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Comparison of CA125, HE4, and ROMA index for ovarian cancer diagnosis

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ABSTRACT

Objective: In view of the high rate of misdiagnosis of ovarian cancer, our study aimed to compare the performances of serum levels of human epididymis secretory protein 4 (HE4) and cancer antigen 125 (CA125), as well as ROMA index in the diagnosis of ovarian cancer.

Methods: Three hundred and seventy-three patients who suffered ovarian cancer were selected in Tianjin Medical University Cancer Institute and Hospital from July 2016 to July 2017. Patients were divided into premenopause group and postmenopause group. Based on the results of pathologic examinations, patients were divided into malignant, benign, and borderline groups, which were further divided into different pathologic type groups. HE4 and CA125 serum levels in each patient were detected and the ROMA index was analyzed. ROC curve analysis was conducted to compare the performances of serum CA125, serum HE4, and ROMA index in the diagnosis of ovarian cancer.

Results: Proportion of postmenopausal patients in malignant group (65.2%) was significantly higher than that in the benign group (34.3%). Serum levels of CA125 and HE4, and ROMA index were higher in patients with different types of malignant tumor than those in corresponding benign group. Serum HE4, serum CA125, and ROMA index had better

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performance in the diagnosis of postmenopausal ovarian cancer than that of premenopausal ovarian cancer. The overall performance of ROMA and HE4 was better than that of CA125, but it was affected by pathologic types.
Conclusions: Serum HE4, serum CA125, and ROMA can be used to predict ovarian cancer. HE4 and ROMA have better performance than CA125 in most cases, but pathologic types can also affect them.

Introduction

As a type of malignant tumor that develops in ovaries, ovarian cancer affects more than 20,000 new cases and causes about 140,000 deaths in females worldwide every year.[1] Early screening was considered to be a promising way to identify ovarian cancers at early stages. However, almost all currently available screening methods have been proved to be ineffective.[2-4] The emergences of various treatment strategies such as chemotherapy have significantly improved the treatment outcomes of patients with ovarian,[5-7] however, resistance develops during treatment and following observation studies have found that all the treatment methods failed to further improve the survival rate of patients during last several decades.[7,8] The early symptoms of ovarian cancer are usually not obvious and the tumor metastasis is common in this disease. Therefore most patients that have ovarian cancers are diagnosed at the advanced stages, leading to the unacceptable high mortality rate.[9] Thus, early diagnosis and treatment of ovarian cancer is indispensable for the treatment of this disease.

Various biomarkers have been developed to facilitate the early diagnosis of ovarian cancer. Cancer antigen 125 (CA-125), which is also known as MUC16 or mucin 16, is a component of the respiratory tract, the ocular surface, and female reproductive tract epithelia in human. With high degree of glycosylation, CA-125 can create a hydrophilic environment to serve as a lubricating barrier against infectious agents and foreign particles.[10] Level of CA-125 is usually increased in blood of patients with certain types of cancers, which makes it a biomarker for the diagnosis of those diseases.[11] CA-125 has also been widely used in the ovarian cancer diagnosis. However, the application of CA-125 as a biomarker for the ovarian cancer diagnosis is still challenged by low specificity as well as sensitivity, and high false negative, or false positive rates.[12] Human epididymis secretory protein 4, a.k.a. HE4, is also considered to be a promising biomarker for ovarian cancer, but the increased levels of HE4 were not observed in all types of ovarian cancer.[13] Risk of ovarian malignancy algorithm (ROMA) is another index that used in the diagnosis, which usually shows balanced diagnostic performance.[14] In view of the different indices that used in the ovarian cancer diagnosis, it will be helpful to compare the performance of those indices.

In this study, patients who suffered ovarian tumor were selected. They were divided into difference groups based on menopause and pathologic types. Serum HE4 and serum CA125 as well as ROMA were used in the diagnosis of ovarian cancer and the performances were compared.

