Diagnostic Accuracy and Optimal Cutoff Value of Serum HE4 to Predict Ovarian Cancer in Thai Women with Pelvic Masses

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Background: *Serum human epididymis protein 4 (HE4) is highly expressed in women with ovarian cancers (OCs), but data about its clinical application of HE4 for Thai women is limited.*
Objective: *To evaluate the diagnostic accuracy and optimal cutoff for HE4 in distinguishing benign lesions, borderline ovarian tumor (BOTs), and OCs compared with CA125 in Thai women at Rajavithi Hospital.*
Material and Method: *The cross-sectional study was conducted in Thai women aged older than 18 years old with pelvic masses whom underwent elective surgery at Rajavithi Hospital between 2012 and 2013. Preoperative serum HE4 and CA125 levels were measured and pathologic specimens were reviewed.*
Results: *Of the 518 participants evaluated, 316 had benign lesions, 43 had BOTs, and 159 had OCs. Between non-cancers and OCs, area under receiver operating characteristic curve (ROC-AUC) for HE4 hardly differed from CA125 (0.85 vs. 0.83, p = 0.402) but was significantly lower in postmenopausal women (0.79 vs. 0.86, p = 0.049). The optimal cutoff value of HE4 was 72 pM/L for all menopausal status. Lower HE4 was seen in 30.8% of mucinous carcinoma and 31.7% of clear cell carcinoma. The HE4 ROC-AUC was significantly higher than CA125 ROC-AUC in distinguishing benign diseases and BOTs (0.71 vs. 0.53, p<0.001), HE4 in 70% of BOTs was 51 to 95 pM/L.*
Conclusion: *Although the 72 pM/L cutoff for HE4 was appropriate in distinguishing between non-cancers and OCs for both pre- and postmenopausal women, the limitation for postmenopausal women, mucinous carcinomas, and clear cell carcinomas require to be complemented with CA125.*

Keywords: *Pelvic mass, Ovarian cancer, Human epididymis protein 4, Cutoff value*

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Ovarian cancer (OC) is a common and lethal malignancy among women worldwide. Its incidence rate increases with age and in postmenopausal women. Pre-operative prediction of OC is the key to successful treatment of women present with pelvic or adnexal masses. The extreme difference of survival rates for OC diagnosed in early and advanced stages indicate the need for biomarkers with higher diagnostic accuracy to discriminate early malignancies from benign pelvic mass.

Serum carbohydrate antigen 125 (CA125) is the most widely used biomarker in diagnosis and monitoring OC, but CA125 measurement has many limitations. Despite its utility in OC diagnosis, CA125 elevation has also been noted in several other conditions, both benign and malignant, such as endometriosis, first trimester of pregnancy, breast cancer, and lesions that promote peritoneal irritation[1]. Serum CA125 level is elevated in less than half of women with early-stage OC; approximately 20% of OC patients have normal or only marginally elevated serum CA125[2].

Various biomarkers have been investigated over the last decade to replace or complement CA125, among these, serum human epididymis protein 4 (HE4) seems to be an intriguing biomarker for improving OC diagnosis[3,4]. HE4 is a stable 4-disulfide core protein associated with the WFDC2 gene. It was originally found in the epithelial cells of the human distal epididymis, and has low expression in normal tissue, higher in non-ovarian malignancies e.g., pulmonary adenocarcinoma and highest expression in OC[5].

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The HE4 reportedly promotes invasion and metastasis of epithelial ovarian cancers (EOCs) by binding interactions with annexin II activated adhesion signaling pathways[6]. In EOCs, HE4 is overexpressed in serous and endometrioid carcinoma[7]. Unlike CA125, HE4 is also elevated in patients with mucinous carcinoma[8]. The HE4 is reportedly as accurate as CA125 as an indicator of ovarian malignancy and their combined use is more accurate still. However, data on serum HE4 as a biomarker for Asian women have been relatively limited.

The present study evaluated the diagnostic accuracy and optimal cutoff value of HE4 for distinguishing among benign diseases, borderline ovarian tumors (BOTs), and OCs compared with CA125 in Thai women at Rajavithi Hospital.

Material and Method

This cross-sectional study was conducted at the Department of Obstetrics and Gynecology of Rajavithi Hospital between January 1, 2012 and December 31, 2013. After obtaining approval of the Institutional Review Board of Rajavithi Hospital (No. 55014), Thai women of at least 18 years of age with clinically diagnosed pelvic or adnexal masses scheduled elective gynecologic surgery were prospectively enrolled, all enrollees gave written informed consent.

