

# Using HE4, RMI, ROMA and CPH-I in the differential diagnosis of adnexal masses

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**Objectives:** In order to improve the early diagnosis of ovarian cancer (OC), we evaluated human epididymis secretory protein 4 (HE4), the risk of malignancy index (RMI), the risk of ovarian malignancy algorithm (ROMA), and the Copenhagen Index (CPH-I) in their ability to predict the risk of adnexal masses being malignant, and in discriminating between serous and mucinous ovarian tumors. **Methods:** A study consisting of 509 patients who were diagnosed with benign, borderline, and malignant adnexal tumors was conducted. We analyzed the values of HE4, RMI, ROMA, and CPH-I. In addition, CA125, CEA, CA125/CEA ratio, CA199, and CA125/CA199 ratio were also assessed to determine their predictive values to diagnoses serous or mucinous ovarian tumors. **Results:** To differentiate between benign and borderline ovarian tumors, we evaluated RMI and CPH-I scores which showed high AUC (0.7593, and 0.7128, respectively), but neither of them showed both high sensitivity and specificity. When discriminating benign and malignant tumors, RMI and CPH-I performed the best for premenopausal women; whereas ROMA and HE4 performed the best for postmenopausal women. Patients with values above the cutoff for CA125 ( $> 328.3$  U/mL), CA125/CEA ( $> 82.26$ ), and CA125/CA199 ( $> 5.633$ ), were more likely to be diagnosed with serous carcinoma. Whereas those with higher values for CEA ( $> 3.7$   $\mu$ g/L) and CA199 ( $> 27.81$  ng/mL) were more likely to have a mucinous carcinoma. **Conclusion:** RMI and CPH-I for premenopausal women, ROMA and RMI for postmenopausal women were reliable indicators to differentiate between women with benign versus malignant tumors. In addition, the ratios CA125/CEA and CA125/CA199 could be used to distinguish serous from mucinous ovarian carcinomas.

## Keywords

CPH-I; HE4; Ovarian tumors; RMI; ROMA

## 1. Introduction

Ovarian cancer (OC) is the seventh most commonly diagnosed cancer among women in the world population. It is also the most lethal gynecologic cancer worldwide and the second most lethal gynecologic cancer in China [1, 2]. Adnexal masses are usually found in the gynecologic clinic during general physical examination. Earlier stage OC results in better survival outcome, whereas more than two-thirds of

the cases have advanced stage at diagnosis [3], who need to be referred to specialty centers for gynecologic cancer surgery by the experts from special multiple department teams (SMDT). Early identification of OC and borderline ovarian tumors (BOT), which are defined as having low malignant potential, is very critical to determine different treatments, such as (i) the extent and method of surgery, (ii) the need to preserve fertility, and (iii) the need of postoperative chemotherapy and infertility treatments [4]. Thus, precise preoperative evaluation of adnexal masses is important for early referral and optimal treatment, requiring specific and sensitive methods. While it is challenging to differentiate between benign, borderline and malignant tumors, patients with ovarian cancer are usually asymptomatic or with non-specific symptoms until in advanced stage.

Although imaging could differentiate ovarian cancer from benign tumors well [5], the diagnostic accuracy of ultrasound for BOT of all stages is only 69% and the specificity of MRI for diagnosis of BOT is only 45.4% [4]. However, the evaluation using imaging requires considerable experience that might not be available at the facilities which are not specialized in this area. Thus, patients could not be referred to specialized gynecologic oncology centers on time.

Concerning laboratory exams, several tumor biomarkers have been evaluated. CA125 (Carbohydrate Antigen 125) has been used as the traditional indicator of ovarian cancer for a long time. However, this measurement is not very sensitive in the early phases of ovarian cancer since it is only elevated in 23 to 50% of stage I cases [6]. In addition, CA125 levels are also elevated in other physiological or pathological conditions, such as menstruation, pregnancy, endometriosis, and inflammatory diseases of the peritoneum [6], which leads to a low specificity (78%) for detecting ovarian cancer [7]. Human epididymis secretory protein 4 (HE 4), has also been studied extensively with regard to the differential diagnosis of ovarian masses. HE4 exhibits relatively normal levels in benign gynecological pathologies, it has better specificity than the

CA 125 in the diagnosis of early stage OC [8]. However, it is not very sensitive, and its level is affected by age, smoking and contraceptive [9, 10]. For these reasons, algorithms, such as the risk of malignancy index (RMI), the risk of ovarian malignancy algorithm (ROMA) and the Copenhagen Index (CPH-I) were developed in an attempt to improve the inherent weaknesses of these biomarkers, combined with CA125, HE4, age, menopause and ultrasound manifestation [11, 12]. However, there is some controversy regarding the advantages of these indices and there are only few studies related to the differential diagnosis between benign, borderline and malignant ovarian tumors, especially in establishing the cut off points between benign and BOT. This study was conducted explicitly to address these issues.

