HE4 and ROMA Index in Czech Postmenopausal Women

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Abstract. *Aim: The first aim of the project was to evaluate the benefits of the determination of human epididymis protein 4 (HE4) and the risk of ovarian malignancy algorithm (ROMA) index for primary detection of ovarian cancer in a population of Czech women. The second aim was to study the advantages HE4, cancer antigen 125 (CA125) and ROMA index for distinguishing between benign and malignant tumors. Aware of the age distribution of ovarian cancer, we focused on postmenopausal patients. Patients and Methods: Our group of patients consisted of 256 females, 21 with ovarian cancer and 235 with benign ovarian tumors. All diagnoses were histologically verified. We determined the serum levels of HE4 and CA125 and calculated the ROMA2 index for postmenopausal women. Serum levels of the analytes were measured using an Architect 1000i instrument. Serum samples were collected prior to surgery or any other form of treatment and the results of the two groups of patients were compared (malignant vs. benign). Results: There was a significant difference in the serum levels for all parameters studied between the groups of patients with malignant and those with benign diagnoses (Wilcoxon test, $p<0.0001$). When all parameters were evaluated at 95% specificity, the HE4 cut-off was 112 pmol/l at a sensitivity of 71.42%, a positive predictive value (PPV) of 55.56%, a negative predictive value (NPV) of 97.14% and an area under the curve (AUC) of 0.9152. The CA125 cut-off was 81 IU/l at a sensitivity of 80.95%, a PPV of 58.62%, a NPV of 98.23% and an AUC of 0.9731. ROMA2 index had a cut-off 37.70% at a sensitivity of 85.71%, a PPV of 62.06%, a NPV of 98.65% and an AUC of 0.9803. The highest diagnostic efficiency was achieved by the ROMA2 index. Conclusion: Determination of HE4 along with CA125 and ROMA2 index calculation is a suitable method for the improvement of the primary detection of ovarian cancer. This approach also improves the differential diagnostic possibilities for distinguishing between malignant and benign tumors.*

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According to the statistics for 2009, the incidence of ovarian cancer in the Czech Republic was 22.5/100,000 women (1). Mortality, despite the decline observed in the last 10 years, is still high. Ovarian cancer is the leading cause of gynecological cancer death, representing 5% of all cancers in women and 23% of all gynecological cancers. Ovarian cancer has a poor prognosis, mainly because of late detection at advanced stages (2). Worldwide attention is therefore focused on the potential of research and the subsequent treatment of this cancer. Unfortunately, no tumor marker approaching the ideal marker has yet been discovered. Until recently, the main tumor marker for ovarian cancer was cancer antigen 125 (CA125). Its use is sensitive but with low specificity (3). The tumor marker human epididymis protein 4 (HE4) has been in clinical use since the year 2003 (4). In the last few years, the effort to increase the efficiency of the diagnostic process has led to the implementation of the risk of ovarian malignancy algorithm (ROMA) index. The ROMA index is calculated using the measured values of HE4 and CA125, while the menopausal status of patients is also taken into account. Conclusions regarding the sensitivity and specificity of HE4 and the ROMA index have been very optimistic in recent studies (5, 6, 7). These developments led to this study being carried out on a female Czech population.

Patients and Methods

Group of patients. The patient study group consisted of 256 females with abnormalities in the pelvis. We divided the patients into two groups. The first consisted of 21 females with ovarian cancer and the second of 235 females with benign ovarian tumors. The age characteristics of these groups are presented in Table I. Serum parameters of HE4 and CA125 were measured and the ROMA2 index was calculated for both groups. Calculation of the ROMA2 index was made according to the following equation: ROMA index %=exp(PI)/[1+exp(PI)] ×100, where the predictive

index, PI=–8.09+1.04×LN[HE4]+0.732 ×LN[CA125] (8). The serum samples were analyzed at the Laboratory of Immunoanalysis, Faculty of Medicine in Pilsen, (Czech Republic) from 2010 to 2011. The menopausal status of patients was determined by doctors at the gynecological clinic according to the methodology of Moore *et al*. (8), but always with emphasis on the actual clinical status of patients.

Serum samples. Serum samples were collected prior to surgery or any other form of treatment. The time of collection was between 7-10 AM for all women. Samples of venous blood were collected using the VACUETTE blood collection system (Greiner Bio-one Company, Kremsmünster, Austria). Blood was centrifuged for 10 min at 1700 ×*g*. Serum samples were immediately frozen to –80˚C. Samples were thawed only once, just prior to analyses.

Methods used. HE4 and CA125 serum levels were measured using the Architect 1000i System (Abbott, Libertyville, IL, USA). Serum samples were collected prior to surgery and samples of the two subgroups of patients were compared (malignant *vs*. benign).

Statistical methods. SAS 9.2 (Statistical Analysis Software release 9.2; SAS Institute Inc., Cary, NC, USA) was used for all statistical analysis. A summary of statistical findings for age and serum levels of each of the analytes is presented. The Wilcoxon test was used to compare distributions of values between benign and malignant tumors.

Results

The number of females and the age characteristics of both groups of patients is shown in Table I. Serum levels of HE4 and CA125 are shown in Table II, together with the calculated results of the ROMA2 index. According to the Wilcoxon test ($p<0.0001$), the results for the beign and malignant groups of patients was significantly different. The results of the next statistical evaluation are summarized in Table III where area under the curve (AUC), cut-off, specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) are presented.

