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RESEARCH ARTICLE

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Diagnostic performance of CA 125, HE4, and risk of Ovarian Malignancy Algorithm for ovarian cancer

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Objective: We evaluated the diagnostic performance of CA 125, HE4, and ROMA for ovarian cancer in Koreans and set optimal cutoffs.

Method: Serum levels of HE4 and CA 125 and the ROMA score were determined in 762 patients with benign gynecological disease and 70 with ovarian cancer. Receiver operating characteristic curves were constructed to calculate the areas under the curve (AUC). CA 125, HE4, and ROMA exhibiting maximum Youden index were determined, respectively, as the optimal cutoffs, and sensitivity and specificity were evaluated by applying those cutoffs.

Results: In benign diseases, CA 125 significantly increased in patients with uterine myoma, adenomyosis, endometrial pathology, or endometriosis, but HE4 only increased in patients with adenomyosis. For the diagnosis of ovarian cancer, the combination of CA 125, HE4, and age showed the highest AUC value of 0.892 in the premenopausal group, and ROMA demonstrated the best diagnostic performance, with an AUC of 0.935 in postmenopausal patients. When the optimal cutoff values for CA 125 and HE4 were applied, the sensitivities of CA 125, HE4, and ROMA in premenopausal women were all the same at 0.714, while the specificities were 0.841, 0.974, and 0.972, respectively. In the postmenopausal group, the sensitivities of these markers were 0.857, 0.804, and 0.929, and the specificities were 0.836, 0.887, and 0.800, respectively.

Conclusion: Although all markers demonstrated good diagnostic performance, they varied depending on the pathologic types of benign diseases and ovarian cancer. For accurate diagnosis of ovarian cancer, CA 125, HE4, and ROMA should be used complementarily.

KEYWORDS
CA 125, HE4, ovarian cancer, ROMA, tumor marker

1 | INTRODUCTION

Ovarian cancer is a common malignant disease and is reported to be the fifth leading cause of cancer-related death in women.[1] In Korea, it was reported to be the tenth most common cancer in women, and the 5-year survival rate of ovarian cancer was 64.1%, according to domestic statistics from the National Cancer Information Center for 2010-2014.[2] The prognosis of this tumor is known to be relatively good if diagnosed in early stages;[3,4] however, about 3/4 of ovarian cancer patients are diagnosed in advanced stages, and the survival rate is as low as 10%-20% in these cases. Therefore, the early detection of ovarian cancer is important for improving patient prognosis.[5]

CA 125 is a widely used tumor marker for diagnosis and monitoring of ovarian cancer, but is not increased in some histological types

of ovarian cancer.[6] It also has a high false positive rate in benign gynecological diseases such as ovarian cysts and uterine myomas.[7] Therefore, CA 125 alone is not sufficient for screening and differential diagnosis of ovarian cancer.[8] Given these circumstances, many studies have introduced human epididymis protein 4 (HE4) as a new tumor marker to help diagnose ovarian cancer.[9-11] In 2010, the US FDA approved the Risk of Ovarian Malignancy Algorithm (ROMA) equation using both CA 125 and HE4 levels and patient menopausal state as a new biomarker for the diagnosis of ovarian cancer.[12-14] Since then, many studies have reported that the simultaneous testing of HE4 and CA 125 with calculation of ROMA is valuable in the diagnosis of ovarian cancer.

In Korea, the HE4 test has been utilized as a new biomarker for ovarian cancers since 2014. Although many studies have been conducted regarding the diagnostic performance of HE4, CA 125, and ROMA in different countries and races,[12,15,16] there is little research on the utility of HE4 and ROMA in Korean women.[17] Therefore, it is necessary to investigate the clinical utility of HE4 and CA 125 and to identify the optimal ROMA cutoff for the diagnosis of ovarian cancer in Koreans.

In this study, we evaluated the diagnostic performance of CA 125, HE4, and ROMA for ovarian cancer in Koreans and set the optimal cutoff for each tumor marker.