During the present study period, the authors prospectively enrolled 548 women with pelvic or adnexal masses. The authors excluded four patients who were pregnant, six with histories of previous adnexal surgery, two with histories of breast cancer, one with a history of cervical cancer, one with colon cancer, 12 with no pre-surgery HE4 results, and four who cancelled surgery after admission, which left 518 participants. These 518 patients included 359 women in the non-cancer group (165 benign ovarian tumors, 151 benign gynecologic diseases and 43 BOTs) and 159 women in the OC group (134 EOCs and 25 non-EOCs).

All participants were hospitalized for the preoperative preparation at least 24 hours prior to surgery. Clinical data were collected and serum CA125 and HE4 levels were measured preoperatively. The authors excluded women who were pregnant, had previous histories of OC or any known malignancy, had previous histories of adnexal surgery, had no biomarker results or who cancelled surgery. In post-operative period, pathologic slides were reviewed by two pathologists with 10 and 15 years of experience, respectively. They were blinded to patients' clinical information and serum biomarkers.

Five ml blood sample from each participating patient was drawn by peripheral venous puncture within 48 hours prior to surgery, which were processed immediately or stored at -20°C until needed. Clotted blood tubes were centrifuged at 800 g for 10 minutes to separate serum. Serum biomarker concentrations were analyzed using Elecsys HE4 and Elecsys CA125 II reagent kits (Cobas 6000 analyzer series, Roche Diagnostics, Indianapolis, IN). These laboratory assays were qualified by internal control and external control by Interlaboratory Comparison with other hospitals. Suggested cutoff values for HE4 were 70 pM/L for premenopausal and 140 pM/L for postmenopausal women, according to the manufacturer's instructions. The cutoff for CA125 was 35 U/mL, as recommended by the National Institute for Health and Clinical Excellence (NICE)[9]. Additional cutoffs for HE4 at 70 pM/L for all women[10] and for CA125 at 200 U/mL for premenopausal and 35 U/mL for postmenopausal women, as recommended by the American College of Obstetricians and Gynecologists[11] were also evaluated. Postmenopausal women were defined as those older than 45 years who had not menstruated for more than one year, or those over 55 years of age. If menopausal status could not be identified from clinical data, FSH level was tested, women who expressed more than 25 IU/L were considered menopausal.

Sample size calculation was based on the formula for one sample comparison of proportion using 2-tail alpha equal 0.05 and acceptable error at 0.075. The study of Moore et al[4] was used to calculate the sensitivity of HE4 in diagnosing OC. As the estimated prevalence of OC in women with a pelvic mass at Rajavithi Hospital was 28%, at least 486 subjects were needed, and 540 subjected initially required to compensate for the expected 10% dropout rate.

Statistical analysis was undertaken using STATA 14 (StataCorp, College Station, TX). Quantitative biomarker assays were initially transformed into log scale because of the wide range and non-normal distribution of data points. Participants' baseline characteristics were described using frequency and percentages for categorical data, and mean, standard deviation, median and range for continuous data. Analysis of variance (ANOVA) and Kruskal-Wallis tests were used to compare serum biomarker levels and categorical variables followed by post-hoc analysis of multiple comparisons as appropriate. Using pathologic diagnoses as classification references,

receiver operating characteristics (ROC) curves were plotted and areas under the curve (AUC) were calculated to compare overall performance of each biomarker for predicting OC, and sensitivity (Sn), specificity (Sp), positive and negative likelihood ratios (LR+ and LR-, respectively), diagnostic odds ratio (dOR), and positive and negative predictive values (PPV and NPV, respectively). The optimal cutoff value was identified by Youden Index method[12]. The *p*-value <0.05 was considered statistically significant.

Results

Baseline characteristics and histopathologic distribution were summarized in Table 1. Mean age (± standard deviation) was 46.7 (±13.9) years, mean body mass index (BMI) was 24.5 (±5.0) kg/m^2, median parity was 1 (range 0 to 10), 34.8% had underlying diseases (most common was hypertension), and 41.3% was postmenopausal women. Participants came from all regions of Thailand, mostly Central Thailand (44.4%).

Endometriotic cysts were the most common benign gynecologic diseases (20.7%), mucinous cystadenomas and mucinous BOTs were the most common benign ovarian tumors (12.4%) and BOTs (6.6%), respectively, whereas clear cell carcinomas (CCCs) were the most common OCs (7.9%). There were 25 patients diagnosed with non-EOCs and included six yolk sac tumors, two immature teratomas, one mixed malignant germ cell tumor, three granulosa cell tumors, one fibrosarcoma, five metastatic colorectal adenocarcinomas, one Krukenberg tumor, one gastrointestinal stromal tumor, one metastatic neuroendocrine tumor, two metastatic sarcomas, and two malignant lymphomas. OCs were found in 45.3% of the postmenopausal women, and 20.4% of the premenopausal women. Among the EOC patients, 43.3% had stage I disease, 14.9% had stage II, 39.6% had stage III, and 2.2% had stage IV.

