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Comparison of HE4, CA125, and ROMA Diagnostic Accuracy

A Prospective and Multicenter Study for Chinese Women With Epithelial Ovarian Cancer

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Abstract: Risk of Ovarian Malignancy Algorithm (ROMA) combing human epididymis secretary protein 4 (HE4) and CA125 showed better diagnostic accuracy for epithelial ovarian cancer (EOC) when compared with HE4 or CA125 alone; however, other studies showed no or worse diagnostic accuracy. We aim to conduct a prospective and multicenter clinical trial to compare the diagnostic accuracy of HE4, CA125, and ROMA for EOC.

A prospective and multicenter (n = 9) trial including 2481 individuals was performed in Chinese women. HE4, CA125, and ROMA diagnostic accuracy were evaluated according to different menopausal status and stages of EOC. Their diagnostic values were evaluated by the area under curve (AUC) and compared by the Z scores. Diagnostic specificity of other kinds of participants (n = 1098) was also evaluated.

For discriminating between healthy control (HC) and EOC, only CA125 showed significant difference for discriminating HC and EOC in all the individuals when compared with HE4 and ROMA ($P < 0.001$ and $P = 0.02$, respectively), at the cutoff value of 31.5, the sensitivity (SN) and specificity (SP) were 88.6% and 97.1%. For discriminating between benign pelvic mass (BPM) and EOC, ROMA showed significant difference for discriminating BPM and EOC in the all individuals ($P = 0.01$ and $P = 0.02$, respectively) and the postmenopausal individuals ($P = 0.03$ and $P = 0.04$, respectively), at the cutoff value of 27.3 and 34.5, the SNs were 97.0% and 89.4%, SPs were 81.4% and 82.5%, separately. Within all kinds of diseases, there was no significant difference in specificity between CA125 and HE4.

In conclusions, when HE4, CA125, and ROMA were compared with each other according to different menopausal status, and stages. Only CA125 showed significant difference for discriminating HC and EOC in all the individuals, and ROMA for discriminating BPM and EOC in the all individuals and postmenopausal individuals when compared with HE4 or CA125. HE4 has showed no significant difference in specificity with all kinds of diseases when compared with CA125.

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Abbreviations: AUC = areas under the curve, BPM = benign pelvic mass, EOC = epithelial ovarian cancer, HC = healthy control, HE4 = human epididymis secretary protein 4, NPV = negative predictive value, PPV = positive predictive value, ROC = receiver operating characteristics, ROMA = Risk of Ovarian Malignancy Algorithm score, SN = sensitivity, SP = specificity.

INTRODUCTION

Ovarian cancer is the 1 of the most common cancers among women in the worldwide. In 2014, an estimated 21,980 new cases were diagnosed with ovarian cancer, with an estimated 14,270 deaths.[1] In China, on basis of the criteria of data quality from the National Central Cancer Registry from 72 registries' data in 2009, the crude incidence in Chinese Cancer Registration areas was 7.95/100,000.[2,3] Less than 25% of cases are limited to the ovary alone at the time of diagnosis. Early diagnosis is very important for the treatment and prognosis of ovarian cancer. Although there are lots of diagnosis methods including clinical examination, imaging modalities, and serum biomarker in clinical practice[4]; however, they lack adequate sensitivity (SN) and specificity (SP).[5] CA125 is the most widely used serum biomarker in clinical practice, but its clinical value is far from ideal. It is elevated in <50% of early stage ovarian

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cancer, resulted in limited sensitivity.[6] In addition, it is also elevated in benign gynecological diseases and nonovarian gynecologic cancer, resulted in limited specificity.[7] Biomarkers for the diagnosis of ovarian cancer are pressing needed in clinical practice.

Recently, human epididymis secretary protein 4 (HE4) emerged as 1 of the most promising biomarker for the diagnosis of epithelial ovarian cancer (EOC).[8] It is demonstrated to be overexpressed in ovarian carcinomas but not in the normal ovary tissue. Its SN was similar to CA125, but an increased diagnostic SP.[9] Previous studies evaluated the clinical utility of the HE4 and CA125 combination in order to assess the risk of EOC patients with pelvic mass.[10,11] In 2011, HE4 in combination with CA125 in a Risk of Ovarian Malignancy Algorithm (ROMA) score was approved for differential diagnosis and malignancy likelihood assessment in women with pelvic mass. However, some studies found that the differential value of HE4 and ROMA were controversial when compared with CA125.[12–14] Up to now, most of the previous studies were based on Europe and the United States population, there is little large-scale and multicenter study was performed to evaluate the diagnostic value of HE4, CA125, and ROMA for EOC in Chinese population. In addition, previous studies demonstrated that the histological subtype, menopausal status, and surgical stage greatly affected the levels of HE4 and ROMA,[15,16] but there were little study to systematically evaluate their effect. At last, most of the studies chosen the diagnostic SN and SP as the primary indicators; however, SN and SP which were chosen as measures of accuracy had limitation. They depended on a diagnostic criterion for positivity which is often chosen arbitrarily. One widely used measurement indicator is the area under the curve (AUC) of a receiver operating characteristic (ROC).[17,18]

To understand the diagnostic value of HE4, CA125, and ROMA for EOC in Chinese population better, we aimed to perform a large-scale, prospective and multicenter clinical trial to systematically evaluate their diagnostic value for EOC. The flowchart of our experimental design is shown in Figure 1.

METHODS

Study Population

Our study was prospective and multicenter (included 9 centers), and registered with the National Institute of Health clinical trial registry (No. NCT01738269). After written informed consents were obtained, all individual in our study was enrolled from October 2012 to February 2013. The general exclusion criteria of our study were listed as we previously described.[19] Briefly, <18 years, missing clinical examination results, <0.5 mL blood sample, the temperature of storing or shipping was >0°C, icteric, lipemic, hemolytic appearance or particles blood sample, pregnant, with a family history of OC, receiving chemotherapy, radiation therapy, and other treatments.