Materials and methods

Patients

A total of 373 patients with ovarian tumor were selected in Tianjin Medical University Cancer Institute and Hospital from July 2016 to July 2017. Patients that combined with disease in liver,

lung, or other important organs were excluded. Postoperative pathologic examinations were performed, and 175 cases were found to be with benign tumor, 181 cases with malignant tumor, and 17 cases with borderline ovarian tumor. None of the patients received treatment before the study. Patients were further divided into premenopause group and postmenopause group according to the following criteria of menopause: ① age ≥60 years old; or ② age <60 years, natural amenorrhea ≥12 months and levels of FSH and estrogen were in the range of menopause. There are 4 cases of stromal tumor, 1 case of germ cell tumor and 4 cases of metastatic tumor (1 case primary GIT). The 175 benign cases included serous (n = 19), mucinous (n = 17), endometrioid (n = 31), clear cell (n = 1), and others (n = 107). All the patients were treated according to the guidelines of NCCN. In the study, we focused on the diagnostic role of CA125, HE4, and ROMA in ovarian tumor. Baseline characteristics of the patients: Age, 51(12-27); Stage I, 41(cases); II, 22(cases); III, 95(cases); Unclassified, 187(cases), among these cases, 175 cases were benign, and another 12 cases were borderline. Median and range for 3 serum indices: CA125 (U/mL), 62.24(2.84-12,494); HE4 (pM/L), 67.14(8.19-3270); ROMA, 16.21(1.04-99.9). And we test serum CEA(ng/mL) in those patients with median and range as: Benign 1.93(1.42-2.89), Borderline 2.20(1.57-2.91), and Malignancy 2.41(1.86-4.01).

Detection method

Before treatment, fasting venous blood (3 mL) was extracted from each patient, which was followed by centrifugation at 3500 r/min for 5 min to separate serum. HE4 and CA125 serum levels were analyzed by Roche cobas 6000 analyzer using reagents that provided by Roche (Basel, Switzerland). Roman index was calculated by Roche ROMA index that generated from ovarian cancer risk assessment software (Roche, Basel, Switzerland). HE4 serum level >140 pmol/L, CA125 serum level >35U/mL, Premenopausal ROMA index >11.4 and Postmenopausal ROMA index >29.9 were defined as high risk.

Statistical analysis

SPSS19.0 (SPSS Inc., Chicago, IL) was used for all statistical analyses. Normal distribution data were recorded by ($x \pm s$), and comparisons between 2 groups were performed by t test. The sensitivity and specificity was evaluated by software Sigmaplot. Benign group was set as negative control to evaluate the predicted role of HE4, ROMA, and CA125 in malignancy group. Nonparametric Mann-Whitney U test was employed for comparisons of nonnormal distribution data and *P* value that less than 0.05 was considered to be of statistical significance.

Results

General information

Patients were diagnosed as benign tumor (175 cases), malignant tumor (181 cases), and borderline ovarian tumor (17 cases). No significant differences in age were found among 3 groups ($P = 0.5$). Proportion of postmenopausal patients in malignant group (65.2 %) was significantly higher than that in benign group (34.3%) or borderline group (41.2%) ($P < 0.05$). Highest median level of HE4, CA125, and ROMA was found in malignant group, followed by borderline group and benign group ($P < 0.05$). Those data suggest that CA125 and HE4 serum levels as well as ROMA index were affected by ovarian cancer. See Table 1 for details.

Table 1
Comparison of serum levels of CA125 and HE4, and ROMA index among groups.

	Benign	Borderline	Malignancy
Age	45(18-75)	50(12-72)	55(13-77)
Menopause			
pre-M	115(65.7%)	10(58.8%)	63(34.8%)
post-M	60(34.3%)	7(41.2%)*	118(65.2%)*,†
CEA (ng/mL)	1.93(1.42-2.89)	2.20(1.57-2.91)	2.41(1.86-4.01)
CA125 (U/mL)	21.269(2.84-994.9)	64.69(3.81-2053)*	368(6.77-12494)*,†
CA125 > 35 U/mL (n)	115	6	23
HE4 (pM/L)	49.01(8.19-137.3)	63.97(41.45-236.3)*	245.9(30.97-3270)*,†
ROMA	8.28(1.04-57.62)	11.7(4.81-81.52)*	83.41(3.15-99.9)*,†

Notes:
* Compared with benign group, $P < 0.05$
† Compared with borderline group, $P < 0.05$; pre-M, premenopause; post-M, postmenopause.