Discussion

Current practice in our hospital suggests that a patient with abnormal findings in the pelvis has samples taken at their first visit for testing of blood markers HE4 and CA125, and the ROMA index is calculated as well. At the same time, a comprehensive gynecological examination is performed, including ultrasound, and the menopausal status of patients is evaluated as well. Thus, during one visit, patients in the clinic receive comprehensive information about the nature of their abnormal pelvic findings. In this situation, clinicians request reliable laboratory parameters which would quickly extend the set of tools available and improve the diagnostics of ovarian cancer.

Table I. *Age characteristics of the patient groups*

Diagnosis	N	Age (years)			
		Mean	Median	Min.	Max.
Ovarian cancer	21	64.37	63	47	82
Benign ovarian tumor	256	65.28	64	48	93

Tumor markers are currently used for follow-up and therapy effect monitoring. In evaluating data, we have focused on the possibilities of using HE4, CA125 and ROMA index for the primary diagnostics of ovarian cancer. The second aim was to study the value of HE4, CA125 determination and the ROMA index for distinguishing benign from malignant tumors.

Table II shows the serum levels of both markers and the ROMA2 index results, calculated for both groups of females. There was a significant difference in the serum levels of all parameters between the groups with malignant and benign diagnoses, as shown by the Wilcoxon test, ($p<0.0001$). Further statistical parameters are shown in Table III. We have evaluated all parameters at cut-off levels which are currently used in our hospital. These results are presented in the first line of Table III. For a more extensive picture of changes in the cut-off levels of each parameter, we also evaluated all three parameters at 95% specificity, shown in the second line of Table III. The highest diagnostic efficiency was achieved by the ROMA2 index. If we focus on the cut-off of all parameters, a rise is observed, in comparison to the cut-off levels which are currently used in our hospital. In 2009, Moore *et al*. published, the results of a prospective multicenter study on 531 patients. In their study, the ROMA2 index had a cut-off of 27.7%, a sensitivity of 92.3%, with a specificity of 74.7%, and NPV of 92.6% (9). In 2011, the same author published the results of a further set of 472 patients. In this study, the ROMA2 index reached a sensitivity of 90.2% with a specificity of 76% and a NPV of 95.8% (8). Our findings are consistent with the findings of these studies.

If we consider the results of receiver operating characteristic (ROC) analysis, we see that the best values for the AUC were achieved in the ROMA2 index, followed by CA125 and then HE4. Our data are consistent with the work by Partheen *et al*., from Sweden, whose study evaluated a group of 394 patients (10).

Before implementation of HE4, only the tumor marker CA125 was used for ovarian cancer detection at our hospital. The combination of both markers is an improvement compared to the results achieved by CA125 alone. In addition, around 20% of cases of epithelial ovarian cancer has little, if any, elevation of CA125. For

Table II. *Postmenopausal women, cancer vs. benign tumor.*

Parameter (units)	Diagnosis	N	Mean	Median	Range	*p*-Value, Wilcoxon test
HE4 (pmol/l)	Cancer	21	649.35	312	17.10-1842	<0.0001
	Benign	256	51.72	39.50	26.70-3590	
CA125 (IU/l)	Cancer	21	2349	295	32.80-44850	<0.0001
	Benign	256	65.80	16.20	3.60-2331	
ROMA2 (%)	Cancer	21	70.91	87.20	22.40-100	<0.0001
	Benign	256	13.75	9.72	1.81-99.60	

HE4: Human epididymis protein 4; CA125: cancer antigen 125; ROMA2: risk of ovarian malignancy algorithm.

Table III. *Postmenopausal women, cancer vs. benign tumor.*

Parameter (units)	AUC	Cut-off	Specificity	Sensitivity	PPV	NPV
HE4 (pmol/l)	0.91518	89.00	87.55	71.42	34.09	97.14
		112.00	94.85	71.42	55.56	97.35
CA125 (IU/l)	0.97315	36.00	84.68	95.23	35.71	99.50
		81.00	94.89	80.95	58.62	98.23
ROMA2 (%)	0.98031	26.30	87.88	95.24	41.67	99.51
		37.70	95.04	85.71	62.06	98.65

HE4: Human epididymis protein 4; CA125: cancer antigen 125; ROMA2: risk of ovarian malignancy algorithm; AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value.

more than 50% of these malignancies, elevated levels of HE4 can be observed, and combinations of these markers may therefore optimize the potential for a successful diagnosis of ovarian malignancies in these patients. Another factor supporting a combination of both markers and the calculation of the ROMA index is that elevated levels of CA125 can be observed as a result of physiological conditions such as menstruation or pregnancy, as well as in benign ovarian tumor, inflammation and the presence of endometriosis and fibroids. This false positivity in the group of premenopausal patients may cause problems in routine clinical practice. Therefore, a combination of HE4 and CA125, together with the calculation of the ROMA index, increases the specificity and sensitivity of testing and is an interesting tool in ovarian cancer diagnostics.

Conclusion

Ovarian cancer is, with its high incidence and mortality, a worldwide problem. One reason for this is the lack of symptoms. The second reason is practically non-existant screening for ovarian cancer. Until recently, the only routinely used marker for ovarian abnormalities was CA125. Determination of HE4 levels, together with those of CA125 and the calculation of the ROMA2 index, is a suitable method for improving primary detection of ovarian cancer. This approach also broadens the range of differential diagnostic possibilities for distinguishing between malignant and benign tumors.

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