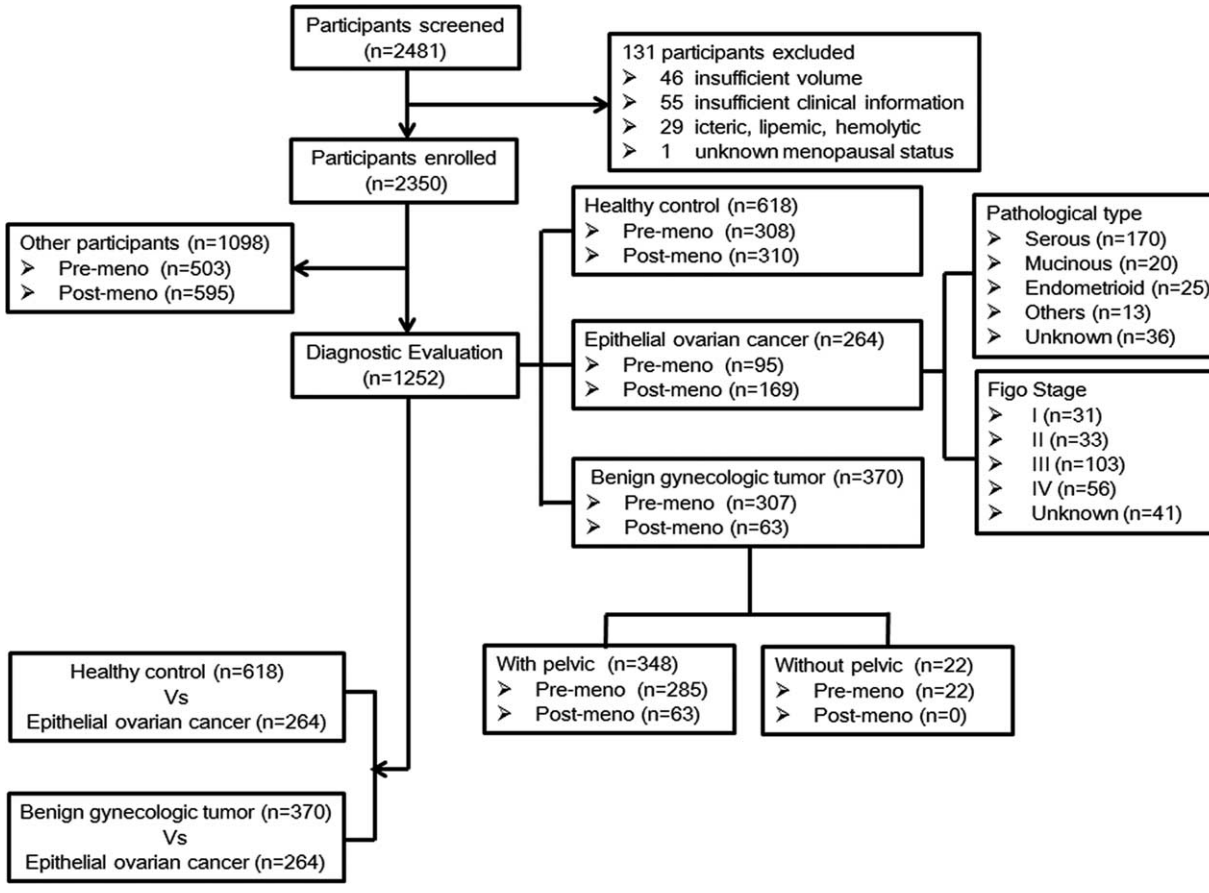

FIGURE 1. Flowchart of our experimental design.

Before surgery, all the patients with pelvic mass were detected by transabdominal and transvaginal ultrasound, and the adnexal lesion was described according to the International Ovarian Tumor Analysis group.[20] After removing the pelvic mass by surgery, the histopathological examination was performed and identified by 3 pathologists to make sure the results.[21] The stage of EOC was identified according to the criteria of International Federation of Gynecology and Obstetrics.[22] The exclusion criteria of healthy control (HC) group were listed as we previously described,[19] and 618 apparently HC individuals were enrolled. The inclusion criteria of the other kinds of participants (n = 1098) were shown as below. The breast cancer, endometrial cancer, gastrointestinal cancer, lung cancer, bladder cancer, and non-EOC patients were identified by the histopathological results. All these individuals were enrolled before surgery, chemotherapy, and radiation therapy. Congestive heart failure, other gynecologic diseases, chronic kidney disease, high blood pressure, hyperthyroidism/hypothyroidism, pneumonia, other nongynecologic diseases, and multi-diseases were identified according to the related results. The clinical characteristics information of individuals enrolled in our study were shown in Table 1.