Comparison of pre and postmenopausal CA125 and HE4 serum levels, and ROMA index among groups

Pre and postmenopausal CA125 and HE4 serum levels, and ROMA index were higher in malignant group than those in borderline group and benign group (P value < 0.01). Besides that, pre and postmenopausal CA125 and HE4 serum levels, and ROMA index in borderline group were all higher than the counterparts in benign group, but significant difference between those groups were only found for ROMA index ($P < 0.01$). Those data suggest that CA125 and HE4 serum levels, and ROMA index were increased with the development of ovarian cancer in pre and postmenopausal patients (Fig 1).

Specificity as well as sensitivity of CA125 and HE4 serum levels, and ROMA index in the ovarian cancer diagnosis

Only benign group and malignant group were considered in this investigation, and HE4 Serum level >140 pmol/L, CA125 serum level >35 U/mL, Premenopausal ROMA index >11.4 and Postmenopausal ROMA index >29.9 were considered to be high risk, otherwise, low risk. As shown in Table 2, before menopause, the CA125, HE4, and ROMA sensitivities in the ovarian cancer diagnosis were 85.71%, 57.14%, and 72.46%, respectively. The specificities were 55.65%, 100.00%, and 79.13%, respectively. After menopause, the sensitivities of HE4, CA125, and ROMA in the diagnosis of ovarian cancer were 71.19%, 88.14%, and 87.29%, respectively. And the specificities were 85.0%, 100.0%, and 91.67%, respectively. CA125 showed significantly higher specificity, HE4 showed significantly higher sensitivity, and ROMA showed both significantly higher specificity and sensitivity in the postmenopausal ovarian cancer diagnosis comparing with premenopausal ovarian cancer ($P < 0.01$).

ROC curve analysis regarding diagnostic values of CA125 and HE4 serum levels, as well as ROMA index for ovarian cancer

ROC curve analysis was first conducted to compare diagnostic values of CA125 and HE4 serum levels, and ROMA index for ovarian cancer (Fig 2). As we could see from Figure 2A, the area under the curve (AUC) of HE4 and CA125, and ROMA were 0.92, 0.88, and 0.93, respectively. The AUC of ROMA and HE4 was significantly bigger than that of CA125 ($P < 0.05$). In premenopausal patients, AUC of HE4 and CA125, as well as ROMA were 0.88, 0.85, and 0.88, respectively. The AUCs of ROMA and HE4 were bigger than that of CA125, but the differences were not significant in statistics ($P = 0.5$). In postmenopausal patients, AUC of HE4, CA125, and