2 | MATERIALS AND METHODS

2.1 | Study subjects

The HE4 assay was requested for a total of 845 patients who visited the Department of Obstetrics and Gynecology, National Health Insurance Service Ilsan Hospital, from March 2015 to August 2017 with suspected gynecological disease. Of these patients, we excluded 13 patients including four cases not tested for CA 125, six patients with malignant disease other than ovarian cancer, and three patients who had follow-up HE4 blood tests. Finally, medical records of 832 patients were reviewed retrospectively. Patients' ages; CA 125 and HE4 levels; menopausal state; final diagnosis based on clinical, histologic, and radiologic findings; and the International Federation of Gynecology and Obstetrics (FIGO) stages of ovarian cancer were recorded. This study was approved by the Institutional Review Board of National Health Insurance Service Ilsan Hospital (IRB no. 2017-01-038).

2.2 | CA 125 and HE4 assays

CA 125 and HE4 tests were performed with a Cobas E 602 immunoassay analyzer using Elecsys CA 125 II and Elecsys HE4 test reagents (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. Both assays utilize the electrochemiluminescence immunoassay (ECLIA) principle. CA 125 levels were measured by the Department of Laboratory Medicine at National Health Insurance Ilsan Hospital, and HE4 concentrations were determined by the Green Cross Reference Laboratory (Yongin-si, Gyeonggi-do, Republic of Korea). The manufacturer suggests a CA 125 cutoff of >35 U/mL and provides the reference limit for each age group in HE4. However, the cutoff of HE4 for diagnosing cancer was not presented; therefore, we used the default cutoff of HE4 set by the Green Cross Reference Laboratory (>92.1 pmol/L for premenopausal women and >121.1 pmol/L for postmenopausal women).

2.3 | Calculation of ROMA value

Using the concentrations of CA 125 and HE4, we calculated ROMA according to the mathematical equations presented below.

1. Premenopausal: predictive index (PI) = −12.0 + 2.38 × LN[HE4] + 0.0626 × LN[CA 125]
2. Postmenopausal: PI = −8.09 + 1.04 × LN[HE4] + 0.732 × LN[CA 125], where LN = natural log function.
3. ROMA (%) = exp(PI)/[1 + exp(PI)] × 100, where exp(PI) = e^{PI}.

The cutoff value of the ROMA proposed by the manufacturer was ≥11.4% in premenopausal women and ≥29.9% in postmenopausal women.

2.4 | Statistical analyses

All statistical analyses were performed by Analyse-it for Microsoft Excel Method Evaluation Edition version 3.76.1 (Analyse-it Software, Ltd., Leeds, UK) and IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, US). For continuous variables, the Mann-Whitney *U* test and Kruskal-Wallis test were used for comparisons between two groups and among three or more groups, respectively. The Steel multiple comparison test was performed to compare the control with several other groups to compensate for alpha error. The Chi-square test was used to compare the categorical variables between the study groups. To analyze the diagnostic performance of each tumor marker and the ROMA value in ovarian cancer, receiver operating characteristic (ROC) curves were constructed, and the area under the curve (AUC) of each marker was calculated according to the method proposed by DeLong et al[18] From the results of ROC curve analyses, the CA 125, HE4, and ROMA with maximal values of Youden index were determined, respectively, as the optimal cutoff values. Sensitivity, specificity, and positive and negative predictive values (PPV and NPV) were evaluated when diagnosing ovarian cancer by applying default and optimal cutoff values. Diagnostic accuracy was calculated as (sensitivity + specificity)/2. Multivariate analysis was performed with binary logistic regression with the diagnosis of ovarian cancer as a binary-dependent variable and patient age, menopausal state, and each test's values as independent variables to identify variables significantly correlated with diagnosis of ovarian cancer. The estimated probabilities calculated from the combination of independent variables were used in ROC curve analyses to evaluate the diagnostic performance of the combination of variables. For all statistical analyses, a *P*-value≤0.05 was considered significant.