TABLE 1. Clinical Characteristic of Individuals in Study

Groups	n	n (Premeno)	Age (Premeno)	n (Postmeno)	Age (Postmeno)
Healthy control	618	308	34.0 (28.0, 42.0)	310	63.5 (57.0, 72.0)
Pregnancy	108	108	28.0 (26.0, 31.0)	0	0 (0, 0)
Breast cancer	178	90	42.0 (38.0, 46.0)	88	57.0 (53.0, 63.8)
Congestive heart failure	43	4	48.5 (43.5, 49.0)	39	73.0 (62.0, 80.0)
Endometrial cancer	135	51	47.0 (41.0, 49.0)	84	58.5 (55.0, 63.8)
Gastrointestinal cancer	139	49	41.0 (34.0, 47.5)	90	62.0 (57.0, 68.0)
Lung cancer	145	33	45.0 (40.0, 49.0)	112	60.5 (56.0, 65.0)
Bladder cancer	66	20	43.5 (34.3, 46.8)	46	65.5 (56.8, 74.5)
Other gynecologic diseases	48	43	30.0 (26.0, 36.0)	5	57.0 (53.0, 68.5)
Chronic kidney disease	69	31	37.0 (33.0, 41.0)	38	62.0 (55.5, 72.0)
High blood pressure	19	5	42.0 (29.5, 46.5)	14	67.5 (58.0, 73.0)
Hyperthyroidism/hypothyroidism	15	10	31.0 (26.8, 40.3)	5	54.0 (52.5, 63.5)
Pneumonia	10	0	0 (0, 0)	10	77.0 (60.8, 79.5)
Other nongynecologic diseases	85	35	37.0 (25.0, 45.0)	50	63.0 (57.0, 71.3)
Nonepithelial ovarian cancer	23	18	41.0 (30.5, 45.25)	5	59.0 (55.0, 64.0)
Multi-diseases	15	6	34.5 (31.3, 45.0)	9	64.0 (60.0, 68.5)
Benign gynecologic tumor	370	307	41.0 (35.0, 46.0)	63	57.0 (54.0, 68.0)
Uterine myoma	150	132	43.0 (37.0, 47.0)	18	55.0 (54.5, 64.0)
Adenomyosis	24	24	43.0 (40.0, 46.3)	0	0 (0, 0)
Ovarian/pelvic cyst	47	40	37.0 (26.0, 43.8)	7	57.0 (56.0, 63.0)
Serous	17	11	33.0 (29.0, 45.0)	6	54.5 (50.3, 61.0)
Mucinous	8	2	32.5 (25.0, –)	6	57.5 (53.8, 69.3)
Endometrioid	4	4	30.5 (27.5, 41.8)	0	0 (0, 0)
Teratoma (mature)	40	35	35.0 (26.0, 42.0)	5	68.0 (52.5, 68.5)
Fibroma/thecoma	16	3	50.0 (42.0, –)	13	61.0 (54.0, 70.0)
Cervical intraepithelial neoplasia	5	4	43.5 (39.5, 46.0)	1	57.0 (57.0, 57.0)
Endometriosis/endometriotic cyst	52	47	40.0 (35.0, 44.0)	5	52.0 (48.0, 67.0)
Tubo abscess/hydrosalpinx	7	5	37.0 (24.5, 42.5)	2	65.0 (60.0, –)
Benign gynecologic tumor	370	307	41.0 (35.0, 46.0)	63	57.0 (54.0, 68.0)
With pelvic mass	348	285	41.0 (35.0, 46.0)	63	57.0 (54.0, 46.0)
Without pelvic mass	22	22	38.0 (31.5, 43.0)	0	0 (0, 0)
Epithelial ovarian cancer	264	95	43.0 (38.0, 47.0)	169	59.0 (54.0, 65.0)
Serous	170	61	44.0 (38.5, 47.5)	109	60.0 (54.0, 65.0)
Mucinous	20	13	43.0 (28.5, 47.0)	7	61.0 (54.0, 65.0)
Endometrioid	25	7	44.0 (41.0, 48.0)	18	59.5 (53.8, 70.3)
Other kinds	13	6	42.5 (37.8, 46.8)	7	57.0 (51.0, 65.0)
Unknown	36	8	42.0 (37.3, 47.0)	28	59.0 (55.3, 63.5)
Figo stage	264	95	43.0 (38.0, 47.0)	169	59.0 (54.0, 65.0)
I	31	19	38.0 (31.0, 46.0)	12	57.0 (51.8, 64.0)
II	33	11	42.0 (40.0, 47.0)	22	60.5 (57.0, 64.3)
III	103	30	44.5 (40.7, 47.3)	73	60.0 (54.0, 65.5)
IV	56	20	44.0 (41.3, 48.8)	36	59.0 (55.0, 65.0)
Unknown	41	15	40.0 (32.0, 47.0)	26	59.0 (52.8, 63.5)

Age was shown as median (25th percentile, 75th percentile).

Sample Collection, Processing, and Storage

Peripheral blood samples (10 mL each) were collected in tubes that contained separating gel and clot activator. After centrifuging at 3400 rpm for 7 min, serum was aliquoted and stored at −80°C until detection.

HE4, CA125, and ROMA Detection

Levels of HE4 and CA125 were measured by Roche Elecsys Cobas 601 platform and the matched reagents Roche Diagnostics (Basel, Switzerland). The detection mechanism of HE4 and CA125 were electrochemiluminescence immunoassay (ECLIA), and the detection range were 15.0 to 1500 pmol/L and 0.600 to 5000 U/mL. In order to make the results of the 9 centers in our study comparable, all the centers had to pass the need of the External Quality Assessment (EQA)/ISO 15189. The formulas of predictive index (PI) which were described in the previous studies for premenopausal and postmenopausal EOC were shown as below.[10,23]

$$Premeno\,pausal:\ PI = -12.0 + 2.38*LN[HE4] + 0.0626*LN \times [CA125]$$

$$Postmeno\,pausal:\ PI = -8.09 + 1.04*LN[HE4] + 0.732*LN \times [CA125]$$

$$ROMAvalue\,(\%) = \exp(PI)/[1+\exp(PI)]*100$$

According to the manufacturer's instructions, for the premenopausal women, ROMA $\geq$ 11.4% indicated high risk of EOC, and <11.4% indicated low risk of EOC. For the postmenopausal women, ROMA $\geq$ 29.9% indicated high risk of EOC, and <29.9% indicated low risk of EOC.