Serum HE4 and CA125 levels compared among disease groups (benign diseases, BOTs, and OCs) were shown in Table 2 and Fig. 1. Because tests of homogeneity of variance showed heteroscedasticity, the Kruskal-Wallis test was used to compared biomarker levels among these groups, medians of log(HE4) and log(CA125) significantly differed among the three disease groups (p<0.001, both). Multiple pairwise comparisons were run using the Mann-Whitney U test at adjusted alpha value determined as 0.017 (0.05/3 paired) to avoid type I error. Serum HE4

Table 1. Baseline characteristics and histopathology of women who presented with pelvic or adnexal masses

Variables	Total (n = 518)
Clinical characteristics	
Age (years), mean (SD)	46.7 (13.9)
Weight (kg), mean (SD)	58.7 (12.7)
Height (cm), mean (SD)	154.9 (6.3)
BMI (kg/m2), mean (SD)	24.5 (5.0)
Parity, median (IQR)	1 (2)
Underlying diseases, n (%)	180 (34.8)
- Hypertension	123
- Diabetic mellitus	46
- Other	44
Menopausal status, n (%)	
- Premenopausal	304 (58.7)
- Postmenopausal	214 (41.3)
Domicile, n (%)	
- Bangkok Metropolitan	110 (21.2)
- Central*	230 (44.4)
- Northern	39 (7.5)
- Northeastern	25 (4.8)
- Eastern	37 (7.1)
- Western	51 (9.9)
- Southern	26 (5.0)
Histopathology	
Benign diseases, n (%)	316 (61.0)
- Benign ovarian tumors	165 (31.9)
Teratomas	56 (10.8)
Serous cystadenomas	34 (6.6)
Mucinous cystadenomas	64 (12.4)
Brenner tumors	2 (0.4)
Fibrothecomas	9 (1.7)
- Benign gynecologic diseases	151 (29.2)
Endometriotic cysts	107 (20.7)
Tuboovarian abscesses	10 (1.9)
Functional/simple/paratubal cysts	19 (3.7)
Pseudocysts	2 (0.4)
Leiomyomas	13 (2.5)
Borderline tumors, n (%)	43 (8.3)
- Serous borderline tumors	9 (1.7)
- Mucinous borderline tumors	34 (6.6)
Cancer, n (%)	159 (30.7)
- EOCs	134 (25.9)
Low grade serous carcinoma	8 (1.5)
Mucinous carcinoma	11 (2.1)
Endometrioid carcinoma	29 (5.6)
Clear cell carcinoma	41 (7.9)
High grade serous carcinoma	25 (4.8)
Mixed epithelial carcinoma	9 (1.7)
Adenocarcinoma, NOS	9 (1.7)
- Non-EOCs	25 (4.8)
Malignant germ cell tumors	9 (1.7)
Malignant sex-cord tumors	4 (0.8)
Metastatic tumors	12 (2.3)

BMI = body mass index; EOC = epithelial ovarian carcinoma; IQR = interquartile range; NOS = not otherwise specified; SD = standard deviation

* Excluded Bangkok Metropolitan

Table 2. Comparison of serum HE4 and CA125 levels with disease groups of pelvic or adnexal masses

Diseases	n	Log(HE4)			Log(CA125)		
		Median	IQR	*p*-value*	Median	IQR	*p*-value*
Benign diseases	316	1.72	0.20	Ref.	1.55	0.74	Ref.
BOTs	43	1.84	0.21	<0.001†	1.57	0.60	0.590
Ovarian cancers	159	2.17	0.77	<0.001†	2.40	1.01	<0.001†

BOT = borderline ovarian tumor; CA125 = cancer antigen 125; HE4 = human epidermis protein 4; IQR = interquartile range
* Kruskal-Wallis test, significant at $p<0.001$
† Multiple comparison by Mann-Whitney U test, significant at adjusted $p<0.017$ (0.05/3)

