endometrial cancer type I and type II (2, 6). These findings initiated great interest in HE4 a potentially useful marker for patients with this diagnosis (4, 6, 11, 12). Our data correspond with the statement that median serum HE4 levels in patients with endometrial cancer are statistically significantly higher when compared with a healthy group (10). HE4, unlike CA125, has higher sensitivity in patients with endometrial cancer and this is fully in concordance with recently published data (10, 13).

For statistical evaluation of the results between patients with stage IA and IB disease, with myometrial invasion depth <50% vs. >50%, respectively, study of a larger group of patients will be required.

## Conclusion

In our pilot study, we found that parallel assessment of HE4 and CA125 biomarkers may improve pre-biopsy verification of ultrasound findings of endometrial abnormalities. In specific cases, these markers may help in precise preoperative staging of endometrial cancer, as added value to the borderline cases from obligatory routine examinations. These findings are insufficient for the proposal of routine clinical examinations of asymptomatic patients with abnormal ultrasound findings and for scheduling of preoperative examinations. This pilot study is, however, ongoing and we expect that results of study on a larger population may enable HE4 measurement to be included in routine practice.

## Acknowledgements

This study is supported by Ministry of Health, Czech Republic - conceptual development of research organization (Faculty Hospital in Pilsen - FNPl, 00669806, CZ.1.07/2.3.00/20.0040).

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*Received October 17, 2013*

*Revised November 19, 2013*

*Accepted November 21, 2013*