Statistical Analysis

All the statistical analyses were conducted by MedCalc 12.7.0.0 (MedCalc Software, Mariakerke, Belgium) and IBM SPSS Statistics 19.0 (IBM, Armonk, NY). Mann–Whitney *U* test was used to compare levels of HE4, CA125, and ROMA. After establishing the ROC curves of HE4, CA125, and ROMA in different groups, the differences of AUC were evaluated by Z scores statistics. Youden index was calculated to choose the optimal threshold.[24] According to the different menopausal status, the threshold which was recommended by the company and the threshold which was calculated from the Youden index of HE4, CA125, and ROMA values were determined for the all, premenopausal, and postmenopausal groups. McNemar test was used to compare the specificity. The SN, SP, positive predictive value (PPV), and negative predictive value (NPV) were calculated as we previously described.[19] A 2-tailed *P* value of <0.05 showed significant difference.

RESULTS

Diagnostic Accuracy for Discriminating Between HC and EOC Group

As shown in Table 2, the diagnostic value of HE4, CA125, and ROMA for all stage, early stage, and advanced stage in all, premenopausal, and postmenopausal individuals for discriminating between the HC and EOC were evaluated. According to the Youden index cutoff and reference value, their diagnostic SN, SP, PPV, and NPV were also shown.

In the all individuals (including premenopausal and postmenopausal), for discriminating between HC and EOC group, as shown in Supplementary Figure 1A, http://links.lww.com/MD/A585. The AUC of CA125 the most, it was 0.956 (0.940, 0.968), it was significantly higher when compared with the HE4 and ROMA ($P < 0.001$ and $P = 0.02$, respectively), at the cutoff value of 31.5, the SN and SP were 88.6% and 97.1%, respectively. For discriminating between HC and early stage EOC, HC and advanced stage EOC, as shown in Supplementary Figure 1B and C, http://links.lww.com/MD/A585, the AUCs of CA125 were the most, they were 0.927 (0.905, 0.945) and 0.966 (0.951, 0.978). They were significantly higher when compared with the HE4 ($P = 0.01$ and $P = 0.04$), but not ROMA.

In the premenopausal individuals, for discriminating between HC and EOC group, HC and early stage EOC group, as shown in Supplementary Figure 1D and E, http://links.lww.com/MD/A585, the AUCs of CA125 were the most, they were 0.919 (0.888, 0.944) and 0.917 (0.883, 0.945), but they were no significantly higher when compared with HE4 and ROMA. For discriminating between HC and advanced stage EOC group, as shown in Supplementary Figure 1F, http://links.lww.com/MD/A585, the AUC of ROMA was the most, it was 0.924 (0.891, 0.949), but it was no significantly higher when compared with HE4 and CA125.

In the postmenopausal individuals, for discriminating between HC and EOC, HC and advanced stage EOC, as shown in Supplementary Figure 1G and I, http://links.lww.com/MD/A585, the AUCs of CA125 were the most, they were 0.977 (0.959, 0.988) and 0.986 (0.969, 0.995). They were significantly higher when compared with the HE4 ($P < 0.001$, $P = 0.01$), but not ROMA. For discriminating between HC and early stage EOC, as shown in Supplementary Figure 1H, http://links.lww.com/MD/A585, the AUC of ROMA was the most, it was 0.947 (0.917, 0.968), it was significantly higher when compared with HE4 ($P = 0.01$), but not CA125.

Diagnostic Evaluation for Discriminating Between the BPM and EOC Group

As shown in Table 3, the diagnostic value of HE4, CA125, and ROMA for all stage, early stage, and advanced stage in all, premenopausal, and postmenopausal individuals for discriminating between the benign pelvic mass (BPM) and EOC were evaluated. According to the Youden index cutoff value and reference value, their diagnostic SN, SP, PPV, and NPV were also shown.

In all the individuals (including premenopausal and postmenopausal), for discriminating between BPM and EOC, as shown in Supplementary Figure 2A, http://links.lww.com/MD/A585, the AUC of ROMA the most, it was 0.919 (0.894, 0.939). It was significantly higher when compared with the HE4 and CA125 ($P = 0.01$ and $P = 0.02$, respectively), at the cutoff value of 27.3, the SN and SP were 97.0% and 81.4%, separately. For discriminating between BPM and early stage EOC, as shown in Supplementary Figure 2B, http://links.lww.com/MD/A585, the AUC of ROMA was the most, it was 0.871 (0.835, 0.902), but it was no significantly higher when compared with HE4 and CA125. For discriminating between BPM and advanced stage EOC, as shown in Supplementary Figure 2C, http://links.lww.com/MD/A585, the AUC of ROMA was the most, it was 0.948 (0.925, 0.966), it was significantly higher when compared with HE4 ($P = 0.013$), but not CA125.