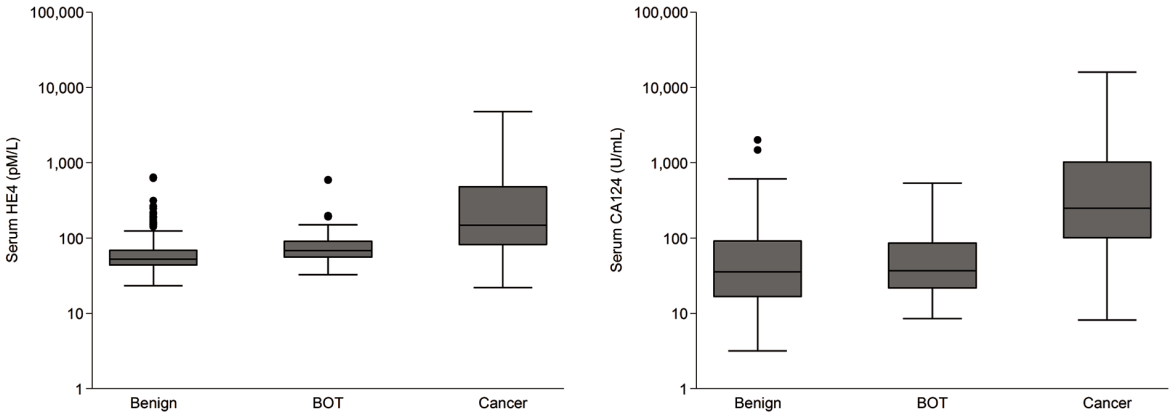

Fig. 1 Comparison of serum HE4 and CA125 levels in diseases associated with pelvic or adnexal masses (BOT = borderline ovarian tumor; CA125 = cancer antigen 125; HE4 = human epidermis protein 4).

levels compared between pairs of the three disease groups differed significantly ($p<0.001$, all three pairs). Serum CA125 levels significantly differed between a pair of benign disease and OC groups ($p<0.001$), and a pair between BOT and OC groups ($p<0.001$), but not between benign disease and BOT groups ($p = 0.590$).

Evaluation of the diagnostic performance of HE4 and CA125 for discrimination between benign diseases, BOTs, and OCs using ROC analysis were shown in Table 3. Serum HE4 and CA125 levels did not significant differ in distinguishing between non-cancerous lesions (benign diseases and BOTs) and OCs (0.85 vs. 0.83, $p = 0.402$). When stratified by menopausal status, the ROC-AUC for HE4 in premenopausal women was slightly higher than for CA125 (0.88 vs. 0.85, $p = 0.428$), but significantly lower than that of CA125 for postmenopausal women (0.79 vs. 0.86, $p = 0.049$). When BOTs were included with OCs, the discrimination between benign diseases and non-benign tumor (BOTs and OCs) was poorer than when BOTs were groups with benign diseases against OCs. In differentiating between benign diseases and BOTs, the ROC-AUC of HE4 was significantly higher than that of CA125 (0.71 vs. 0.53, $p<0.001$). At the cutoff level of 51 pM/L, HE4 in these distinctions had 88.4% Sn and 46.5% Sp. The HE4 also had slightly lower ROC-AUC than serum CA125 in differentiating between BOTs and OCs (0.77 vs. 0.84, $p = 0.111$).

Comparison of various cutoff values for HE4 and CA125 were shown in Table 4. The optimal cutoff values in all, pre- and postmenopausal women were 72, 63, and 126 pM/L for HE4 and 110, 123, and 57 U/mL for CA125, respectively. The optimal and recommended HE4 cutoff values for all menopausal states were slightly different; use of this single cutoff increased Sn and decreased Sp compared with using separate cutoffs for pre- and postmenopausal women in both optimal and recommended types. However, the single optimal cutoff value at 72 pM/L had higher dORs than the recommended cutoff value at 70 pM/L and separate cutoffs for pre- and postmenopausal women. In contrast, the optimal cutoffs of CA125 for all women had lower Sn and higher Sp than using separate cutoffs for pre- and postmenopausal women. When compared with the recommended cutoffs, the optimal cutoffs stratified by menopausal status had higher dOR.

Distribution of histopathologic diagnosis according to serum HE4 and CA125 levels at optimal

Table 3. Performance and optimal cutoff values of serum HE4 and CA125 for discrimination between benign diseases, borderline ovarian tumor and ovarian cancer