As for the different prognosis and surgical strategy for ovarian malignant tumors, it is crucial to accurately evaluate the tumor pathology types before surgery [13]. Most of the patients with pelvic mucinous carcinomas are diagnosed as having digestive tract tumors after surgery [14], and these patients should be operated on by gastrointestinal surgical experts. Therefore, the patients who are suspected of having mucinous tumors should be recommended for colonoscopy and gastroscopy before surgery. In this study, we also investigated the value of CA125, CEA, CA199, CA125/CEA, and CA125/CA199 as predictors of serous and mucinous pathological types.

Firstly, we aimed to evaluate the performance of CA-125, HE4, RMI, ROMA and CPH-I as the diagnostic tools for differential diagnosis between benign, borderline and malignant ovarian tumors. We also evaluated the proper cutoff point of these indices for this population so that women with adnexal masses can be evaluated and referred to a specialty center as quickly as possible; secondly, we wanted to find tests that can predict whether malignant tumors are more likely to be serous or mucinous. The purpose of this study was to stratify the patients with adnexal mass to achieve optimal treatment in special centers.

## 2. Methods

### 2.1 Patients

A total of 509 women diagnosed with ovarian malignant tumor (231 cases), borderline ovarian tumor (51 cases), or benign ovarian disease (227 cases) were identified and retrospectively analyzed after surgery at the first Affiliated Hospital of University of Science & Technology of China from July 2005 to June 2018. The diagnoses of the patients are shown in Table 1. We included patients who had a diagnosis of an ovarian cyst or a pelvic mass with a planned surgical intervention and a definite diagnosis after surgery. Those cases with previous ovarian cancer treatment, a history of oophorectomy, unresectable mass, another benign or malignant tumors, other gynecological diseases (e.g., pelvic infection), liver disease, renal disease, lung disease, coronary heart disease, autoimmune diseases, incomplete required data, or pregnancy were excluded from our study group. The post-

menopausal state was defined as more than 1 year of amenorrhea at the time of diagnosis, and patients who had undergone hysterectomy were considered postmenopausal at the age of 50 or older [15].

Before the operation, all subjects were required to have a sample of fasting venous blood collected in the morning. Electrochemiluminescence immunoassay (ELICA) was applied in measuring the levels of CA125, HE4, CEA and CA199 with the Cobas e 601 analyzer from Roche Diagnostics following the manufacturer's instructions. These biomarkers were measured as a part of routine work in cases with adnexal masses in our hospital, the data was acquired from patients' medical records. The normal standard cutoff value for serum CA125 was 35 U/mL for premenopausal patients and 20 U/mL for postmenopausal patients. The cut off values for HE4 was 70 pmol/L, for serum CEA was 5.0  $\mu\text{g/L}$ , and for CA199 was 37 ng/mL. During the study period the principle of the detection of these markers in our center has not changed, which is always electrochemiluminescence immunoassay (ELICA), and the normal reference values have not changed. Although personnel changes are unavoidable, all measurements are carried out according to the manufacturer's instructions leading to minimal variation.

The study was approved for retrospective data analysis by the First Affiliated Hospital of the USTC Expert Commission for Physician Confidentiality, and by the ethics review board of the First Affiliated Hospital of the USTC, file # 2018KY52. All patients underwent ultrasonography before surgery, and CT or MRI was performed on the patients with a solid echo mass.

### 2.2 RMI

RMI was calculated by serum CA125 level, ultrasound scan result (score of 0, 1, or 3), and menopausal status (1 for premenopausal and 3 for postmenopausal). Ultrasound scores were calculated as follows: multilocularity, solid areas, bilaterality, ascites, and intra-abdominal metastases were scored 1, respectively; total scores of 0, 1, and those above 2 points yielded U values of 0, 1, and 3, respectively. The RMI algorithm was the following equation:  $\text{RMI} = \text{U} \times \text{menopausal status} \times \text{CA125}$ . Patients with an RMI greater than 200 were considered to be at a high risk of carcinoma [11].

### 2.3 ROMA

Serum HE4, CA125, and menopausal status were used for ROMA calculation. ROMA calculates the coefficient for the natural log (LN) of serum values and integrates it into a logistic regression formula using the following equation:

premenopausal [Predictive Index (PI) =  $-12.0 + 2.38 \times \text{LN}(\text{HE4}) + 0.0626 \times \text{LN}(\text{CA125})$ ]

postmenopausal [PI =  $-8.09 + 1.04 \times \text{LN}(\text{HE4}) + 0.732\text{LN}(\text{CA125})$ ]. ROMA index =  $\exp(\text{PI}) / [1 + \exp(\text{PI})] \times 100$ . The ROMA cutoff value was 11.4% for premenopausal patients and 29.9% for postmenopausal patients; above those values was defined as high risk [6].