TABLE 2. Diagnostic Accuracy for Discriminating Between HC and EOC

Groups	Indicator	AUC (95% CI)	Value (Cutoff/Reference)	SN, % (Cutoff/Reference)	SP, % (Cutoff/Reference)	PPV, % (Cutoff/Reference)	NPV, % (Cutoff/Reference)
HC vs EOC	HE4	0.901 (0.879, 0.920)*,†	74.4/92.1, 121.0§	83.3/74.2	88.2/95.6	75.1/87.9	92.5/89.7
	CA125	0.956 (0.940, 0.968)†,‡	31.5/35.0§	88.6/86.4	97.1/98.2	92.9/95.4	95.2/94.4
	ROMA	0.929 (0.909, 0.945)*,‡	24.3/11.4, 29.9§	83.7/84.9	97.7/94.3	94.0/86.5	93.4/93.6
HC vs Early EOC	HE4	0.836 (0.806, 0.863)*,†	75.9/92.1, 121.0§	75.0/59.4	88.5/95.8	40.3/58.5	97.2/95.8
	CA125	0.927 (0.905, 0.945)‡	28.2/35.0§	85.9/81.3	95.8/98.2	67.9/82.5	98.5/98.1
	ROMA	0.885 (0.859, 0.908)‡	24.3/11.4, 29.9§	78.1/76.6	97.7/94.3	78.1/58.3	97.7/97.5
HC vs advanced EOC	HE4	0.941 (0.922, 0.956)*,†	98.5/92.1, 121.0§	82.4/78.0	97.1/95.8	87.9/82.7	95.5/94.4
	CA125	0.966 (0.951, 0.978)‡	34.8/35.0§	89.9/89.9	98.2/98.2	92.9/92.9	97.4/97.4
	ROMA	0.955 (0.938, 0.968)‡	27.8/11.4, 29.9§	87.4/90.0	98.9/94.2	95.2/79.9	96.8/97.3
Premeno (HC vs EOC)	HE4	0.879 (0.844, 0.910)†	71.5/92.1§	71.6/60.0	98.4/99.7	93.2/98.3	91.8/89.0
	CA125	0.919 (0.888, 0.944)	31.5/35.0§	84.2/82.1	95.5/97.1	85.1/89.7	95.1/94.6
	ROMA	0.887 (0.852, 0.916)‡	14.8/11.4§	73.7/73.7	97.1/90.3	88.6/70.0	92.3/91.7
Premeno (HC vs early EOC)	HE4	0.836 (0.792, 0.874)†	79.8/92.1§	70.0/56.7	99.0/99.7	87.5/94.4	97.1/95.9
	CA125	0.917 (0.883, 0.945)	34.1/35.0§	86.7/86.7	97.1/97.1	74.3/74.3	98.7/98.7
	ROMA	0.845 (0.802, 0.882)‡	22.8/11.4§	70.0/70.0	99.7/90.3	95.5/41.2	97.2/96.9
Premeno (HC vs advanced EOC)	HE4	0.919 (0.886, 0.945)	71.5/92.1§	80.0/54.0	98.4/100.0	88.9/100.0	96.8/93.1
	CA125	0.920 (0.888, 0.946)	31.5/35.0§	84.0/82.0	95.5/97.1	75.0/82.0	97.4/97.1
	ROMA	0.924 (0.891, 0.949)	17.5/11.4§	80.0/80.0	98.7/90.1	90.9/57.1	96.8/96.5
Postmeno (HC vs EOC)	HE4	0.920 (0.892, 0.943)*,†	105.6/121.0§	78.7/82.3	96.1/91.6	91.7/84.2	89.2/90.4
	CA125	0.977 (0.959, 0.988)‡	26.8/35.0§	92.9/88.8	98.4/99.4	96.9/98.7	96.2/94.2
	ROMA	0.974 (0.955, 0.986)‡	27.5/29.9§	92.3/91.1	97.7/98.4	95.7/96.9	95.9/95.3
Postmeno(HC vs early EOC)	HE4	0.833 (0.789, 0.871)*,†	89.1/121.0§	67.7/61.8	90.3/91.6	43.4/44.7	96.2/95.6
	CA125	0.944 (0.915, 0.966)‡	25.3/35.0§	88.2/76.5	97.4/99.4	78.9/92.9	98.7/97.5
	ROMA	0.947 (0.917, 0.968)‡	27.5/29.9§	85.3/82.4	97.7/98.4	80.6/84.8	98.4/98.1
Postmeno (HC vs advanced EOC)	HE4	0.958 (0.934, 0.975)*,†	117.9/121.0§	85.3/89.0	98.4/91.6	94.9/78.9	95.0/95.9
	CA125	0.986 (0.969, 0.995)‡	27.7/35.0§	95.4/93.6	98.7/99.4	96.3/98.1	98.4/97.8
	ROMA	0.984 (0.967, 0.994)‡	27.8/29.9§	95.4/94.5	98.1/98.4	94.5/95.4	98.4/98.1

AUC = area under curve, CI = confidence interval, EOC = epithelial ovarian cancer, HC = healthy control, HE4 = human epididymis secretary protein 4, NPV = negative predictive value, PPV = positive predictive value, ROMA = Risk of Ovarian Malignancy Algorithm, SN = sensitivity, SP = specificity.

* Compared to CA125.

† Compared to ROMA.

‡ Compared to HE4, AUC showed significant difference.

§ Means reference value recommended by the company; 35 U/mL for CA125; 92.1 U/mL in the premeno women; 121.0 U/mL in the postmeno women for HE4; 11.4 in the premeno women; 29.9 in the postmeno women for ROMA.