Patients (n)	Tests	ROC			Cutoff values	Sn	Sp
		AUC	95% CI	*p*-value			
Benign + BOTs (359) vs. cancer (159)	HE4	0.85	0.81 to 0.89	0.402	72	83.7	74.7
	CA125	0.83	0.79 to 0.87	Ref.	110	74.2	79.4
Premenopausal women	HE4	0.88	0.82 to 0.94	0.428	63	87.1	81.4
Benign + BOTs (242) vs. cancer (62)	CA125	0.85	0.80 to 0.91	Ref.	123	80.7	79.8
Postmenopausal women	HE4	0.79	0.73 to 0.85	0.049*	126	61.9	84.6
Benign + BOTs (117) vs. cancer (97)	CA125	0.86	0.81 to 0.91	Ref.	57	81.4	74.4
Benign (316) vs. BOTs + cancer (202)	HE4	0.83	0.79 to 0.87	0.008*	72	75.3	78.2
	CA125	0.77	0.72 to 0.81	Ref.	110	62.9	79.4
Premenopausal women	HE4	0.84	0.80 to 0.89	0.013*	65	72.0	86.0
Benign (222) vs. BOTs + cancer (82)	CA125	0.75	0.69 to 0.82	Ref.	120	62.2	78.4
Postmenopausal women	HE4	0.77	0.71 to 0.83	0.06	119	58.3	85.1
Benign (94) vs. BOTs + cancer (120)	CA125	0.83	0.78 to 0.89	Ref.	55	75.0	76.6
Benign (316) vs. BOTs (43)	HE4	0.71	0.63 to 0.78	<0.001*	51	88.4	46.5
	CA125	0.53	0.44 to 0.61	Ref.	14	93.0	19.6
Premenopausal women	HE4	0.69	0.57 to 0.81	0.003*	56	65.0	68.5
Benign (222) vs. BOTs (20)	CA125	0.45	0.33 to 0.57	Ref.	25	80.0	31.1
Postmenopausal women	HE4	0.63	0.51 to 0.75	0.496	51	91.3	24.5
Benign (94) vs. BOTs (23)	CA125	0.68	0.56 to 0.79	Ref.	14	95.7	35.1
BOTs (43) vs. cancer (159)	HE4	0.77	0.70 to 0.84	0.111	95	66.7	81.4
	CA125	0.84	0.76 to 0.90	Ref.	123	72.3	83.7
Premenopausal women	HE4	0.83	0.74 to 0.92	0.183	90	59.7	100.0
BOTs (20) vs. cancer (62)	CA125	0.90	0.82 to 0.97	Ref.	123	80.7	95.0
Postmenopausal women	HE4	0.73	0.63 to 0.83	0.241	155	54.6	87.0
BOTs (23) vs. cancer (97)	CA125	0.80	0.68 to 0.87	Ref.	197	53.6	95.7

AUC = area under the curve; BOT = borderline ovarian tumor; CA125 = cancer antigen 125; CI = confident interval; HE4 = human epidermis protein 4; ROC = receiver operating characteristic; Sn = sensitivity; Sp = specificity
* Significant at $p<0.05$

Table 4. Comparison of sensitivity, specificity, and positive and negative predictive values for serum HE4 and CA125 in different cutoff values in predicting ovarian malignancy in women with pelvic or adnexal masses

Tests	Cutoff types	Cutoff values	Sn	Sp	LR+	LR-	dOR	PPV	NPV
HE4 (pM/L)	Optimal	72 for All	83.7	74.7	3.30	0.22	15.16	59.4	91.2
		63 for PreM/126 for PostM	71.7	82.5	4.10	0.34	11.94	64.4	86.8
	Recommended	70 for All	82.4	74.9	3.28	0.23	13.97	59.3	90.6
		70 for PreM/140 for PostM	64.8	88.0	5.40	0.40	13.50	70.6	85.0
CA125 (U/mL)	Optimal	110 for All	74.2	79.4	3.60	0.33	11.09	61.5	87.4
		123 for PreM/57 for PostM	81.1	78.0	3.69	0.24	15.21	62.6	90.3
	Recommended	35 for All	89.9	49.6	1.78	0.20	8.76	44.1	91.8
		200 for PreM/35 for PostM	75.5	80.5	3.87	0.30	12.72	63.2	88.1

All = premenopausal and postmenopausal women; CA125 = cancer antigen 125; dOR = diagnostic odds ratio; HE4 = human epidermis protein 4; LR- = negative likelihood ratio; LR+ = positive likelihood ratio; NPV = negative predictive value; PPV = positive predictive value; PreM = premenopausal women; PostM = postmenopausal women; Sn = sensitivity; Sp = specificity

cutoff values were shown in Table 5. Although 63% of the benign disease group expressed lower levels of serum HE4 and CA125, 26.1% of patients with benign ovarian tumors and 29.5% of those with other benign gynecologic diseases had higher HE4 whereas only 13.1% of the endometriosis patients had high HE4. In

Table 5. Distribution of histopathologic diagnosis with serum HE4 and CA125 levels at optimal cutoffs