**Table 1. Characteristics of the patients.**

	Benign	Borderline	Malignant	$P_{12}$ value <sup>h</sup>	$P_{13}$ value <sup>i</sup>	$P_{23}$ value <sup>j</sup>
<b>Numbers</b>	231	51	227			
<b>Mean age, years</b>	37.97 ± 16.18	43.65 ± 17.11	51.38 ± 14.96	0.091	< 0.001	0.008
<b>Menopausal status</b>						
Pre-menopausal	181 (78.35%)	36 (70.59%)	106 (46.70%)	0.233	< 0.001	< 0.001
Post-menopausal	50 (21.65%)	15 (29.51%)	121 (53.30%)			
<b>FIGO stage</b>						
I	/	/	42 (18.50%)	NA	NA	NA
II	/	/	16 (7.05%)			
III	/	/	138 (60.53%)			
IV	/	/	31 (13.66%)			
<b>Pathologic grade</b>						
Well differentiated	/	/	6 (2.64%)	NA	NA	NA
Moderately differentiated	/	/	9 (3.96%)			
Poorly differentiated	/	/	143 (63.00%)			
NA	/	/	69 (30.40%)			
<b>Histological pathology</b>						
Serous	23 (9.96%)	22 (43.14%)	142 (62.56%)	< 0.001	< 0.001	< 0.001
Mucinous	32 (13.85%)	27 (52.94%)	28 (12.33%)			
Clear cell	0	0	19 (8.37%)			
Endometrial	12 (5.19%)	2 (3.92%)	2 (0.88%)			
Sex cord-stromal	9 (3.90%) <sup>d</sup>	0	3 (1.32%) <sup>e</sup>			
Germ cell	149 (64.50%) <sup>f</sup>	0	23 (10.13%) <sup>g</sup>			
Others	6 (2.60%) <sup>h</sup>	0	10 (4.41%) <sup>i</sup>			

a.  $P_{12}$  value of comparison between benign and borderline ovarian tumors.

b.  $P_{13}$  value of comparison between benign and malignant ovarian tumors.

c.  $P_{23}$  value of comparison between borderline and malignant ovarian tumors.

d. 9 cases of thecoma cell tumor.

e. 3 cases of granular cell tumor.

f. 149 cases of mature cystic teratoma.

g. 2 cases of mixed germ cell tumor; 4 cases of cancellations of cystic mature teratoma; 4 cases of endodermal sinus tumor; 4 cases of immature teratoma; 9 cases of dysgerminoma.

h. Multiple mixed ovarian cystadenoma; tubo-ovarian cyst; ovarian lymphangioma; ovarian serous cystadenoma with thecoma cell tumor; mesosalpinx cyst; leiomyoma of the left adnexa, and mature cystic teratoma of right ovary.

i. Mixed ovarian tumors (endometrial adenocarcinoma and serous adenocarcinoma); mesothelioma; ovarian sarcomatous carcinoma; interovarian variant diffuse large B-cell lymphoma; 2 cases of ovarian small round cell carcinoma; mixed ovarian tumors (mucinous adenocarcinoma and clear cell carcinoma); mixed ovarian tumors (serous adenocarcinoma and clear cell carcinoma) of the left ovary and serous adenocarcinoma of the right ovary; pelvic mesenchymal-origin tumors; vascular-origin tumor.

NA: Not available.

#### 2.4 CPH-I

The Copenhagen Index was calculated by serum CA125, HE4, and the age of the patient. The algorithm of the CPH-I is as follows. The coefficients in CPH-I are:  $CPH-I = -14.0647 + 1.0649 \times \log_2(HE4) + 0.6050 \times \log_2(CA125) + 0.2672 \times \text{age}/10$ . The predicted probability (PP) is:  $PP = e(CPH-I) / (1 + e(CPH-I))$ ; and the patients with the PP greater than 7% were considered at high risk for carcinoma [12].

#### 2.5 Statistical analysis

Data was analyzed using Graph Pad Prism and SPSS Data Editor 18.0. The continuous variables were expressed in mean, standard deviation (SD) and range, and the categorical variables were expressed in counts and percentages. The Mann-Whitney test or analysis of variance (ANOVA) was

used to compare the test values in different groups.  $P$  values for comparisons of the proportion of patients were determined by Chi square test. We made overall consideration of the diagnostic performance of CA-125, HE4, RMI, ROMA and CPH-I by calculating sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV), efficiency, as well as constructing the ROC curve and calculating the area under the curve (AUC). To find the optimal cut-off value, sensitivity and specificity of each cut-off point was generated by computerized program and the most appropriate value was selected by maximizing the Youden index on the basis of AUC. Multivariable logistic regression analyses were performed to find out the possible confounders which are common at this group of patients with factors with