3 | RESULTS

3.1 | Characteristics of study subjects

Of the 832 patients, 762 and 70 were diagnosed with benign gynecological diseases and ovarian cancer, respectively. The clinical characteristics of each group are shown in Table 1. There were significant differences in age, CA 125 and HE4 levels, and proportion of menopausal patients between the two groups. In the benign disease group, 284 (37.3%) had ovarian cysts/tumors, 217 (28.5%) had uterine leiomyomas, 80 (10.5%) had adenomyosis, 77 (10.1%) had endometrial pathology, and 69 (9.1%) had endometriosis of the ovary. In patients with ovarian cancer, endometrioid adenocarcinoma was seen in 12 patients (17.1%), followed by 11 (15.7%) with serous adenocarcinoma, 11 (15.7%) with adenocarcinoma, 4 (5.7%) with mucinous adenocarcinoma, and 4 (5.7%) with clear cell adenocarcinoma. Regarding the FIGO stages of cancer patients, 19 (27.1%) cases were

TABLE 1 Characteristics of study subjects

Parameter	Benign disease (N = 762)	Ovarian cancer (N = 70)	*P*-value
Age (y)[a]	45.0 (36.0-51.0)	64.0 (50.9-77.0)	<0.0001
CA 125 level (U/mL)[a]	17.1 (12.0-35.5)	90.5 (30.9-416.6)	<0.0001
HE4 level (pmol/L)[a]	47.2 (40.9-55.3)	163.9 (88.1-473.9)	<0.0001
No. of menopausal subjects, N (%)	195 (25.6%)	56 (80.0%)	<0.0001
Diagnosis, N (%)	Ovarian cyst(s)/tumor(s), 284 (37.3%)	Ovarian cancer, 70 (100.0%)	-
	Uterine leiomyoma, 217 (28.5%)		
	Adenomyosis, 80 (10.5%)		
	Endometrial pathology, 77 (10.1%)		
	Endometriosis of ovary, 69 (9.1%)		
	Pelvic inflammatory disease, 11 (1.4%)		
	Polycystic ovarian disease, 4 (0.5%)		
	Other benign conditions, 20 (2.6%)[b]		
Pathologic finding, N (%)	Leiomyoma, 164 (21.5%)	Surface epithelial-stromal tumors	-
	Adenomyosis, 65 (8.5%)	Endometrioid adenocarcinoma, 12 (17.1%)	
	Endometrial polyp/hyperplasia, 63 (8.3%)	Serous adenocarcinoma, 11 (15.7%)	
	Endometriosis/endometriotic cyst, 58 (7.6%)	Adenocarcinoma, 11 (15.7%)	
	Mature cystic teratoma, 45 (5.9%)	Mucinous adenocarcinoma, 4 (5.7%)	
	Mucinous cystadenoma, 28 (3.7%)	Clear cell adenocarcinoma, 4 (5.7%)	
	Serous cystadenoma, 23 (3.0%)	Carcinosarcoma, 3 (4.3%)	
	Other benign cyst(s)/tumor(s), 43 (5.6%)	Sex cord-stromal tumors	
	Inflammation, 5 (0.7%)	Granulosa cell tumor, 3 (4.3%)	
	Other benign conditions, 5 (0.7%)	Germ cell tumors	
	No pathologic diagnosis, 9 (1.2%)	Immature teratoma, 3 (4.3%)	
	Biopsy not done, 254 (33.3%)	Malignant, not otherwise specified	
		Metastatic adenocarcinoma, 3 (4.3%)	
		Diffuse large B cell lymphoma, 1 (1.4%)	
		Biopsy/surgery not done, 15 (21.4%)	
FIGO stage, N (%)	-	I, 19 (27.1%)	
		II, 2 (2.9%)	
		III, 31 (44.3%)	
		IV, 18 (25.7%)	

FIGO, the International Federation of Gynecology and Obstetrics.

[a]Data are shown as median (1st to 3rd quartiles).

[b]Cases of dysfunctional uterine bleeding (N = 4), non-gynecological problems (N = 4), uterovaginal prolapse (N = 4), vulvar mass (N = 2), amenorrhea, dysmenorrhea, torsion of ovary, premature ovarian failure, invasive hydatidiform mole, and bacterial vaginosis.

in stage I, 2 (2.9%) were in stage II, 31 (44.3%) were in stage III, and 18 (25.7%) were in stage IV.

3.2 | Tumor marker levels in benign diseases and ovarian cancer

The levels of CA 125 and HE4 in benign gynecological diseases are presented in Table 2. The level of CA 125 was significantly higher in uterine myoma, adenomyosis, endometrial pathology, and endometriosis of the ovary than in the ovarian cyst/tumor group. Meanwhile, HE4 concentration was statistically higher only in the adenomyosis group compared to the ovarian cyst/tumor group.