Histopathology	n	HE4 (pM/L)	<72	<72	≥72	≥72
		CA125 (U/mL)	<123/57	≥123/57	<123/57	≥123/57
Benign diseases	316		200 (63.3%)	46 (14.5%)	47 (14.9%)	23 (7.3%)
Benign tumors	165		111 (67.3%)	11 (6.7%)	32 (19.4%)	11 (6.7%)
Endometriosis	107		60 (56.1%)	33 (30.8%)	5 (4.7%)	9 (8.4%)
Other	44		29 (65.9%)	2 (4.6%)	10 (22.7%)	3 (6.8%)
BOTs	43		22 (51.2%)	0 (0.0%)	11 (25.6%)	10 (23.3%)
Ovarian cancers	159		9 (5.7%)	17 (10.7%)	21 (13.2%)	112 (70.4%)
LGSCs	8		0 (0.0%)	1 (12.5%)	3 (37.5%)	4 (50.0%)
MCs	13		0 (0.0%)	4 (30.8%)	3 (23.1%)	6 (46.2%)
EMCs	29		0 (0.0%)	2 (6.9%)	4 (13.8%)	23 (79.3%)
CCCs	41		6 (14.6%)	7 (17.1%)	5 (12.2%)	23 (56.1%)
HGSCs	25		0 (0.0%)	0 (0.0%)	0 (0.0%)	25 (100%)
Other EOCs	18		1 (5.6%)	0 (0.0%)	2 (11.1%)	15 (83.3%)
Non-EOCs	25		2 (8.0%)	3 (12.0%)	4 (16.0%)	16 (64.0%)
Total	518		231 (44.6%)	63 (12.2%)	79 (15.3%)	145 (28.0%)

HE4 = human epidermis protein 4; CA125 = cancer antigen 125; BOT = borderline ovarian tumor; LGSC = low grade serous carcinoma; MC = mucinous carcinoma; EMC = endometrioid carcinoma; CCC = clear cell carcinoma; HGSC = high grade serous carcinoma; EOC = epithelial ovarian cancer

the benign disease group, 50% of patients with tubo-ovarian abscesses had high HE4 levels. Half of the BOT group had high HE4 expression >72 pM/L, all of whom had tumor masses confined within their ovaries without peritoneal seeding; nevertheless, 69.8% of the BOT group had HE4 levels between 51 and 95 pM/L. Moreover, 76.8% of the BOT group had low serum CA125. In the OC group, higher HE4 was seen in 87.5% of low-grade serous carcinomas (LGSCs), 69.3% mucinous carcinomas (MCs), 93.1% endometrioid carcinomas (EMCs), 68.3% CCCs, 100% high-grade serous carcinoma (HGSCs), 94.4% other EOCs and 80% non-EOCs. However, low levels of both HE4 and CA125 were seen in 14.6% of CCCs and 8.0% of non-EOCs. The MCs varied in HE4 and CA125 expression, but showed no pattern of low levels for both markers.

Discussion

Accurate prediction of the nature of a pelvic or adnexal mass can allow patients to be cared for at the most appropriate level, whether locally or at specialized center for further management without under- or overburdening health resources. Systematic review has shown that OC patients who are managed in specialized centers by gynecologic oncologists have improved survival outcome[13]. HE4 is one of the most promising biomarkers to complement CA125 for improving diagnostic performance of ovarian malignancy. However, incomplete information on diagnosis accuracy and optimal cutoff value for HE4 limits it clinical applicability to Thai women.

The present study investigated the accuracy of HE4 in evaluating the likelihood of ovarian malignancy in Thai women. This was a cross-sectional study of all women aged at least 18 years with pelvic or adnexal masses scheduled to undergo elective surgery, and who were prospectively enrolled, regardless of final pathologic diagnosis of the mass. This allowed the study population to reflect real clinical situations in using of biomarkers would help determine the nature of the mass.

Of 352 women with pelvic or adnexal masses in the present study, 69.3% were in the non-cancer group and 30.7% were in the cancer group. The BOTs were included in the non-cancer group owing to low malignant potential and indolent behavior. The performance of biomarkers was supported to include those in the non-cancer group. Endometriotic cysts were most common diseases in the non-cancer group (20.7%) and in premenopausal women (31.6%), CCCs were the most common disease in the cancer group (7.9%). The BOTs were found in 8.3% and were mostly of mucinous type. This reflects the variation in the study population and histopathologic distribution. Asian women tend to have higher incidences of BOTs and CCCs than Caucasian women, which effects the biomarkers' diagnostic performance. Compared with previous studies[4,14-16], the present study had

higher prevalence of BOTs (10.2% vs. 3.9 to 6.5%) and CCCs (7.4% vs. 1.5 to 1.9%).