TABLE 3. Diagnostic Accuracy for Discriminating Between BPM and EOC

Groups	Indicator	AUC (95% CI)	Value (Cutoff/Reference)	SN, % (Cutoff/Reference)	SP, % (Cutoff/Reference)	PPV, % (Cutoff/Reference)	NPV, % (Cutoff/Reference)
BPM vs EOC	HE4	0.902 (0.875, 0.924)*	74.4/92.1, 121.0§	83.3/74.2	87.4/93.7	83.3/89.9	87.4/82.7
	CA125	0.890 (0.863, 0.914)*	85.6/35.0§	78.8/86.4	88.2/71.8	83.5/69.9	84.6/87.4
	ROMA	0.919 (0.894, 0.939)†,‡	27.3/11.4, 29.9§	97.0/84.9	81.4/79.0	93.4/75.4	90.3/87.3
BPM vs early EOC	HE4	0.838 (0.799, 0.873)	75.9/92.1, 121.0§	75.0/59.4	87.9/93.7	53.3/63.3	95.0/92.6
	CA125	0.837 (0.798, 0.872)	70.1/35.0§	71.8/81.3	85.9/71.8	48.4/34.7	94.3/95.4
	ROMA	0.871 (0.835, 0.902)	27.3/11.4, 29.9§	75.0/76.6	93.4/79.3	67.6/40.5	95.3/94.9
BPM vs advanced EOC	HE4	0.941 (0.916, 0.960)	98.5/92.1, 121.0§	82.4/83.0	95.4/93.7	89.1/85.7	92.2/92.4
	CA125	0.919 (0.891, 0.941)*	97.5/35.0§	84.9/89.9	89.9/71.8	79.4/59.3	92.9/94.0
	ROMA	0.948 (0.925, 0.966)‡	34.5/11.4, 29.9§	85.5/89.9	96.0/79.3	90.7/66.5	93.6/94.5
Premeno (BPM vs EOC)	HE4	0.848 (0.807, 0.882)*	71.5/92.1§	71.6/60.0	90.9/97.2	72.3/87.7	90.6/87.9
	CA125	0.858 (0.818, 0.892)	61.6/35.0§	77.9/82.1	85.3/72.3	63.8/49.7	92.0/92.4
	ROMA	0.854 (0.813, 0.887)†	20.7/11.4§	69.5/73.7	93.3/79.3	77.6/54.3	90.2/90.0
Premeno (BPM vs early EOC)	HE4	0.808 (0.760, 0.850)	79.8/92.1§	70.0/56.7	93.7/97.2	53.8/68.0	96.7/95.5
	CA125	0.853 (0.808, 0.890)	47.5/35.0§	83.3/86.7	79.0/72.3	29.4/24.8	97.8/98.1
	ROMA	0.815 (0.767, 0.856)	24.2/11.4§	70.0/70.0	95.4/79.7	61.8/26.6	96.8/96.2
Premeno (BPM vs advanced EOC)	HE4	0.894 (0.855, 0.925)	71.5/92.1§	80.0/70.0	90.9/97.2	60.6/81.4	96.3/94.9
	CA125	0.866 (0.825, 0.901)	105.6/35.0§	72.0/82.0	93.3/72.3	65.5/34.2	95.0/95.8
	ROMA	0.896 (0.858, 0.926)	16.3/11.4§	80.0/80.0	90.5/79.7	59.7/40.8	96.3/95.8
Postmeno (BPM vs EOC)	HE4	0.892 (0.845, 0.929)*	151.4/121.0§	71.0/82.3	96.8/77.8	98.4/90.8	55.5/62.0
	CA125	0.902 (0.856, 0.937)*	66.0/35.0§	84.0/88.8	82.5/69.8	92.8/88.8	65.8/69.8
	ROMA	0.919 (0.876, 0.950)†,‡	34.5/29.9§	89.4/91.1	82.5/77.8	93.2/91.7	74.3/76.6
Postmeno(BPM vs early EOC)	HE4	0.796 (0.702, 0.871)	77.5/121.0§	76.5/61.8	74.6/77.8	61.9/60.0	85.5/79.0
	CA125	0.819 (0.728, 0.890)	66.0/35.0§	70.6/76.5	82.5/69.8	68.6/57.8	83.9/84.6
	ROMA	0.845 (0.757, 0.911)	32.3/29.9§	79.4/82.4	81.0/77.8	69.2/66.7	87.9/89.1
Postmeno (BPM vs advanced EOC)	HE4	0.933 (0.885, 0.965)	151.4/121.0§	81.7/89.0	96.8/77.8	97.8/87.4	75.3/80.3
	CA125	0.937 (0.890, 0.968)	110.0/35.0§	88.1/93.6	85.7/69.8	91.4/84.3	80.6/86.3
	ROMA	0.950 (0.906, 0.977)	65.3/29.9§	80.0/94.5	93.7/77.8	95.9/88.0	78.7/89.1

AUC = area under curve, BPM = benign pelvic mass, CI = confidence interval, EOC = epithelial ovarian cancer, HE4 = human epididymis secretary protein 4, NPV = negative predictive value, PPV = positive predictive value, ROMA = Risk of Ovarian Malignancy Algorithm, SN = sensitivity, SP = specificity.

* Compared to ROMA.

† Compared to HE4.

‡ Compared to CA125, AUC showed significant difference.

§ Means reference value recommended by the company; 35 U/mL for CA125; 92.1 U/mL in the premeno women; 121.0 U/mL in the postmeno women for HE4; 11.4 in the premeno women; 29.9 in the postmeno women for ROMA.

In the premenopausal individuals, for discriminating between BPM and EOC, as shown in Supplementary Figure 2D, http://links.lww.com/MD/A585, the AUC of CA125 was the most, it was 0.858 (0.818, 0.892), but it was no significantly higher when compared with HE4 and ROMA. For discriminating between BPM and early stage EOC, BPM and advanced stage EOC, as shown in Supplementary Figure 2E and F, http://links.lww.com/MD/A585, the AUCs of CA125 and ROMA were the most, but HE4, CA125, and ROMA were no significantly difference when compared with each other.

In the postmenopausal individuals, for discriminating between BPM and EOC, as shown in Supplementary Figure 2G, http://links.lww.com/MD/A585, the AUC of ROMA was the most, it was 0.919 (0.876, 0.950). It was significantly higher when compared with the HE4 and CA125 ($P = 0.03$ and $P = 0.04$, respectively), at the cutoff value of 34.5, the SN and SP were 89.4% and 82.5%, separately. For discriminating between BPM and early stage EOC, BPM and advanced stage EOC, as shown in Supplementary Figure 2H and I, http://links.lww.com/MD/A585, the AUCs of ROMA were the most, they were 0.845 (0.757, 0.911) and 0.950 (0.906, 0.977), but they were no significantly higher when compared with HE4 and CA125.