The levels of CA 125 and HE4 according to pathologic findings and FIGO stage of ovarian cancer are shown in Table 3. Within the surface epithelial-stromal tumor group, the level of CA 125 was not statistically different between the endometrioid adenocarcinoma and other subgroups of surface epithelial-stromal tumor. However, the level of HE4 was significantly lower ($P = 0.0249$) in clear cell adenocarcinoma compared to endometrioid adenocarcinoma as a control group. In the comparison between surface epithelial-stromal tumor and other pathologic types, the level of CA 125 was statistically lower in the germ cell tumors ($P = 0.0270$); however, the level of HE4 showed no significant difference. In addition, the levels of both tumor markers increased significantly as the FIGO stage progressed.

3.3 | Diagnostic performance of each tumor marker for ovarian cancer

To investigate the independent variable significantly related to the diagnosis of ovarian cancer, multivariate logistic regression analysis was performed. For premenopausal patients, patient age and the level of CA 125 were significantly associated with ovarian cancer. For postmenopausal patients, both CA 125 and HE4 levels were related to diagnosis of ovarian cancer. When evaluating all patients regardless of menopausal state, the levels of CA 125 and HE4 and menopausal state were significant independent variables for the diagnosis of ovarian cancer (Table 4).

The diagnostic performance of each tumor marker, ROMA values, and the combinations of these tumor markers are summarized in Table 5. In the premenopausal patient group, the combination of CA 125 + HE4 + age showed the highest AUC value of 0.892; however, it was not statistically different from that of CA 125 (0.831). In the postmenopausal patient group, the ROMA value showed the highest AUC value of 0.935, and it was significantly different from that of CA 125 (0.889, $P = 0.0231$), but not different from that of HE4 (0.882) or CA 125 + HE4 combination (0.927). In all patients regardless of menopausal state, the AUC values of HE4, and the AUCs for the combinations of CA 125 + HE4, CA 125 + HE4 + age, CA 125 + HE4 + menopausal state, and CA 125 + HE4 + age + menopause state were 0.896, 0.909, 0.892, 0.931, and 0.923, respectively, and these values were significantly higher than that of CA 125 alone (0.811).

The sensitivity, specificity, PPV, and NPV from the ROC curve analysis using the default cutoff and the optimal cutoff of each tumor marker are presented in Table 6. In the premenopausal patient group, when the optimal cutoff (>71.7 U/mL) rather than the default cutoff (>35 U/mL) was applied, the sensitivity was the same, at 0.714; however, the specificity increased from 0.695 to 0.841 for CA 125. The diagnostic accuracy also increased from 0.705 to 0.778. For HE4, the specificity decreased from 0.986 to 0.974; however, the sensitivity increased from 0.571 to 0.714 when the optimal cutoff (>83.0 pmol/L) was used rather than the default cutoff (>92.1 pmol/L). The diagnostic accuracy of HE4 also increased from 0.779 to 0.844. Regarding ROMA values, the sensitivity was the same, at 0.714, but the specificity improved from 0.875 to 0.972.

In the postmenopausal patient group, the sensitivity of CA 125 was enhanced from 0.714 to 0.857, but the specificity decreased

TABLE 2 CA 125 and HE4 levels according to benign disease

Benign diseases	CA 125 (U/mL)	*P*-value[a]	HE4 (pmol/L)	*P*-value[a]
Ovarian cyst/tumor (N = 284)	14.3 (10.2-22.2)	-	46.0 (39.7-55.0)	-
Uterine myoma (N = 217)	**16.7 (12.1-30.2)**	**0.0017**	47.6 (42.7-53.6)	0.7021
Adenomyosis (N = 80)	**91.2 (38.8-151.8)**	**<0.0001**	**53.1 (45.7-61.2)**	**0.0004**
Endometrial pathology (N = 77)	**16.4 (13.6-26.9)**	**0.0116**	48.9 (43.4-60.5)	0.0792
Endometriosis of ovary (N = 69)	**42.0 (24.8-86.9)**	**<0.0001**	43.2 (38.2-49.2)	0.1644
Pelvic inflammatory diseases (N = 11)	15.3 (10.4-24.4)	1.0000	50.9 (35.2-63.6)	1.0000
Polycystic ovarian disease (N = 4)	12.4 (11.2-18.8)	1.0000	34.4 (30.0-39.9)	0.0773
Others (N = 20)	12.6 (7.7-25.3)	0.9993	46.0 (39.2-53.3)	1.0000

Data are shown as median (1st to 3rd quartiles).