Wang et al[17] conducted a meta-analysis of the diagnostic accuracy of serum HE4 and CA125 for OCs, using 32 studies. Comparable with the present study, area under summary ROC (sROC) curves for HE4 were 0.89 (95% CI 0.86 to 0.92) and CA125 0.87 (95% CI 0.88 to 0.93). However, the pooled sensitivity (78%, 95% CI 77 to 79) and specificity (86%, 95% CI 85 to 87) for HE4 differed from the present study as these results depended on cutoff value. In the present meta-analysis, the sROC-AUC for serum CA125 was higher than for HE4 among postmenopausal patients (0.88, 95% CI 0.85 to 9.91 for HE4; and 0.92, 95% CI 0.89 to 0.94 for CA125), but was similar in the premenopausal subgroup (0.85, 95% CI 0.82 to 0.88 for HE4; and 0.85, 95% CI 0.82 to 0.88 for CA125). These results were consistent with the present study in postmenopausal but not premenopausal patients. The ROC-AUC was slightly higher for HE4 than CA125 in the premenopausal women (0.88 vs. 0.85, $p = 0.428$) with 87.1% Sn and 81.4% Sp. Serum HE4 was shown not be elevated in endometriosis and this may have contributed to the better performance of HE4 in premenopausal women[18,19].

There are conflicting reports regarding the optimal cutoff of serum HE4. The use of different cutoffs also plays a role in the outcome of different studies. The manufacturer recommended separate cutoffs for pre- and postmenopausal women. Nevertheless, a recent publication showed that HE4 levels increase with age, not with menopausal status[20]. Using the Youden index, this current study suggested the optimal HE4 cutoffs at 72 pM/L for all menopausal status demonstrated higher LR+ and lower LR- than using separate cutoffs for pre- and postmenopausal women.

Recently, ECOs are divided in two broad categories of type I and type II, based on difference in morphology, immunohistochemistry, molecular genetic events, and clinical behavior[21]. Type I tumors include all major subtypes (LGSCs, EMCs, MCs, and CCCs) but exhibit low-grade nuclear and architectural features, generally indolent, present in early stage and can be associated with benign ovarian precursor lesions. Type II tumors comprise HGSCs, high grade EMCs, and undifferentiated carcinomas. They are aggressive and present in advanced stage. Serum HE4 levels vary among different histological subtypes of EOCs with the highest values for type II tumors[7]. In the present study, serum HE4 had the highest level in EOCs compared with other groups, and had significantly higher levels in SCs and EMCs, but lower levels in CCCs and MCs, corresponding to type II and type I respectively.

At the optimal HE4 cutoff 72 pM/L, the authors found false negatives (lower HE4 levels) in 16.4% of OCs overall, including 30.8% of MCs and 31.7% of CCCs. Type I EOC is a group of different tumors that require attention but can be difficult to diagnose. The CCCs and MCs are quite aggressive, particularly at late stages, and have even higher mortality than type II. Finding early markers that are specific to all histology subgroups is a future challenge.

At the optimal HE4 cutoff 72 pM/L, 25.3% of non-cancerous lesions were seen as false positives. Endometriosis had a 13% false positive rate, and HE4 was elevated in many non-cancerous lesions, including about half of BOTs, quarter of benign tumors and 30% of benign gynecologic diseases other than benign tumor and endometriosis (half of tubo-ovarian abscess [TOA]). These findings are inconsistent with that of Moore et al[18] who found elevated HE4 in 10% of TOAs and 8% of benign ovarian tumors.

Whether BOTs should be classified as non-cancer or cancer is unclear. The present study found 69.8% of BOTs had serum HE4 levels of 51 to 95 pM/L, and 76.8% of BOTs had serum CA125 levels lower than optimal cutoff values. However, HE4 expression in BOTs was not associated with histologic type, age, CA125 level, or disease stage[22]. All BOTs with elevated HE4 in the present study were confined within the ovary without peritoneal seeding. Further study of other predictive factors that complement HE4 for BOT diagnosis could be interesting.

Serum HE4 is a novel biomarker, but most published data are based on Caucasian women. This diagnostic study evaluated effectiveness of HE4 and optimal cutoff value in Thai women, which could be clinically applicable. Measurement bias was limited by blinding reviewers of pathologic slides to clinical data and biomarker levels. The present study has a limitation in its application to the country as a whole (generalization). Although the participants were in every region of Thailand, those were a hospital-based population from a super-tertiary center and were not representative all Thai women.

Conclusion

The optimal cutoff for serum HE4 is 72 pM/L, regardless of all menopausal status. Although the accuracy of serum HE4 is strongest for OCs (especially

EOCs), its use should be complemented with serum CA125, because of its limitations in predicting of mucinous carcinomas and CCCs and lower performance in postmenopausal women.

What is already known on this topic?

Serum HE4 and CA125 showed similar performance in discrimination between non-cancerous lesions and OCs (ROC-AUC 0.85 vs. 0.83, $p = 0.402$). The performance of serum HE4 and CA125 in the present study was consistent with a meta-analysis study of Wang et al[17], area under summary ROC (sROC) curve of HE4 was 0.89 (95% CI 0.86 to 0.92) and CA125 0.87 (95% CI 0.88 to 0.93). Otherwise, the performance of serum HE4 in postmenopausal women was significantly poorer than CA125 (ROC-AUC 0.79 vs. 0.86, $p = 0.049$) as this meta-analysis.

