

The diagnostic value of serum HE4 and CA-125 and ROMA index in ovarian cancer

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Received January 19, 2016; Accepted May 19, 2016

DOI: 10.3892/br.2016.682

Abstract. Ovarian cancer is a common malignancy of the female reproductive system. Tumor markers serve as tools in the diagnosis of the disease. The aim of the present study was to determine the diagnostic value of sera levels of carbohydrate antigen-125 (CA-125), human epididymis protein 4 (HE4) as well as the area under the curve of the receiver operating characteristic (ROC) and the risk of ovarian malignancy algorithm (ROMA) index in ovarian cancer. The sera were measured using an electrochemiluminescence immunoassay on 158 individuals (64 patients with ovarian cancer, 64 with ovarian benign tumor and 30 healthy individuals) between September 2013 and May 2015. The results showed that levels of HE4 and CA-125 in the sera of the ovarian benign tumor group as well as their ROMA index were significantly higher ($P<0.05$) than those of the ovarian benign tumor and control groups, regardless of pre- or postmenopausal status. However, the level of CA-125 was significantly higher ($P<0.05$) in the ovarian benign tumor group compared with the healthy group, while the level of HE4 was similar in the two groups. The sensitivity of the ROMA index was higher ($P<0.01$) with detection of HE4 and CA-125. In the ovarian cancer group, the areas under ROC curves of ROMA, HE4 and CA-125 were 0.994, 0.990 and 0.941, respectively. The specificity and positive predictive value of HE4 in the premenopausal ovarian cancer group reached 98.36 and 95%, respectively. In conclusion, the results showed that the serum level of HE4 and the ROMA index are important indicators in the diagnosis of ovarian cancer. However, in addition to HE4 and CA-125 detection, the ROMA index is extremely valuable in improving the diagnostic efficiency of ovarian cancer.

Introduction

Ovarian cancer is one of the three most common malignant tumors in the female reproductive system. It has an insidious onset with a difficult early diagnosis (1). In approximately, 70% of all cases of ovarian cancer, the disease is not diagnosed before reaching an advanced stage (2). The 5-year survival rate associated with ovarian cancer is $<30\%$ (3). Over 90% of all cases of ovarian masses detected in premenopausal and $\leq 60\%$ in postmenopausal women, are benign (4). The early diagnosis of ovarian malignant tumor becomes a key factor in improving the survival rate of patients. Tools currently in use for differentiating between low- and high-risk patients with ovarian cancer are the tumor markers carbohydrate antigen-125 (CA-125) and the human epididymis protein 4 (HE4), as well as the index value of risk of ovarian malignancy algorithm (ROMA) (5).

The tumor marker CA-125 has been used for 30 years for the monitoring of ovarian cancer, diagnosis, effective evaluation, and recurrence (6). Although clinical application of CA-125 has been extensive, its specificity as a marker of malignant tumor or early diagnosis of ovarian cancer requires reassessment (7). In premenopausal women, the detection of CA-125 in ovarian cancer sensitivity and specificity is not ideal because of the menstrual cycle, pregnancy and other effects (8).

The introduction of HE4, a type of gynecological tumor marker, has attracted much attention. HE4 has shown a sensitivity and specificity of 72.9 and 95%, respectively, for differentiating between types of ovarian masses, which is better than that of CA-125 detection (9). HE4 is highly expressed in ovarian cancer, endometrial cancer tissues and in the adjacent tissues, normal tissues and benign tumors (10). Consequently, as an ideal tumor marker, HE4 has received increased attention. It has been confirmed (10-14) that HE4 has an obvious difference in the expression level between benign gynecological diseases such as ovarian cyst, uterine fibroids, endometriosis, endometrial polyps and other ovarian cancers, including endometrial and cervical cancer, which can be used for the differential diagnosis of the disease. However, in order to utilize the value of existing detection and to further improve the accuracy of early diagnosis of ovarian cancer while simultaneously assessing the risk of ovarian cancer and combining the research results and the relevant statistical analysis, the ROMA index value (11-16) has been introduced (17). The ROMA index value is an algorithm that takes into account the levels