Diagnostic Specificity of HE4, CA125, and ROMA for Other Kinds of Participants

As shown in Figure 2, we analyzed the specificity of HE4, CA125, and ROMA for other kinds of participants (n = 1098). First, we analyzed their specificity for premenopausal other

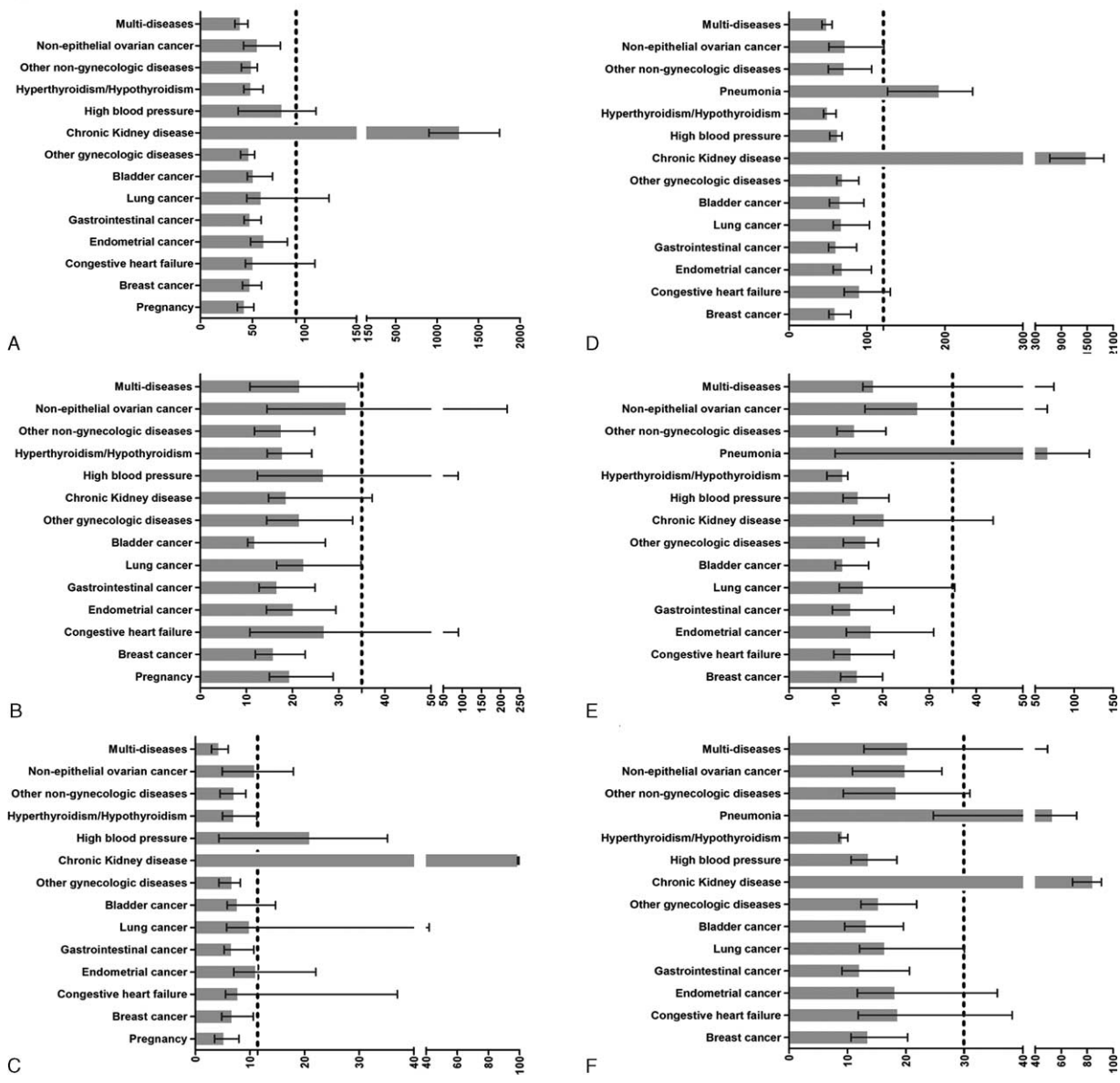

FIGURE 2. Diagnostic specificity of HE4, CA125, and ROMA for other kinds of participants. (A) HE4 in premeno EOC; (B) CA125 in premeno EOC; (C) ROMA in premeno EOC; (D) HE4 in postmeno EOC; (E) CA125 in postmeno EOC; (F) ROMA in postmeno EOC. EOC = epithelial ovarian cancer, HE4 = human epididymis secretary protein 4, ROMA = Risk of Ovarian Malignancy Algorithm.

participants (n = 503). As shown in as shown in Figure 2A, B, and C, HE4 showed the best diagnostic specificity. It showed significant improvement when compared with ROMA ($P < 0.001$), but not CA125. Second, their specificity for postmenopausal other participants (n = 595) were also analyzed, as shown in Figure 2D, E, and F. CA125 showed the best diagnostic specificity. It showed significant improvement when compared with ROMA ($P = 0.04$), but not HE4. Third, their specificity for all other participants (n = 1098) were analyzed. HE4 showed the best diagnostic specificity. It showed significant improvement when compared with ROMA ($P < 0.001$), but not CA125.