What this study adds?

Serum HE4 levels were significant different among three groups of benign gynecologic diseases, BOTs, and OCs ($p<0.001$). For discrimination between non-cancerous lesions and OCs in Thai women, serum HE4 had the optimal cutoff value at 72 pM/L for all menopausal status. Thirty-one percent of mucinous carcinoma and 31.7% of clear cell carcinoma expressed lower HE4 levels. HE4 showed significantly better ability for distinction of benign diseases and BOTs than CA125 (ROC-AUC 0.71 vs. 0.53, $p<0.001$). Seventy percent of BOTs had serum HE4 levels at 51 to 95 pM/L.

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Potential conflicts of interest

None.

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ความแม่นยำในการวินิจฉัยและค่าตัดที่เหมาะสมของซีรัม HE4 เพื่อทำนายมะเร็งรังไข่ในสตรีชาวไทยที่มีก้อนอุ้งเชิงกราน

มรุต ญาณารณพ, จิติมา ติยายน, ศรัญญู นาครังษี, บัณฑิต ถิ่นคำรพ

ภูมิหลัง: *ซีรัม human epididymis protein 4 (HE4) พบการแสดงออกอย่างสูงในสตรีผู้ป่วยมะเร็งรังไข่ แต่ข้อมูลเกี่ยวกับการใช้ประโยชน์ HE4 ทางคลินิกในคนไทยมีจำกัด*

วัตถุประสงค์: *เพื่อประเมินความแม่นยำในการวินิจฉัยและค่าจุดตัดที่เหมาะสมของ HE4 ในการแยกรอยโรคไม่อันตราย เนื้องอกรังไข่ก้ำกึ่ง และมะเร็งรังไข่ เปรียบเทียบกับ CA125 ในสตรีชาวไทยที่โรงพยาบาลราชวิถี*

วัสดุและวิธีการ: *การศึกษาแบบตัดขวางในสตรีไทยอายุ 18 ปีขึ้นไป ที่มีก้อนในอุ้งเชิงกรานผู้ซึ่งเข้ารับการผ่าตัดแบบนัดที่โรงพยาบาลราชวิถี ระหว่าง พ.ศ. 2555 ถึง พ.ศ. 2556 ระดับซีรัม HE4 และ CA125 ได้ถูกวัดก่อนผ่าตัดและชิ้นเนื้อทางพยาธิวิทยาถูกทบทวน*

ผลการศึกษา: *ผู้ร่วมการศึกษา 518 ราย ถูกประเมิน 316 ราย เป็นรอยโรคไม่อันตราย 43 ราย เป็นเนื้องอกรังไข่ก้ำกึ่ง และ 159 ราย เป็นมะเร็งรังไข่ ระหว่างโรคที่ไม่เป็นมะเร็งและมะเร็งรังไข่ พื้นที่ใต้โค้ง receiver operating characteristic (ROC-AUC) ของ HE4 แตกต่างจาก CA125 เล็กน้อย (0.85 และ 0.83, p = 0.402) แต่ต่ำกว่าอย่างมีนัยสำคัญในสตรีวัยหมดประจำเดือน (0.79 และ 0.86, p = 0.045) ค่าจุดตัดที่เหมาะสมของ HE4 คือ 72 pM/L สำหรับทุกสถานะประจำเดือน HE4 ระดับต่ำพบในมะเร็งชนิด mucinous ร้อยละ 30.8 และมะเร็งชนิด clear cell ร้อยละ 31.7 HE4 พบ ROC-AUC สูงกว่า CA125 อย่างมีนัยสำคัญในการแยกโรคไม่ร้ายแรงกับเนื้องอกรังไข่ก้ำกึ่ง (0.71 และ 0.53, p<0.001) ร้อยละ 70 ของเนื้องอกรังไข่ก้ำกึ่งมีระดับซีรัม HE4 51-95 pM/L*

สรุป: *แม้ว่า HE4 ที่ค่าจุดตัด 72 pM/L ในการแยกระหว่างรอยโรคที่ไม่ใช่มะเร็งและมะเร็งรังไข่ เหมาะสมสำหรับสตรีทั้งวัยก่อนและหลังหมดประจำเดือน แต่การใช้ HE4 มีข้อจำกัดในสตรีวัยหมดประจำเดือน มะเร็งชนิด mucinous และมะเร็งชนิด clear cell ดังนั้นการใช้ร่วมกับ CA125 เป็นสิ่งจำเป็น*