[a]*P*-value was calculated using the Steel multiple comparison with the ovarian cyst/tumor group as a control.

Bold values imply statistically significant results.

TABLE 3 CA 125 and HE4 levels according to pathologic classification and FIGO stage of ovarian cancer

Classification	CA 125 (U/mL)	*P*-value[a]	HE4 (pmol/L)	*P*-value[a]
Pathology				
Surface epithelial-stromal tumor (N = 45)	94.0 (38.9-442.9)	-	198.5 (83.2-421.5)	-
Endometrioid adenocarcinoma (N = 12)	90.5 (49.3-859.7)	-	179.4 (83.9-345.3)	-
Serous adenocarcinoma (N = 11)	126.3 (59.2-592.6)	0.9999	317.7 (107.8-1379.0)	0.8450
Adenocarcinoma (N = 11)	415.2 (250.5-1167.0)	0.9370	225.0 (147.8-1243.6)	0.7293
Mucinous adenocarcinoma (N = 4)	72.2 (29.5-201.3)	0.8190	121.6 (57.5-186.7)	0.7613
Clear cell adenocarcinoma (N = 4)	31.0 (21.0-62.3)	0.7125	**37.8 (35.3-47.0)**	**0.0249**
Carcinosarcoma (N = 3)	28.1 (13.9-46.7)	0.7782	212.8 (126.8-350.9)	1.0000
Sex cord-stromal tumor (N = 3)	25.4 (13.8-42.0)	0.1318	95.1 (89.3-171.4)	0.8733
Germ cell tumor (N = 3)	**9.1 (7.2-16.7)**	**0.0270**	103.9 (97.3-109.4)	0.8046
Malignant, not otherwise specified (N = 4)	93.4 (51.9-170.2)	0.9903	95.8 (68.5-687.0)	0.9568
Biopsy/surgery not done (N = 15)	270.4 (39.6-1404.7)	0.9083	165.0 (88.0-754.8)	0.9918
FIGO stage				
I (N = 19)	28.1 (17.1-54.9)	-	84.6 (45.6-108.1)	-
II (N = 2)	72.2 (50.4-94.0)	0.2358	127.0 (41.2-212.8)	0.9401
III (N = 31)	**237.9 (53.9-429.3)**	**0.0008**	**213.5 (105.0-933.0)**	**<0.0001**
IV (N = 18)	**388.9 (79.3-1459.9)**	**0.0002**	**285.9 (99.7-620.6)**	**0.0004**

FIGO, the International Federation of Gynecology and Obstetrics.
Data are shown as median (1st to 3rd quartiles).
[a]*P*-value was calculated using the Steel multiple comparison with the surface epithelial-stromal tumor group as the control when comparing groups according to the pathologic classification, and the endometrioid adenocarcinoma group was used as a control for comparing groups belonging to surface epithelial-stromal tumors. In addition, the stage I group was used as a control to compare CA 125 and HE4 levels according to FIGO stage of ovarian cancer.
Bold values imply statistically significant results.

from 0.903 to 0.836 with the optimal cutoff (>22.5 U/mL). Furthermore, the diagnostic accuracy improved to 0.847 from 0.809. For HE4, despite the decrease in specificity from 0.969 to 0.887, the sensitivity increased from 0.571 to 0.804 when the optimal cutoff (>85.5 pmol/L) was used. Therefore, the diagnostic accuracy improved from 0.770 to 0.846. The sensitivity of ROMA was enhanced from 0.696 to 0.929, while the specificity was reduced from 0.913 to 0.800 when the optimal cutoff was applied. Overall, the diagnostic accuracy increased from 0.805 to 0.865.

In all subjects regardless of menopausal state, the sensitivity of CA 125 improved from 0.714 to 0.814, but the specificity decreased from 0.748 to 0.665 when using the optimal cutoff of >26.6 U/mL. As a result, the diagnostic accuracy changed from 0.731 to 0.740. The sensitivity, specificity, and diagnostic accuracy of HE4 were 0.800, 0.938, and 0.869, respectively, and HE4 showed a higher specificity and diagnostic accuracy than CA 125 when the optimal cutoff for HE4 (>79.6 pmol/L) was used.