**Table 2. Comparison of HE4, RMI, ROMA Index and CPH-I in the assessment of adnexal mass.**

	HE4 (pmol/L)	RMI	ROMA (%)	CPH-I (%)
Benign	35.29 ± 66.68	232.54 ± 1687.22	6.81 ± 14.30	2.24 ± 8.03
BOT	43.51 ± 51.72	479.55 ± 838.99	10.86 ± 14.73	5.26 ± 9.25 <sup>a</sup>
Malignant	294.04 ± 404.96 <sup>a,b</sup>	5474.71 ± 13230.96 <sup>a,b</sup>	51.55 ± 36.98 <sup>a,b</sup>	42.37 ± 40.83 <sup>a,b</sup>

a. Compared with the group of benign ovarian tumors,  $P < 0.05$ .

b. Compared with the group of BOT,  $P < 0.05$ .

BOT: Borderline ovarian tumor.

$P$  value of  $< 0.1$  by univariate analyses. A level of  $P$  value  $< 0.05$  was considered statistically significant.

### 3. Results

#### 3.1 Clinical characteristics of patients

A total of 509 eligible cases previously treated in our hospital were enrolled in this study. The characteristics and the histopathology classifications of the cases in this study are shown in Table 1. Epithelial ovarian cancer made up most of the malignant tumors i.e.170 cases (74.8%), and serous ovarian cancer accounted for 62.56% of the total malignant tumors in this study. The patients with malignant ovarian tumors tend to be older and more are menopausal than those with benign and borderline ovarian tumors. Then we conducted multivariate regression analysis to find out the possible confounders which are common at this age of patients with factors of  $P$  value  $< 0.1$  at univariate analyses and serum biomarkers (Table S1). We found that age was significantly associated with the differential diagnosis between benign and borderline, whereas age had not significantly associated with benign vs. malignant, and BOT vs. malignant. Menopausal status was not found to be helpful in differentiating between the three groups.

#### 3.2 Comparison of HE4, RMI, ROMA, and CPH-I in the assessment of the adnexal tumors

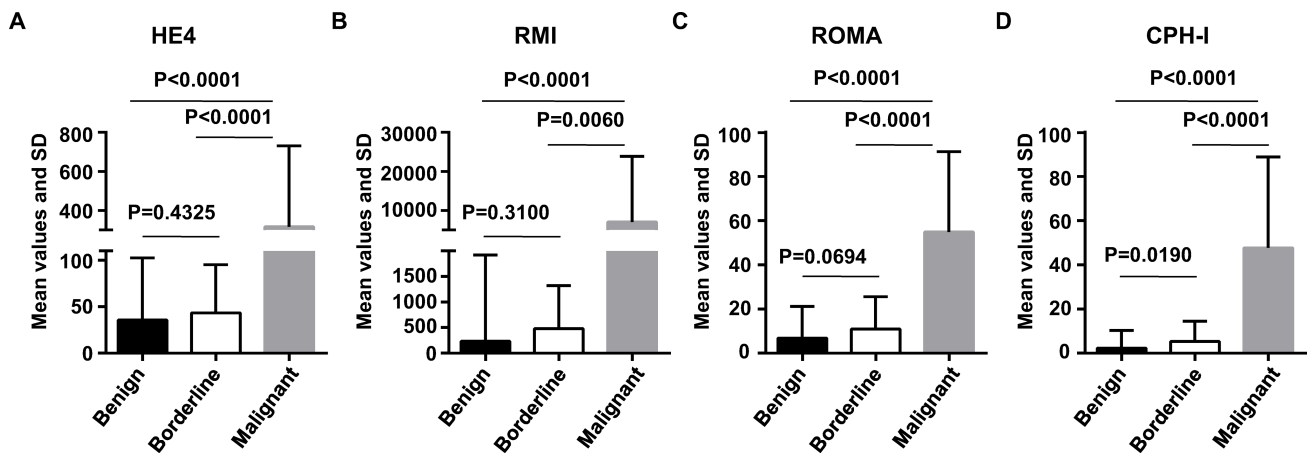
The means and SDs of HE4, RMI, ROMA, and CPH-I levels of patients with benign, borderline, and malignant ovarian tumors are shown in Table 2 and Fig. 1. According to our statistical data, we found that there was no difference between the benign and borderline tumors in the values of HE4, RMI, and ROMA. While the value of CPH-I was significantly elevated in BOT which was higher than that of benign ovarian tumors ( $P = 0.0190$ ). We then conducted ROC curves for HE4, RMI, ROMA and CPH-I (Fig. 2), with the speculation that RMI and CPH-I might be suitable to differentiate benign and borderline ovarian tumors (AUC = 0.7593, and AUC = 0.7128, respectively). However, after the evaluation of diagnostic performance of RMI and CPH-I, by calculating sensitivity, specificity, PPV, NPV and accuracy at standard and optimal cut-off values (Table 3), we found that neither index showed both high sensitivity and specificity. We also evaluated CPH-I in different age groups (Table S2) and found it performed best among those under 30 years with the highest AUC of 0.9194 (sensitivity of 83.33% and specificity of 91.21% at optimal cut-off point).