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Key words: carbohydrate antigen-125, epididymis secretory protein 4, risk of ovarian malignancy algorithm index, ovarian cancer

of CA-125 and HE4 together with menopausal status using quantitative and objective parameters (18). The sensitivity and specificity of ROMA are 88.7 and 74.7%, respectively, when applied in cohorts of pre- and postmenopausal women (17). Previous investigations on the application of HE4 and ROMA in ovarian cancer with results indicated improvement in the diagnostic accuracy of ovarian cancer. In the present study, we evaluated the values of these tools in the global and differential diagnosis of ovarian cancer. We thus analyzed the sera levels of HE4, CA-125 and determined the values of the ROMA index combined with menopausal status in patients suffering ovarian carcinoma, as confirmed by surgical treatment in Xuzhou Central Hospital (Jiangsu, China). The period of the study was from October 2013 to May 2015.

Subjects and methods

Clinical data. In total, the present study included 158 cases, which were divided into the ovarian cancer, benign ovarian disease and healthy control groups. Selected patients did not receive chemotherapy or hormonal therapy, or a combination thereof for other tumors or serious heart, liver and kidney disease, or diabetes. A total of 64 patients in the ovarian cancer group were selected between October 2013 and May 2015 in Xuzhou Central Hospital with pelvic mass, which was examined and confirmed by postoperative pathological findings. There were 14 cases of papillary serous cystadenocarcinoma, 1 case of clear cell carcinoma, 7 cases of mucinous cystadenocarcinoma and 42 cases of serous cystadenocarcinoma.

According to the staging method of the International Federation of Gynaecology and Obstetrics, 10 cases were stage I, 18 cases of stage II, 23 cases of stage III and 13 cases of stage IV. The patients were aged 30-51 years with an average age of 55 ± 11.9 years. Twenty-seven patients were in premenopausal status (aged 30-51 years, average age 43.8 ± 6.08 years) while 37 patients were in postmenopausal status (aged 47-81 years, average age 63 ± 7.9 years). The 64 patients were in the benign ovarian disease group (6 cases of ovarian serous cystadenoma, 14 cases of ovarian mucinous cystadenoma, 30 cases of mature ovarian teratoma, 5 cases of theca cell tumor and 9 cases of ovarian endometriosis cyst). The patients were aged 24-82 years, with an average age of 47.81 ± 13.9 years. Of the 64 patients, 40 patients were in premenopausal status (aged 24-47 years, average age 38.9 ± 6.8 years), while 24 patients were in postmenopausal status (aged 50-82 years, average age 62.7 ± 9.2 years). Thirty normal females in the healthy control group identified during the same period with no liver and kidney disease and no tumor history, were included. The patients were aged 30-63 years, with an average age of 45.2 ± 8.25 years. Of the 30 cases, 21 cases were at a premenopausal status, aged 30-49 years with an average age of 40.8 ± 5 years. Nine cases were of postmenopausal status with an age of 51-63 years and an average age of 55.7 ± 3.4 years.

All the subjects provided written inform consent. The study was approved by the Ethics Committee of the Xuzhou Central Hospital.

Sample collection. Samples were collected from all the patients prior to surgery and 3 ml blood was collected. Serum was centrifuged at 2000 x g and stored at -20 and -80°C until use.

Table I. Sera levels of HE4, CA-125 and ROMA index of three groups.

Parameters	Healthy control group	Benign tumor group	Ovarian cancer group
Cases	30	64	64
HE4	39.04 ± 8.38	54.76 ± 42.35	$739.03 \pm 860.04^{a,b}$
CA-125	15.08 ± 5.28	49.07 ± 175.61^a	$868.85 \pm 1204.08^{a,b}$
ROMA index	6.18 ± 2.21	10.15 ± 11.98	$76.30 \pm 28.57^{a,b}$

The three parameters were significantly increased in the ovarian cancer group while only CA-125 was significantly increased in the benign tumor group relative to the healthy control group. ^aCompared with those of the control group, $P < 0.05$; ^bCompared with those of the benign tumor group, $P < 0.05$. HE4, human epididymis protein 4; CA-125, carbohydrate antigen-125; ROMA, risk of ovarian malignancy algorithm.