4 | DISCUSSION

In this study, we analyzed the diagnostic performance of CA 125, HE4, and the combination of these two markers in the differential diagnosis of benign gynecological diseases and ovarian cancer in Korean patients. In addition, we tried to derive the optimal cutoff suitable for a Korean population. A previous domestic study set the 95th percentile of CA 125, HE4, and ROMA from 1,809 healthy people as the normal cutoff, and then these cutoffs were applied to differentiate 140 patients with ovarian cancer and 123 with benign gynecological diseases. In the same study, the sensitivity and specificity for CA 125 were 0.563 and 0.735 in patients less than 50 years of age and 0.859 and 0.762 in patients 50 years of age or older, respectively. The sensitivity and specificity of HE4 were 0.359 and 0.951, respectively, in patients under the age of 50 and 0.718 and 0.952 in subjects 50 years or older. In addition, the sensitivity and specificity of ROMA were 0.391 and 0.961, respectively, in premenopausal women and 0.845 and 0.800 in postmenopausal patients.[17] Although the specificity in a certain group was high, the sensitivity was lower than 0.4; therefore, this makes it difficult to apply normal cutoff values in actual clinical situations. In contrast, we calculated the optimal cutoff values from gynecological diseases and ovarian cancer patients and applied these optimal cutoff values to diagnose ovarian cancer. As a result, the sensitivity increased to higher than 0.7 in premenopausal patients and higher than 0.8 in postmenopausal women (Table 6).

TABLE 4 Multivariate analysis of patient age and CA 125 and HE4 levels for the diagnosis of ovarian cancer

Group	Variables	Odds ratio (95% CI)	*P*-value
Premenopause	Age (y)	**1.166 (1.011-1.344)**	**0.0352**
Case N = 14	CA 125 (U/mL)	**1.005 (1.001-1.010)**	**0.0142**
Control N = 567	HE4 (pmol/L)	1.005 (0.999-1.010)	0.0761
Postmenopause	Age (y)	1.014 (0.982-1.047)	0.3946
Case N = 56	CA 125 (U/mL)	**1.006 (1.001-1.011)**	**0.0227**
Control N = 195	HE4 (pmol/L)	**1.007 (1.000-1.013)**	**0.0366**
Total	Age (y)	1.027 (0.998-1.057)	0.0728
Case N = 70	CA 125 (U/mL)	**1.006 (1.003-1.009)**	**0.0004**
Control N = 762	HE4 (pmol/L)	**1.005 (1.002-1.009)**	**0.0046**
	Menopause	**7.220 (2.527-20.630)**	**0.0002**

CI, confidence interval.
Bold values imply statistically significant results.

Meanwhile, we analyzed the levels of CA 125 and HE4 in various benign gynecological diseases. The level of CA 125 was significantly higher in uterine myomas, adenomyosis, endometrial pathologies, and endometriosis of the ovary than in the ovarian cyst/tumor group. On the other hand, the level of HE4 was statistically high only in the adenomyosis group. In our previous study, Park et al[19,20] also reported that the levels of CA 125 and HE4 increased in various benign gynecological diseases, especially in adenomyosis. The increase in CA 125 and HE4 concentrations differed according to disease, and an increase in CA 125 was more frequent than an increase in HE4 in benign conditions, as our previous and current studies showed.[19,20] Therefore, it is considered that the specificity of HE4 is higher than that of CA 125. Furthermore, the degrees of increase in the levels of these tumor markers also varied according to the pathologic type of ovarian cancer. In this study, the level of CA 125 was low in germ cell tumor, whereas the level of HE4 was low in clear cell adenocarcinoma. Fujiwara et al differentiated low-grade serous/endometrioid carcinoma and every grade of clear cell, mucinous, and transitional carcinoma as type I ovarian cancer, while high-grade serous/endometrioid carcinoma and malignant mixed mesodermal tumor were classified as type II ovarian cancer. In their study, the AUCs of CA 125 for type I and type II cancer were 0.76 and 0.92, respectively, which were different from each other, and those of HE4 were 0.82 and 0.95. These results also demonstrate that the diagnostic performance of tumor markers differed according to the pathologic type of ovarian cancer.[16] As CA 125 and HE4 levels vary in benign gynecological diseases and pathologic types of ovarian cancers, it is desirable to use both tumor markers complementarily for diagnosing ovarian cancer.