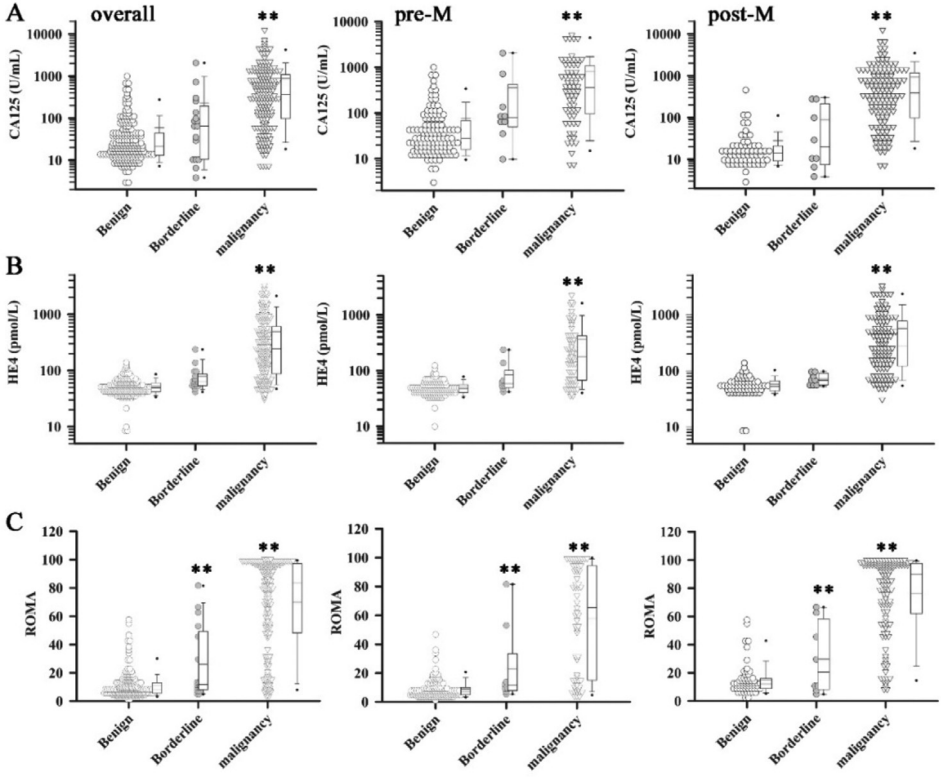

Fig. 1. Comparison of pre and postmenopausal serum levels of CA125 and HE4, and ROMA index among groups. (A) comparison of serum levels of CA125 among groups; (B) comparison of serum levels of HE4 among groups; (C) comparison of ROMA index among groups.
Notes: **compared with benign group, $P < 0.01$.

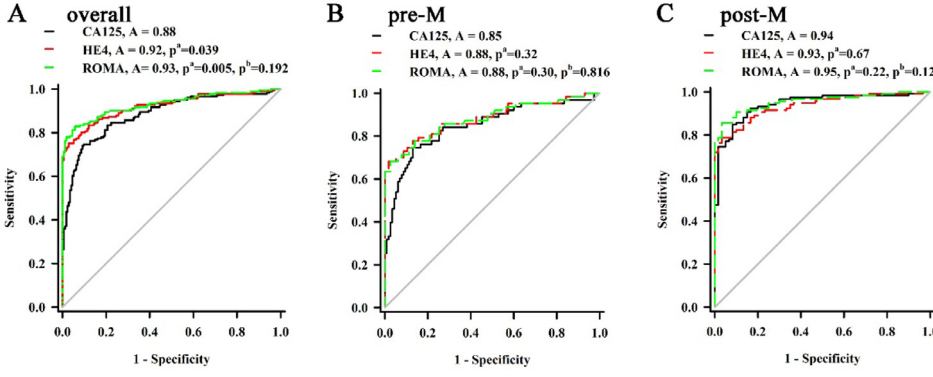

Fig. 2. ROC curve analysis of the diagnostic values of serum levels of CA125 and HE4, and ROMA index for ovarian cancer. (A) ROC curve analysis of the diagnostic values of serum levels of CA125 and HE4, and ROMA index for ovarian cancer in all patients; (B) ROC curve analysis of the diagnostic values of serum levels of CA125 and HE4, and ROMA index for ovarian cancer in premenopausal patients; (C) ROC curve analysis of the diagnostic values of serum levels of CA125 and HE4, and ROMA index for ovarian cancer in postmenopausal patients.

Table 2
Specificity and sensitivity of serum levels of CA125 and HE4, and ROMA index in the diagnosis of ovarian cancer.

Groups		Cases	Low risk (cases)	High risk (cases)	Sensitivity (%)	Specificity (%)
CA125						
Overall	Benign	175	115	60		
	Malignant	181	23	158	87.2	65.71
pre-M	Benign	115	64	51		
	Malignant	63	9	54	85.71	55.65
post-M	Benign	60	51	9		
	Malignant	118	14	104	88.14	85.00*
HE4						
Overall	Benign	175	175	0		
	Malignant	181	61	120	66.3	100
pre-M	Benign	115	115	0		
	Malignant	63	27	36	57.14	100
post-M	Benign	60	60	0		
	Malignant	118	34	84	71.19*	100
ROMA						
Overall	Benign	175	146	29		
	Malignant	181	28	153	84.53	83.43
pre-M	Benign	115	91	24		
	Malignant	63	13	50	72.46	79.13
post-M	Benign	60	55	5		
	Malignant	118	15	103	87.29*	91.67*

Notes:
* Compared with premenopausal values, $P < 0.05$.

ROMA were 0.93, 0.94, and 0.95, respectively. There were no significant differences among them ($P = 0.5$). Those data suggest that the overall performance of ROMA and HE4 is better than that of CA125.