All of the values of HE4, RMI, ROMA, and CPH-I in malignant ovarian tumors were significantly higher compared to those in benign ovarian tumors ( $P < 0.0001$ ) (Fig. 1). The ROC curves for HE4, RMI, ROMA, and CPH-I are shown in Fig. 3 for premenopausal and postmenopausal patients, respectively. We also observed that RMI (AUC = 0.9513) and ROMA (AUC = 0.9345) showed the highest AUC in the discrimination of benign and malignant ovarian tumors among pre- or postmenopausal patients, respectively. We also evaluated the diagnostic performance of these four indicators (HE4, RMI, ROMA, and CPH-I) at both standard and optimal cut-off values (Table 4). For the premenopausal patients, RMI (optimal cutoff value = 101) showed the highest sensitivity of 87.74% with a specificity of 90.06%; whereas, CPH-I (optimal cutoff value = 2.27%) performed the best in specificity (94.48%) with a sensitivity of 72.64%. For the postmenopausal patients, ROMA (optimal cutoff value = 18.51%) showed the highest sensitivity of 93.39%, with a specificity of 84.00%; whereas, HE4 (optimal cutoff value = 64.5 pmol/L) had a specificity of 92.00% with a sensitivity of 78.50%.

#### 3.3 CA125, CEA, CA199, CA125/CEA, and CA125/CA199 in the classification of serous and mucinous pathological types of ovarian cancer

We also evaluated the efficacy of CA125, CEA, CA199, CA125/CEA, and CA125/CA199 for the prediction of tumor histology (Table 5). For patients with serous ovarian tumors, the levels of CA125, CA125/CEA, and CA125/CA199 levels were significantly higher than those with mucinous ovarian tumors ( $P < 0.0001$ ). We then constructed ROCs for CA125, CEA, CA199, CA125/CEA, and CA125/CA199 based on the ability to discriminate serous from mucinous ovarian tumors (Fig. 4), and found that CA125/CEA and CA125/CA199 have higher AUCs (AUC = 0.9079, and AUC = 0.9132, respectively). These results suggested that CA125/CEA and CA125/CA199 were superior in the classification of ovarian mucinous and serous tumors.

According to the cutoff values selected by maximizing the Youden index on the basis of AUC, ovarian cancer patients, with CA125, CA125/CEA, and CA125/CA199 above 328.3 IU/mL, 82.26, and 5.633 respectively, are more likely to be serous subtypes. While the cutoff values for consideration of mucinous subtypes were  $> 3.7 \mu\text{g/L}$  and  $> 27.81 \text{ ng/mL}$  of CEA and CA199, respectively.



**Fig. 1. HE4, RMI, ROMA, and CPH-I in the discrimination of adnexal mass.**

(A) HE4 in the discrimination of ovarian tumors from benign, borderline, and malignant; (B) RMI in the discrimination of ovarian tumors from benign, borderline, and malignant; (C) ROMA in the discrimination of ovarian tumors from benign, borderline, and malignant; (D) CPH-I in the discrimination of ovarian tumors from benign, borderline, and malignant. SD: standard deviations.

**Table 3. HE4, RMI, ROMA Index and CPH-I in the discrimination of benign vs. BOT adnexal mass.**

	AUC	Cutoff Value	Sensitivity (%)	Specificity (%)	Positive predictive values (%)	Negative predictive values (%)	Accuracy (%)
<b>RMI</b>							
SCV	0.7593	200	41.18	90.04	47.73	87.39	81.21
OCV		54.42	78.43	63.64	32.26	93.04	66.31
<b>CPH-I</b>							
SCV	0.7128	7%	23.53	93.51	44.44	84.71	80.85
OCV		1.048%	64.71	76.62	37.93	90.77	74.47

AUC: Area under the curve; SCV: Standard cut-off value; OCV: Optimal cut-off value.

**Table 4. HE4, RMI, ROMA Index and CPH-I in the discrimination of benign vs. malignant adnexal mass.**

Parameter	Optimal cutoff values <sup>a</sup>						Standard cutoff values			
	AUC	Cutoff Value	Sensitivity (%)	Specificity (%)	Accuracy (%)	Cutoff Value	Sensitivity (%)	Specificity (%)	Accuracy (%)	
<b>Pre-menopausal</b>	HE4	0.8092	49.5	68.87	89.5	81.88	70 pmol/L	60.38	95.58	82.58
	RMI	0.9513	101.0	87.74	90.06	89.20	200	80.19	95.03	89.90
	ROMA	0.8155	7.61%	69.81	89.5	83.62	11.4%	62.26	93.37	81.88
	CPH-I	0.8288	2.27%	72.64	94.48	86.41	7%	58.49	95.03	81.53
<b>Post-menopausal</b>	HE4	0.8865	64.5	78.50	92.00	82.46	70 pmol/L	77.69	92.00	81.87
	RMI	0.9005	538.9	79.34	90.00	82.46	200	91.74	70.00	85.38
	ROMA	0.9345	18.51%	93.39	84.00	90.06	29.9%	87.60	88.00	87.13
	CPH-I	0.8516	3.32%	79.34	86.00	81.29	7%	72.73	88.00	77.19

a. The optimal cut-off values were selected by maximizing the Youden index on the basis of AUC.