In a meta-analysis of 28 studies, Wang et al reported that the sensitivity of HE4 for diagnosing ovarian cancer was 0.763, which was slightly lower than that of CA 125 (0.792); however, the specificity of HE4 was 0.936 and was significantly higher than that of CA 125 (0.821). In addition, they reported the sensitivity and specificity of ROMA as 0.853 and 0.824, respectively.[21] Similar to the meta-analysis, the sensitivity and the specificity of CA 125 in our study were 0.857 and 0.836, respectively, and the sensitivity

TABLE 5 Area under the receiver operating characteristics curve for the diagnosis of ovarian cancer

Group	Biomarker	AUC (95% CI)	*P*-value[a]
Premenopause	CA 125 (U/mL)	0.831 (0.711-0.951)	-
Case N = 14	HE4 (pmol/L)	0.820 (0.656-0.984)	0.8802
Control N = 567	ROMA (premenopause)	0.824 (0.664-0.983)	0.9152
	CA 125 + HE4	0.847 (0.724-0.970)	0.3902
	CA 125 + HE4 + age	0.892 (0.805-0.978)	0.1866
Postmenopause	CA 125 (U/mL)	0.889 (0.836-0.942)	-
Case N = 56	HE4 (pmol/L)	0.882 (0.821-0.943)	0.8582
Control N = 195	ROMA (postmenopause)	**0.935 (0.902-0.969)**	**0.0231**
	CA 125 + HE4	0.927 (0.887-0.967)	0.1674
	CA 125 + HE4 + age	0.878 (0.818-0.939)	0.6852
Total	CA 125 (U/mL)	0.811 (0.753-0.869)	-
Case N = 70	HE4 (pmol/L)	**0.896 (0.842-0.951)**	**0.0191**
Control N = 762	CA 125 + HE4	**0.909 (0.866-0.952)**	**0.0008**
	CA 125 + HE4 + age	**0.892 (0.845-0.940)**	**0.0008**
	CA 125 + HE4 + menopause state	**0.931 (0.894-0.969)**	**<0.0001**
	CA 125 + HE4 + age + menopause state	**0.923 (0.890-0.956)**	**<0.0001**

AUC, area under the receiver operating characteristics curve; CI, confidence interval; ROMA, Risk of Ovarian Malignancy Algorithm.
[a]Against AUC value of CA 125.
Bold values imply statistically significant results.