HE4 and CA125 serum levels, as well as ROMA of patients that have different pathologic types

Patients were further divided into different pathologic type subgroups, including serous ovarian cancer group, mucinous ovarian cancer group, endometrioid carcinoma group, clear cell ovarian cancer group, and other ovarian cancer group. As we could see from Figure 3, CA125 and HE4 serum levels, and ROMA were higher in all malignant groups than those in benign groups ($P < 0.01$). Those data indicated that HE4 and CA125, as well as ROMA are related to the development of all types of ovarian cancers. Due to the data limitation, we cannot represent data under malignant lesions as serous and nonserous.

Comparison of ROC curves of HE4 and CA125, as well as ROMA index of patients with different pathologic types

For patients with serous ovarian cancer, AUC of HE4, and CA125, and ROMA were 0.99, 0.96, and 0.99 (Fig 4). Significant difference was found between CA125 and ROMA (P value < 0.05). For patients with mucinous ovarian cancer, AUC of HE4, CA125, and ROMA were 0.77, 0.74, and 0.71. AUC of CA125 and HE4 were bigger than that of ROMA, but no significant differences were found among them ($P > 0.05$). In patients with endometrioid carcinoma, AUC of HE4, CA125, and ROMA were 0.87, 0.83, and 0.89. AUC of ROMA and HE4 were bigger than that of CA125, but no significant differences were found among them (P value > 0.05). For patients with clear cell ovarian cancer, AUC of HE4, CA125, and ROMA were 0.71, 0.73, and 0.72. There were no significant differences among them (P value > 0.05). False positive rate of CA125 and HE4 was 34.29% and 12.70% respectively. And false negative rate of CA125 and HE4 was 0% and 33.70%.

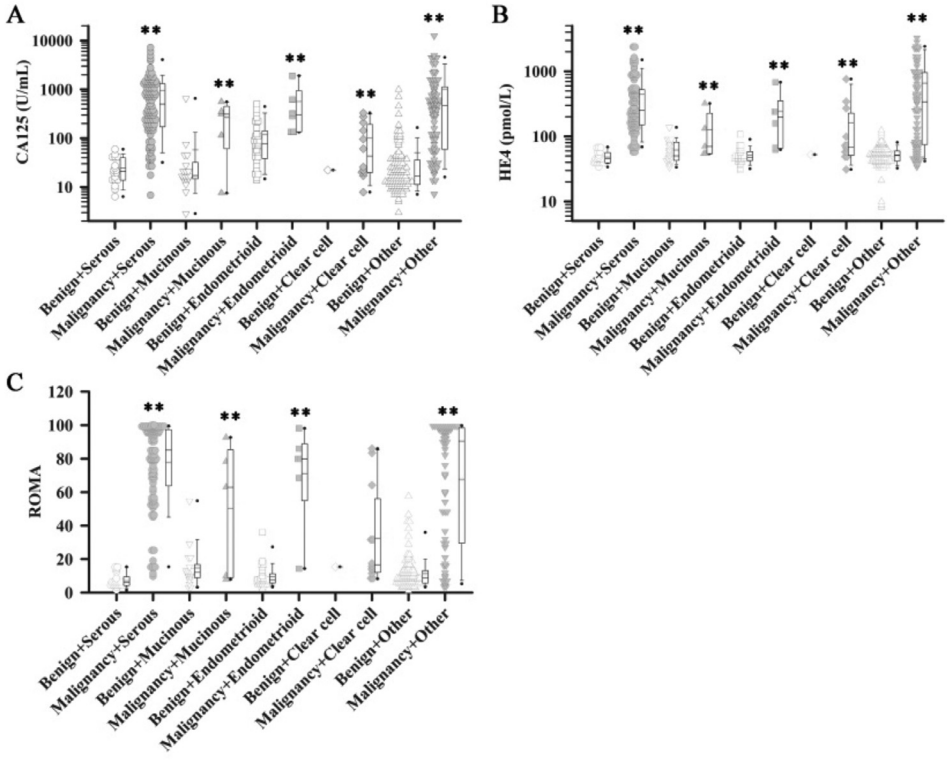

Fig. 3. Serum levels of CA125 and HE4, and ROMA in patients with different pathologic types. (A) Serum levels of CA125 in patients with different pathologic types; (B) Serum levels of HE4 in patients with different pathologic types; (C) Serum levels of ROMA in patients with different pathologic types.