Sample detection. Serum CA-125 and HE4 were detected using the full automatic chemiluminescence analyzer Cobs601 and the corresponding kit according to manufacturer's protocol (Roche Diagnostics, Indianapolis, IN, USA). Briefly, serum HE4 and CA-125 levels were calculated for ROMA index value using the Roche ROMA index of ovarian cancer risk assessment software. Serum HE4 and CA-125 reference range was <140 pmol/l and <35 U/ml, respectively.

ROMA index calculation. The ROMA index was calculated according to the levels of HE4 and CA-125. HE4 and CA-125 values were input to the ovarian cancer risk assessment software, followed by automatic calculation of the corresponding ROMA index. The premenopausal calculation formula of the ROMA index was: $12 + 2.38 \times \text{LN}(\text{HE4}) + 0.0626 \times \text{LN}(\text{CA-125})$. The postmenopausal calculation formula of the ROMA index was: $8.09 + 1.04 \times \text{LN}(\text{HE4}) + 0.732 \times \text{LN}(\text{CA-125})$. When Roche Elecsys specificity was 75%, premenopausal women with a ROMA value ≥ 11.4 , had a higher risk of ovarian cancer. Postmenopausal women with ROMA value ≥ 29.9 had a higher risk of ovarian cancer.

Statistical analysis. SPSS 16.0 statistical software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. HE4, CA-125, ROMA index and other non-normal measurement data were shown as the quartile interval. The count data were shown using rate. The use of the rank sum test (Man-whitney U test) and Chi-square test data were statistically analyzed. The area under curve (AUC) of receiver operating characteristic (ROC) were calculated for a comparison of the three test methods. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of the difference of serum HE4 and CA-125 levels and the ROMA index between groups. The serum levels of HE4, CA-125 and ROMA index in the ovarian cancer group were significantly higher than those in the benign tumor and

Table II. The diagnostic values of CA-125, HE4 and ROMA in ovarian cancer compared with the golden standard.

Characteristics	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Total				
CA-125	85.07 (57/64)	92.31 (84/94)	90.6 (57/67)	89.36 (84/91)
HE4	75 (48/64)	97.87 (92/94)	96 (48/50)	85.19 (92/108)
ROMA index	93.75 (60/64)	92.55 (87/94)	89.55 (60/67)	86.14 (87/101)
Premenopausal				
CA-125	92.59 (25/27)	88.52 (54/61)	78.13 (25/32)	96.43 (54/56)
HE4	70.37 (19/27)	98.36 (60/61)	95.00 (19/20)	88.24 (60/68)
ROMA index	96.3 (26/27)	88.52 (54/61)	78.79 (26/33)	98.18 (54/55)
Postmenopausal				
CA-125	86.49 (32/37)	90.9 (30/33)	91.43 (32/35)	90.90 (30/35)
HE4	78.38 (29/37)	96.97 (32/33)	96.67 (29/30)	80.00 (32/40)
ROMA index	91.89 (34/37)	96.97 (32/33)	97.14 (34/35)	91.43 (32/35)

Sensitivity and specificity of the positive and negative predictive values of HE4, CA-125 and ROMA standardized with pathological diagnosis were comparable between the groups. HE4, human epididymis protein 4; CA-125, carbohydrate antigen-125; ROMA, risk of ovarian malignancy algorithm.

Table III. The diagnostic values of CA-125, HE4 and ROMA in ovarian cancer.

Characteristics	CA-125		HE4	
	Positive	Negative	Positive	Negative
Total				
ROMA index				
Positive	57	10	48	18
Negative	10	81	2	90
χ^2	86.721		89.755	
P-value	<0.001		<0.001	
Premenopausal				
ROMA index				
Positive	25	8	19	13
Negative	7	48	1	55
χ^2	35.41		39.27	
P-value	<0.001		<0.001	
Postmenopausal				
ROMA index				
Positive	32	2	29	5
Negative	3	33	1	35
χ^2	51.47		48.62	
P-value	<0.001		<0.001	

The ROMA index and a comparison of the sera levels of CA-125 and HE4 in the diagnosis of ovarian cancer between the three groups were significantly different. HE4, human epididymis protein 4; CA-125, carbohydrate antigen-125; ROMA, risk of ovarian malignancy algorithm.