TABLE 6 Diagnostic performance of CA 125, HE4, and ROMA

Menopause state	Parameter	Cutoff	Diagnostic performance (95% CI)			
			Sensitivity	Specificity	PPV	NPV
Premenopausal (Case N = 14) (Control N = 567)	CA 125 (U/mL)	Default (>35 U/mL)	0.714 (0.454-0.883)	0.695 (0.656-0.731)	0.055 (0.039-0.076)	0.990 (0.977-0.996)
		Best (>71.7 U/mL)	0.714 (0.454-0.883)	0.841 (0.809-0.869)	0.100 (0.071-0.140)	0.992 (0.981-0.996)
	HE4 (pmol/L)	Default (>92.1 pmol/L)[a]	0.571 (0.326-0.786)	0.986 (0.972-0.993)	0.500 (0.305-0.695)	0.989 (0.981-0.994)
		Best (>83.0 pmol/L)	0.714 (0.454-0.883)	0.974 (0.957-0.984)	0.400 (0.268-0.548)	0.993 (0.984-0.997)
	ROMA (%)	Default (≥11.4%)	0.714 (0.454-0.883)	0.875 (0.845-0.900)	0.123 (0.087-0.173)	0.992 (0.982-0.996)
		Best (≥22.5%)	0.714 (0.454-0.883)	0.972 (0.955-0.983)	0.385 (0.258-0.529)	0.993 (0.984-0.997)
Postmenopausal (Case N = 56) (Control N = 195)	CA 125 (U/mL)	Default (>35 U/mL)	0.714 (0.585-0.816)	0.903 (0.853-0.937)	0.678 (0.571-0.769)	0.917 (0.879-0.943)
		Best (>22.5 U/mL)	0.857 (0.743-0.926)	0.836 (0.778-0.881)	0.600 (0.518-0.677)	0.953 (0.914-0.975)
	HE4 (pmol/L)	Default (>121.1 pmol/L)[a]	0.571 (0.441-0.692)	0.969 (0.935-0.986)	0.842 (0.701-0.924)	0.887 (0.853-0.914)
		Best (>85.5 pmol/L)	0.804 (0.682-0.887)	0.887 (0.835-0.924)	0.672 (0.575-0.756)	0.940 (0.902-0.964)
	ROMA (%)	Default (≥29.9%)	0.696 (0.567-0.801)	0.913 (0.865-0.945)	0.696 (0.585-0.789)	0.913 (0.875-0.940)
		Best (≥18.8%)	0.929 (0.830-0.972)	0.800 (0.738-0.850)	0.571 (0.499-0.641)	0.975 (0.938-0.990)
Total (Case N = 70) (Control N = 762)	CA 125 (U/mL)	Default (>35 U/mL)	0.714 (0.599-0.807)	0.748 (0.716-0.778)	0.207 (0.177-0.240)	0.966 (0.952-0.976)
		Best (>26.6 U/mL)	0.814 (0.708-0.888)	0.665 (0.631-0.698)	0.183 (0.161-0.206)	0.975 (0.960-0.985)
	HE4 (pmol/L)	Best (>79.6 pmol/L)	0.800 (0.692-0.877)	0.938 (0.919-0.953)	0.544 (0.469-0.617)	0.981 (0.970-0.988)

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; ROMA, Risk of Ovarian Malignancy Algorithm.
[a]Laboratory default cutoff values for HE4.

and the specificity of HE4 were 0.804 and 0.887 when optimal cutoffs were applied to the postmenopausal patients. Similar to the meta-analysis described above, the sensitivity and the specificity of ROMA were 0.929 and 0.800, respectively, showing better sensitivity than that of CA 125 and HE4 but lower specificity. Direct comparison of sensitivity and specificity among studies is difficult because of the differences in the types of benign gynecological diseases used as control groups, the stages and pathologic types of ovarian cancer patients, and the cutoff for diagnosing ovarian cancer. However, HE4 would have higher specificity but lower sensitivity than CA 125 based on the results of this and previous studies. Therefore, complementary combinations of the two tumor markers and ROMA would contribute to improving ovarian cancer diagnostic performance.

The aim of this study was to evaluate the diagnostic performance of CA 125, HE4, and ROMA in Korean patients with benign gynecological diseases and ovarian cancer and to search for the ideal combination of CA 125 and HE4 to perform better than conventional ROMA. However, only 70 ovarian cancer patients were included in our study, and little numbers of cases according to the pathologic classifications had to be compared as shown in Table 3. Furthermore, only 14 of them were premenopausal. Therefore, deriving an optimal combination statistically superior to ROMA was difficult due to the small number of cases. In this study, the AUC of the combination of CA 125 + HE4 in the premenopausal patient group was 0.847, which was higher than the AUC of ROMA (0.824), but the difference was not statistically significant ($P = 0.6440$). In the postmenopausal patient group, the AUC of ROMA was 0.935, which was higher than the AUC of CA 125 + HE4 combination (0.927), but the difference was not significant ($P = 0.2851$). In future studies, observing a large number of ovarian cancer patients could allow derivation of an optimal combination of CA 125 and HE4 that could be helpful for the diagnosis of ovarian cancer in Korean women. Furthermore, more researches should be done to confirm the better diagnostic performance of CA125, HE4, and ROMA when optimal cutoffs are applied.

In conclusion, this study evaluated the diagnostic performance of CA 125, HE4, and ROMA for ovarian cancer in Korean patients, and these markers demonstrated good diagnostic performance. The diagnostic performance of each marker varied depending on the type of benign gynecological disease and pathologic type of ovarian cancer. Therefore, CA 125, HE4, and ROMA should be used as complementary tests to improve the diagnosis of ovarian cancer.

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