Those data suggest that the performances of HE4, CA125, and ROMA were different during the diagnosis of different types of ovarian cancer.

Discussion

Due to the functionality of CA-125 in protecting female reproductive tract epithelia from the adverse effects of infectious agents and foreign particles, level of CA-125 is usually increased in patients with ovarian cancer.[10] About 80%-90 % of females with advanced-stage ovarian cancer showed increased level of CA-125 in blood, and higher level of CA-125 usually indicates poor treatment outcomes and prognosis.[15] As a glycoprotein that produced and secreted by serous and endometrioid EOC, level of HE4 is usually increased in serum of patients with different types of ovarian cancers.[16] ROMA is a simple biomarker that calculated based on ovarian malignancy algorithm, which was developed by Moore et al[17] The emergence of this method avoided the use of ultrasound findings under the risk of malignancy index and improved diagnostic outcomes. Bigger ROMA values indicate higher risk of ovarian cancer.[17] In this study, significant higher serum CA-125 and HE4 levels, and ROMA were found in malignant group than those in borderline group and benign group. In addition, CA-125 and HE4 serum levels, as well as ROMA were also higher in borderline group than those in benign group. Those data suggest that CA-125 and HE4 serum levels, and ROMA were increased with the development of ovarian cancer. Besides that, higher serum CA-125 and HE4 levels, as well as ROMA were found in all types of