healthy control groups, and there was significant difference ($P<0.05$). The expression level of HE4 and ROMA index in the benign tumor group was not significantly different. The

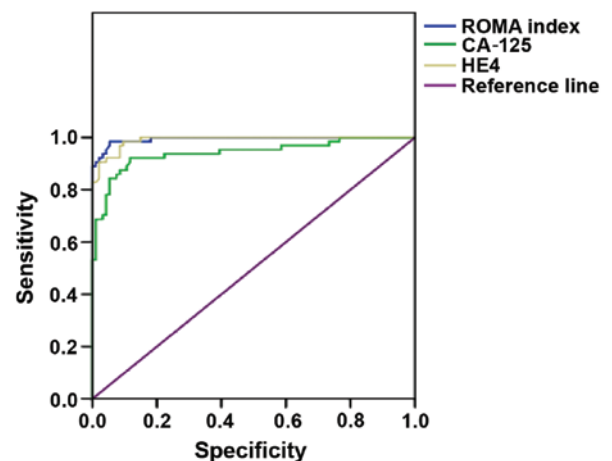


Figure 1. The receiver operating characteristic of risk of ovarian malignancy algorithm (ROMA) index, human epididymis secretory protein 4 (HE4) and carbohydrate antigen-125 (CA-125) in the diagnosis of ovarian cancer.

expression level of CA-125 in serum was significantly higher than that in the healthy control group ($P<0.05$, Table I).

Evaluation of serum HE4, CA-125 and ROMA in the diagnosis of ovarian cancer. The patients in the ovarian benign disease and healthy control groups were further divided into the pre- and postmenopausal groups. The patients with ovarian cancer were divided into the pre- and postmenopausal groups. The serum levels of HE4, CA-125 and ROMA index were detected to evaluate the sensitivity, specificity, positive predictive value and negative predictive value of HE4, CA-125 and ROMA standardized with pathological diagnosis (Table II). The ROMA index, and a comparison of the sera levels of CA-125 and HE4 in the diagnosis of ovarian cancer in each group indicated significant differences between the three groups ($P<0.001$, Table III). The AUC of ROC of the ROMA index,

HE4 and CA-125 in the diagnosis of ovarian cancer gradually decreased to 0.994, 0.990 and 0.941, respectively (Fig. 1).

Discussion

The early diagnosis of ovarian malignancies is one of the key factors for improving the survival rate of patients (19). CA-125 has been used as a tumor marker for the diagnosis and monitoring of ovarian cancer for 30 years, and is also used for efficacy evaluation and monitoring of recurrence (8). Data have shown that the serum levels of CA-125, HE4 and ROMA in ovarian cancer patients were significantly higher than those of the patients with ovarian benign disease and healthy women (20). The specificity and positive predictive value of HE4 for ovarian cancer was the highest, and the sensitivity of ROMA index was the highest. In the present study, the 158 cases were divided into the premenopausal and postmenopausal group to evaluate the three indicators in the diagnostic value of ovarian cancer. The ROMA index demonstrated the highest sensitivity and negative predictive value for ovarian cancer. HE4 had the highest specificity and positive predictive value. The specificity of HE4 for ovarian cancer was higher in the postmenopausal women, as reported elsewhere (21). The sensitivity, specificity, positive predictive value and negative predictive value of the ROMA index in ovarian cancer were the highest (91.89, 96.97, 97.14 and 91.45%), respectively. CA-125 and HE4 were significantly different from the ROMA index, and the ROMA index was significantly better than CA-125 and HE4 in the diagnosis of ovarian cancer. In addition, the ROC curve drawn in this study for the benign tumor of ovary and healthy control groups, identified that the area under the ROC curve of CA-125, HE4 and ROMA index was increased by 0.941, 0.990 and 0.994, respectively. This result confirmed the clinical diagnostic value of the ROMA index (5). It also showed that detection of ROMA index in the diagnosis of ovarian cancer was higher than CA125 and HE4.

In conclusion, application of the ROMA index and HE4 for the diagnosis of ovarian cancer was found to be effective and it has good clinical application value, which may be useful for clinicians.

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