DISCUSSION

Previous studies demonstrated that the histological subtype, menopausal status, and surgical stage were greatly related to the level of HE4.[15] By immunohistochemistry, 93% of serous and 100% of endometrioid EOCs expressed HE4, only 50% and 0% of clear cell carcinomas and mucinous tumors were positive.[7] Most of the studies considered the diagnostic SN and SP as the primary evaluation indicators, but their effect on the diagnostic NPV and PPV were not taken into consideration. For example, in our study, for discriminating BPM versus early stage EOC in the premenopausal individuals, the diagnostic sensitivity of HE4 and CA125 when at the reference value were 56.7% and 86.7%, their specificity were 97.2% and 72.3%, separately. The specificity of HE4 was >24.9% than CA125; however, its NPV were <2.6% than CA125. In clinical practice, this meant that the possibility of true BPM was diagnosed as BPM decrease by 2.6% in the premenopausal individuals. One particularly widely used measure is the AUC.[17,18,25] In our study, we used the AUC to compare the diagnostic value of HE4, CA125, and ROMA.

For discriminating between the HC and EOC, HE4, CA125, and ROMA were compared each other to compare their diagnostic value according to the menopausal status (premenopausal and postmenopausal) and stage EOC (early stage and advanced stage). Their AUCs showed no significant difference, except for CA125 for discriminating HC verse EOC in the all individuals. Many other diseases conditions can also cause an elevation of CA 125 levels, including: endometriosis, liver cirrhosis, normal menstruation, pelvic inflammatory disease, pregnancy, uterine fibroids. Elevation of CA125 can also be seen in cancers other than ovarian cancer, including malignancies of the uterine tubes, endometrium.[26] These diseases' conditions may cause bias for the results of discriminating between the HC and EOC. In addition, because of the prevalence of EOC, larger sample size was needed for the detection of EOC. For clinical application, our results for discriminating between the HC and EOC may provide an auxiliary diagnostic method for the clinical examination, such as, transvaginal ultrasound and imagination methods, and combination the serum biomarker and clinical examination methods may improve the diagnostic value for EOC detection. For discriminating between the BPM and EOC, when HE4, CA125, and ROMA were compared each other to compare their diagnostic value for discriminating BPM and EOC according to different menopausal status and stages of EOC. Their AUCs showed no significant difference, except for ROMA for discriminating BPM and EOC in the all individuals and postmenopausal individuals. Previous studies found that HE4 showed superior specificity in the differentiation of benign and malignant adnexal masses in premenopausal women.[27] In our study, at the reference value in different menopausal status and stages, HE4 showed more specificity compared with the CA125, this was consistent with the previous studies. Its sensitivity were less than CA125, except for BPM versus advanced EOC in the premenopausal individuals, this is mainly because the HE4 levels are less frequently elevated than CA125 in women with benign gynecologic disease, particularly in premenopausal patients.[28] Using AUC analysis method, our results were consistent with some previous studies. Some studies found that ROMA was better in postmenopausal EOC[21,29]; however, others studies found that ROMA showed no significant difference or worse in postmenopausal women.[12,16,30] In our study, ROMA also showed significant difference compared with HE4 and CA125 for discriminating BPM and EOC in the all individuals and postmenopausal individuals. It was consistent with some studies. Studies found that ROMA performed better in EOC group,[31–33] but other studies showed ROMA performed no significant difference or worse.[13,34] We also analyzed the specificity of HE4, CA125, and ROMA in different other kinds of diseases. HE4 has similar diagnostic specificity with all kinds of diseases in our study when compared with CA125. Other kinds of diseases, such as, pregnancy,[35] breast cancer,[36] congestive heart failure,[37] endometrial cancer,[38] lung cancer,[39] chronic kidney diseases,[40] and pneumonia.[41] Chronic kidney diseases had the highest effect on the levels of HE4. Studies found that HE4 suppresses the activity of multiple proteases, including serine proteases and matrix metalloproteinases, and specifically inhibits their capacity to degrade type I collagen. It suggested that HE4 is a potential biomarker of renal fibrosis and a new therapeutic target.[42]

There were several reasons for the difference between our study and the other studies. The number of patients enrolled may greatly affect the results, and the percentage of the subtypes can affect the results, as mentioned previous, clear cell carcinomas and mucinous tumors did not express HE4. Besides the subtype classification, the percentage of women in premenopausal and postmenopausal also can affect the results. At last, the percentage of the early stage and advanced stage can affect the results. So in our study, to avoid the effects and bias of these factors as more as possible, we systematically evaluated their diagnostic value in a relative large and multicenter prospective research in Chinese population according to different subtypes, menopausal status, and stages.

There were also some limitations in our study. First, we had not evaluated the diagnostic value of HE4, CA125, and ROMA in each single research center, because the sample size is relatively small if we studied their diagnostic value in each center. Second, in our study, we used the AUC of HE4, CA125, and ROMA to evaluate their diagnostic value, and we did not evaluate their diagnostic specificity at specific NPV, in our future study, we will perform this study. Third, as mentioned previously, our sample size is more than most of the previous studies (not including the meta-analysis); however, if we evaluated their diagnostic value according to different subtypes, menopausal status, and stages, the sample size is relatively small. More patients are needed in our future study.

In conclusions, we systematically compared the diagnostic accuracy of HE4, CA125, and ROMA in a large and multicenter prospective research in Chinese women according to different menopausal status, and stages. For discriminating between HC and EOC, only CA125 showed significant difference for discriminating HC and EOC in all the individuals when compared with HE4 or ROMA. For discriminating between BPM and EOC, only ROMA showed significant difference for

discriminating BPM and EOC in the all individuals and postmenopausal individuals when compared with HE4 or ROMA. HE4 has similar diagnostic specificity with all kinds of diseases in our study when compared with CA125. In the clinical recommendation, on the one hand, our results for discriminating between the HC and EOC may provide an auxiliary diagnostic method for the clinical examination, such as, transvaginal ultrasound and imaging methods, and combination the serum biomarker and clinical examination methods may improve the diagnostic value for EOC detection. On the other hand, clinical doctors and laboratory examiner should take menopausal status, stages, and population into consideration before the usage of HE4, CA125, and ROMA in the clinical practice.

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