AUC: Area under the curve.

## 4. Discussion

How to precisely assess the risk for women with pelvic masses is still crucial for optimal treatment and to improve the survival rates. In our study, we have compared the ability of HE4, RMI, ROMA, and CPH-I in differentiating ovarian masses between benign, borderline and malignant tumors, which will ultimately optimize patient treatment and referral to the appropriate special gynecological oncology centers when indicated.

It is difficult preoperatively distinguish between ovarian benign and borderline tumors and there are only a couple of

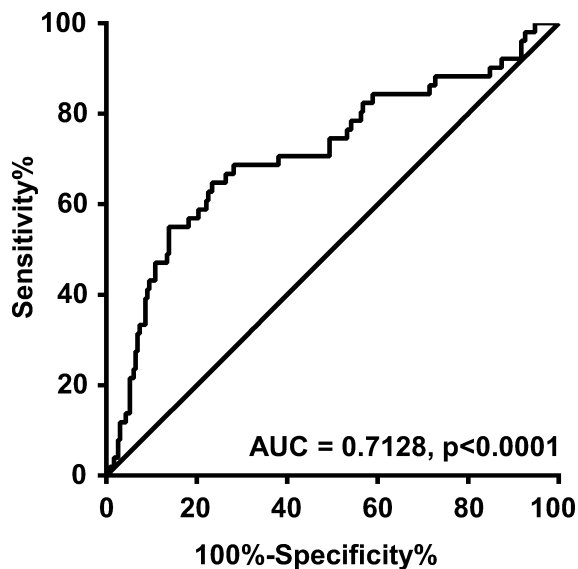
studies regarding the evaluation of the effectiveness of different methods that have been reported. Hada *et al.* compared the diagnostic performance of HE4, RMI and ROMA, in which none of the parameters was the ability to differentiate between benign and borderline tumors [16]. CPH-I was also evaluated but showed poor performance in discrimination of benign and borderline ovarian masses (AUC < 0.7) [17]. Zhang *et al.* found RMI performed better for of distinguishing BOT from benign tumors at the cut-off value of 60, with sensitivity of 63.58% and specificity of 87.60%. Thus, they suggested to use < 60, 60-200, > 200 as warning lines

**Table 5. CA125, CEA, CA125/CEA, CA199 and CA199/CEA in the discrimination of serous and mucinous adnexal mass.**

	n	CA125 (U/mL)	CEA ( $\mu\text{g/L}$ )	CA125/CEA	CA199 (ng/mL)	CA125/CA199
Serous	142	1714.90 $\pm$ 2741.43	3.26 $\pm$ 8.67	1626.60 $\pm$ 3319.70	23.13 $\pm$ 42.74	305.08 $\pm$ 709.41
Mucinous	28	237.65 $\pm$ 343.59	27.98 $\pm$ 56.91	99.28 $\pm$ 257.43	192.66 $\pm$ 284.62	63.97 $\pm$ 307.66
AUC		0.8446	0.8359	0.9079	0.8536	0.9132
OCV <sup>a</sup>		328.3	3.700	82.26	27.81	5.633
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

a. OCV: The optimal cut-off values were selected by maximizing the Youden index on the basis of AUC.

AUC: Area under the curve; OCV: Optimal cut-off value.