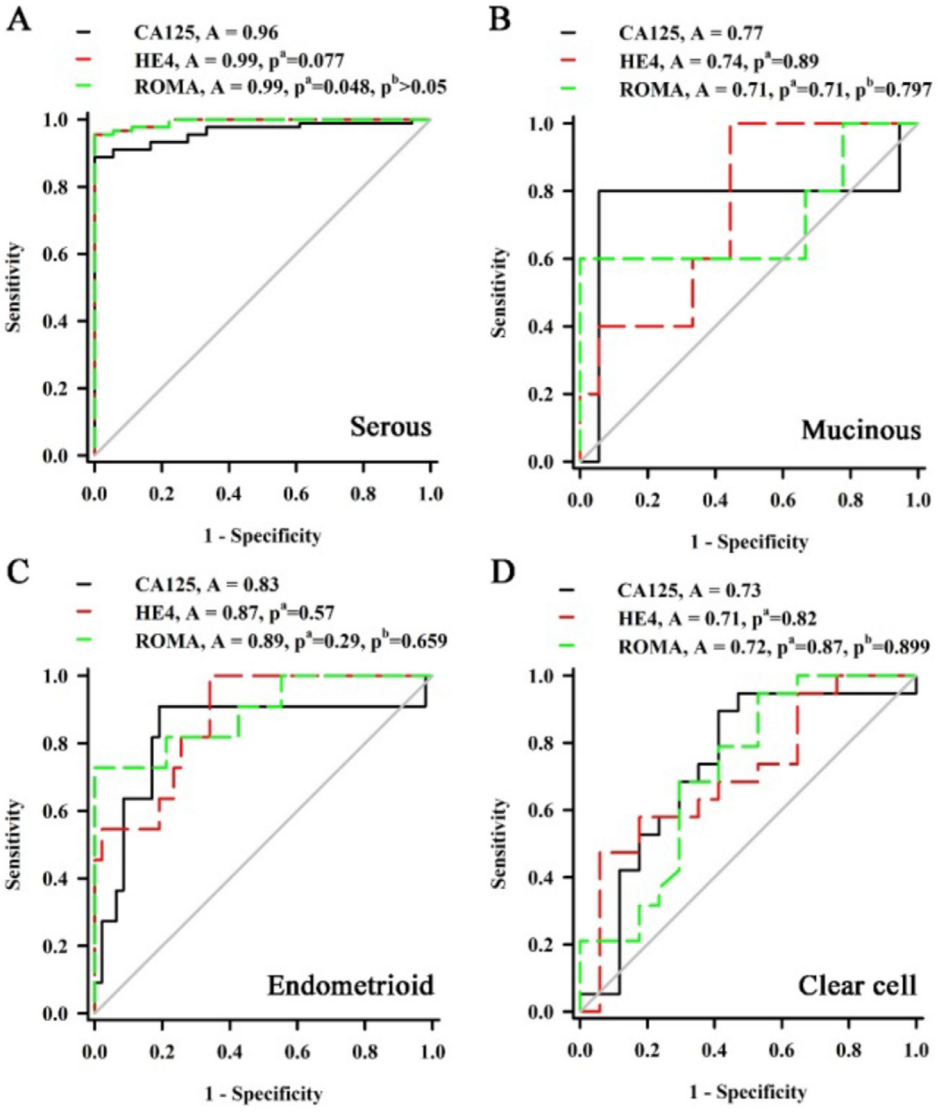

Fig. 4. Comparison of ROC curves of CA125 and HE4, and ROMA index in patients with different pathologic types. (A) Comparison of ROC curves of CA125 and HE4, and ROMA index in patients with serous ovarian cancer; (B) Comparison of ROC curves of CA125 and HE4, and ROMA index in patients with mucinous ovarian cancer; (C) Comparison of ROC curves of CA125 and HE4, and ROMA index in patients with endometrioid carcinoma; (D) Comparison of ROC curves of CA125 and HE4, and ROMA index in patients with clear cell ovarian cancer.

malignant tumor comparing with those in the corresponding type of benign tumor, but the degrees of increases were different. Those data suggest that CA-125 and HE4 serum levels, as well as ROMA can be increased by different types of ovarian cancers with different degrees.

CA-125, HE4, and ROMA are 3 most popularly utilized biomarkers for the ovarian cancer diagnosis. In a recently research, a meta-analysis was performed to compare the accuracy of CA125, HE4, and ROMA in the ovarian cancer diagnosis.[18] In the investigation, HE4 showed significantly better performance than CA125 or ROMA. Consistent results were found in this study,

the specificities of HE4 in the ovarian cancer diagnosis of all patients, premenopausal patients, and postmenopausal patients were 100 %, which were much higher than those of CA125 or ROMA, indicating that application of HE4 can increase the accuracy of the diagnosis for this disease. In this study, performances of ROMA and CA125 were better in postmenopausal subgroup than those in premenopausal subgroup.[18] Similarly, in this study, CA125 showed significantly higher specificity, HE4 showed significantly higher sensitivity, and ROMA showed both significantly higher specificity and sensitivity in postmenopausal ovarian cancer comparing with premenopausal group, indicating that those 3 biomarkers are helpful for the ovarian cancer diagnosis of postmenopausal patients. In this study, ROC curve analysis was conducted to analyze and compare the performances of CA125, HE4, and ROMA. We found that CA125 and ROMA performed much better in the postmenopausal subgroup comparing with the premenopausal subgroup. The performance of HE4 as well as ROMA was better than that of CA125 in all patients and premenopausal, but no significant differences were found in the performances of those 3 biomarkers in postmenopausal. Those data suggest that ROMA and HE4 are more proper for use in the diagnosis of postmenopausal patients with ovarian cancer.

Ovarian cancer can be divided into different pathological types, and different biomarkers may perform differently in the diagnosis of different types due to the different expression patterns.[19,20] In current investigation, the performances of CA125 and HE4 serums as well as ROMA in the diagnosis of different pathological types of ovarian cancer were compared by ROC curve analysis. Although the overall performances of ROMA and HE4 were higher than that of CA125, different pathological types significantly affect the performances of those biomarkers. Those data suggest that the selection of appropriate biomarkers is important for the precise diagnosis regarding different types of ovarian cancers.

There are several cases in subtype of ovarian tumor included in the work, such as 1 case for clear cell subgroup benign ovarian tumor. If further work, more cases need to be recruit. This is a single center study. To make the conclusion more convincing, more cases from other source could be compared.

Conclusions

In conclusion, current study found that CA125 and HE4 serum levels, as well as ROMA index were increased for patients with different types of malignant tumor comparing with that in corresponding benign group. Performance of HE4 and CA125 serum as well as ROMA index were better for the diagnosis of postmenopausal ovarian cancer than that in premenopausal ovarian cancer. For premenopausal patients, it is preferable to apply ROMA or HE4. Overall performance of ROMA and HE4 was higher than that of CA125. Biomarkers should be selected based on different pathological types.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.currproblcancer.2018.06.001.

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