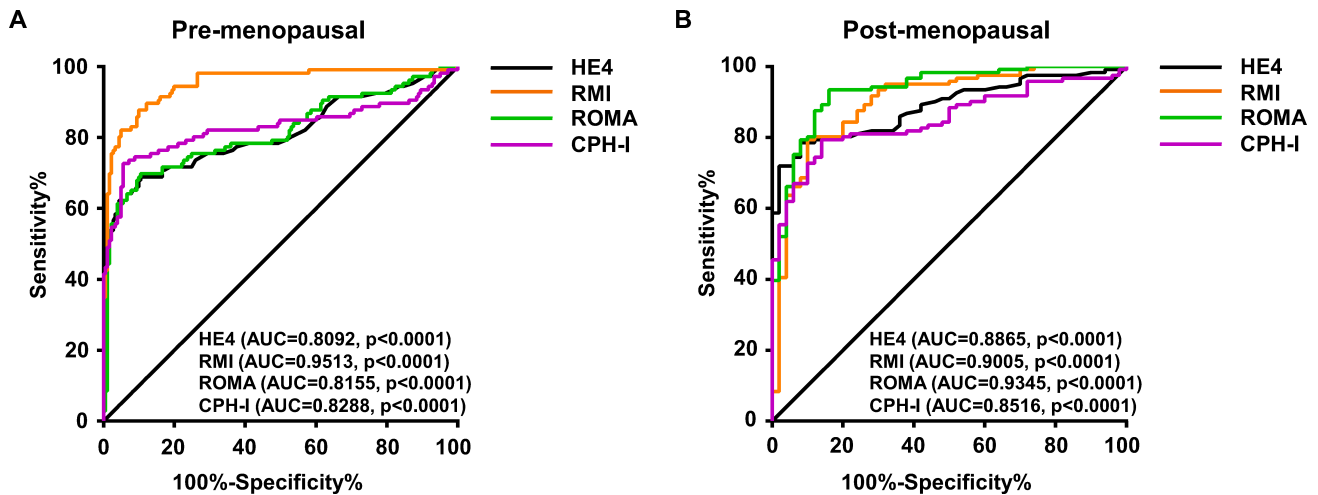
**Fig. 2. The ROC curves of CPH-I in the discrimination of adnexal mass.** P values of ROC curves are listed below the curves. AUC: Area under the curve.

for clinicians to distinguish benign, borderline and malignant ovarian tumors [18]. In other reports, RMI also showed increased values for BOT compared to benign tumors [11, 19]. In our study, RMI and CPH-I had slightly higher value, and higher AUC in the borderline group compared to borderline ovarian tumors. The diagnostic performance was not satisfactory at either the standard or optimal cut-off values. As the CPH-I is dependent on age, we also evaluated CPH-I in different age groups and found that CPH-I had a better diagnostic precision among women under 30 years (AUC = 0.9194). Therefore, we suggest that for women under 30 years of age, CPH-I is more appropriate to identify borderline tumors. However, further research with a more specific cohort and large sample size is required considering the variety of pathological types and other characteristic in this study.

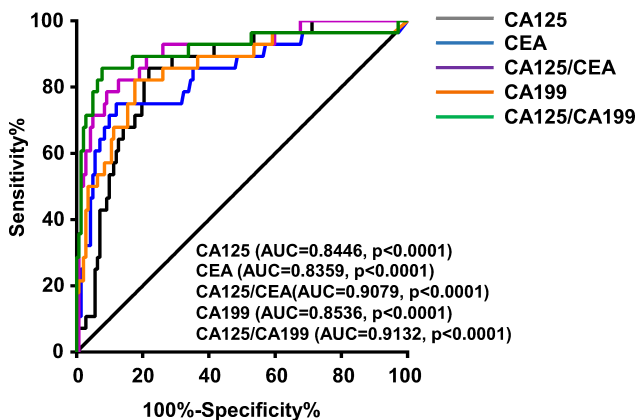
There are many reports on the use of HE4, RMI, ROMA, and CPH-I for the evaluation of patients with ovarian masses and their ability to differentiate benign and malignant tumors as shown in Table S3 [11, 17, 19–51]. RMI and ROMA are reported to have the highest AUC for the predicting malignancy [11, 20–23, 25–27, 29, 31–38, 40–42, 44–46, 48]. Many of the published studies from Europe support that MRI

is the best differentiator between benign and malignant tumors regardless of the patient’s menopausal status, and the AUC values ranged from 0.849 to 0.959 [21, 23, 25, 31, 45]; whereas, several studies support that ROMA is better for postmenopausal women [17, 19, 26, 27, 38, 46, 47]. Similarly, RMI and ROMA have equal prediction ability in studies published from America [11, 24]. In contrast, almost all Asian studies recommend ROMA as the best, especially for postmenopausal women [22, 29, 30, 33–36, 41, 42, 44, 48]. A study from Thailand found that RMI has better performance than HE4 and ROMA [32]. In our study, we found that RMI had the highest AUC and accuracy for premenopausal women, whereas ROMA had the highest AUC and accuracy for postmenopausal women. HE4 tended to have high specificity in the diagnosis of ovarian cancer, especially for premenopausal women, similar to the study of Musalhi *et al.* They reported that HE4 had a higher specificity (93%) for premenopausal patients with adnexal mass [29]. In another research study, HE4 demonstrated higher specificity than of RMI and ROMA for both premenopausal and postmenopausal women [26]. Therefore, we recommend that RMI could be used for premenopausal women with adnexal masses and ROMA could be used for postmenopausal women. The addition of HE4 could help avoid over diagnosis.

As shown in Table S3, we found that there were no regional differences in the use of the standard cutoff values of the four indicators. There were three main standard cutoff values for HE4: 70 pmol/L for all women; 140 pmol/L for all women; 70 pmol for premenopausal women and 140 pmol/L for postmenopausal women. Almost all the studies have used the same standard cutoff value for RMI of 200. There were three main standard cutoff values for ROMA, which were 13.1%/27.7%, 7.4%/25.3%, and 11.4%/29.9%. The standard cutoff values of HE4 are mainly decided by the manufacturer’s instructions, depending on the different detection method, such as immune-enzymatic (EIA) assay, electrochemiluminescent, (ECLIA) or chemiluminescent microparticle immunoassay (CMIA). However, the threshold value of HE4 used, including whether or not the menopausal status is considered, is left to the choice of clinicians [6]. The cutoff values of ROMA are also dependent on laboratory methods used for HE4 and CA125, hormonal status and also differ for pre- and postmenopausal women [52]. Moreover, we



**Fig. 3. The ROC curves of HE4, RMI, ROMA and CPH-I in the discrimination of adnexal mass between benign and malignant.**  
 A: The ROC curves for pre-menopausal patients to discriminate adnexal mass between benign and malignant; B: The ROC curves for post-menopausal patients to discriminate adnexal mass between benign and malignant. *P* values of ROC curves are listed below the curves. AUC: Area under the curve.



**Fig. 4. The ROC curves of CA125, CEA, CA125/CEA, CA199 and CA125/CA199 in the discrimination of serous or mucinous adnexal mass.** *P* values of ROC curves are listed below the curves. AUC: Area under the curve.

found that the optimal cutoff values used by Asian studies, especially Japan, were more similar to ours. The HE4 optimal cutoff values in our study (49.5 pmol/L and 64.5 pmol/L) are similar to those reported by Huy *et al.* (55.4 pmol/L and 59.3 pmol/L), and Fujiwara (44.0 pmol/L and 63.3 pmol/L) [40, 41]. Furthermore, only four groups of researchers reported their optimal cutoff value for RMI, and they were very different from each other [11, 29, 33, 45]. Our ROMA cutoff value (7.61%/18.51%) is similar to that of Fujiwara *et al.* (6.0%/19.6%) [41]. The optimal cutoff values used worldwide show variability which could be related to the heterogeneity of histologic types, characteristic of patients, and different methodology. In our study, the optimal cutoff values used are supported by the higher accuracy of diagnosis, but require further validation.

Thus far, there is no suitable biomarkers for the accurate diagnosis of mucinous or serous tumors before surgery [13].

However, it has been reported that the predictive value of a CA125/CEA < 25 in excluding EOC is 100%, which is helpful in the preoperative differential diagnosis between a primary ovarian cancer and a colorectal origin [14]. In our analysis, we found that CA125/CEA and CA125/CA199 had higher AUC than a single indicator such as CA125, CEA, CA199, suggesting that combining the tests facilitates the identification of mucinous versus serous tumors. We recommend that patients suspected of having mucinous cancer undergo a digestive tract scope before surgery. Only patients with mucinous cancer that is excluded to originate from digestive tract should be sent to gynecologic oncology specialty centers. In this study, we are the first to show that the CA125/CA199 ratio can be used to differentiate between mucinous and ovarian tumors, establishing the foundation for our future research direction.

However, our study has several limitations. First, our research has a monocentric design and is a retrospective study which might bias the results. Second, the results of our study are depended on the characteristic of a particular cohort which cannot be used directly in clinical practice. In order to acquire a more practical result, further clinical multi-center studies with larger sample size are needed. However, we hope that our study provides the foundation and direction for further research to solve critical questions of ovarian cancer screening, especially for clinicians who are making decisions based on these indicators for the optimal treatment of patients.

## 5. Conclusions

RMI and CPH-I for premenopausal women, as well as ROMA and RMI for postmenopausal women are reliable diagnostic indicators to differentiate between women with benign versus malignant adnexal tumors. CA125/CEA and CA125/CA199 can be used to diagnose serous and mucinous

ovarian carcinomas.

### Author contributions

ZS, CZ, LQ, TZ, ML, JZ, DW, YZ, XY: study concept and design and critical revision of the manuscript. ZS, XY: drafting of the manuscript. ZS, CZ, LQ: acquisition of data, analysis, and interpretation of data. ZS, TZ, ML, JZ: statistical analysis. DW, YZ, XY: study supervision. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

The study was approved for retrospective data analysis by the First Affiliated Hospital of the USTC Expert Commission for Physician Confidentiality, and by the ethics review board of the First Affiliated Hospital of the USTC, file # 2018KY52.

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### Conflict of interest

The authors declare no competing interests.

### Supplementary material

Supplementary material associated with this article can be found, in the online version, at <https://ejgo.imrpress.com/EN/10.31083/j.ejgo.2021.01.2192>.

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