Clinical value of serum CA125, CA724, HE4, premenopausal and postmenopausal ROMA index for diagnosis of high-grade serous ovarian cancer

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Research Article

Keywords: High-grade serous ovarian cancer, CA125, CA724, HE4, ROMA, Diagnosis

Posted Date: April 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1334547/v1

Abstract

Background: This study aimed at exploring a panel of novel biomarker for early detection of high-grade serous ovarian cancer (HGSOC).

Methods: The serum levels of CA125, CA724 and HE4 were compared among serum samples collected from patients with HGSOC, borderline malignancy or benign gynecologic pathologies. Univariable and multivariable logistic regression analyses were performed to identify the risk factors of HGSOC. Nomogram was established for predicting HGSOC using the risk factors. Receiver operating characteristic (ROC) curves was plotted and the area under the curve (AUC), sensitivity and specificity of the CA125, CA724, HE4, ROMA and new panel were calculated.

Results: The serum concentration of CA125, CA724 and HE4 were increased among patients with HGSOC. The AUC of CA125, CA724, HE4, ROMA2 and the new panel based on age, CA125 and ROMA2 were 0.732 (0.646-0.818), 0.773 (0.699-0.847), 0.886 (0.831-0.941), 0.835 (0.763-0.907), and 0.933 (0.896-0.969), respectively. Nomogram was constructed to diagnose HGSOC based on CA125, age and ROMA2. The C-indices of this nomogram was 0.88 (95% CI= 0.81-0.95) in training cohort and 0.91 (95% CI= 0.86-0.96) in the validation cohort. The sensitivity and specificity were 89.66% and 82.44% in diagnosing HGSOC, and the positive predictive value and negative predictive value of new algorithm were 69.33% and 94.74%, respectively.

Conclusion: The new index based on age, CA125 and ROMA2 could be a new auxiliary diagnostic indicator in distinguishing HGSOC from borderline malignancy.

Background

Ovarian cancer is the deadliest gynecologic malignancy and the fifth leading cause of cancer-related death in women. It is estimated that more than 300,000 new cases and 200,000 new deaths of ovarian cancer occurred worldwide in 2020 [1]. Although outcomes have improved in recent years, there are no validated screening strategies for early diagnosis of high-grade serous ovarian cancer (HGSOC) is the most prevalent and aggressive subtype of ovarian cancer [2]. Most patients with HGSOC are in the advanced stage of the disease with distant metastases when it was diagnosed, at which point the five-year survival rate was 17% [3]. The standard diagnostic strategy includes clinical examination, serum tumor biomarkers and ultrasound [4]. However, none of these diagnostic methods have ideal specific and sensitive rate when used separately. Thus, there remains an urgent need to develop more accurate diagnostic methods to extend the survival of ovarian cancer.

Carbohydrate antigen 125 (CA125) is a mucinous glycoprotein that expressed by epithelial ovarian cancer cell. It elevated in most epithelial ovarian cancer cases and some benign lesions, making it unreliable in differentiating patients with ovarian cancer from benign disease. Other biomarkers such as carbohydrate antigen 724 (CA724) and human epididymis 4 (HE4) have been employed in diagnosing ovarian cancer. Previous studies showed that clinical usefulness of CA724 in diagnosing epithelial

cancers with sensitivity of 50% in ovarian cancer and 40% in colorectal and gastric cancer [5]. All of these cannot yield high efficiency in both sensitivity and specificity when used separately. Thus, the Risk of Ovarian Malignancy Algorithm (ROMA) was first used by Moore et al based on HE4 and CA125 to calculate the risk of HGSOC [6]. However, the ideal predictive tumor diagnostic panel has not yet been identified for HGSOC. In this study, we assessed the diagnostic values of CA125, CA724, HE4, ROMA and the new algorithm for distinguishing HGSOC from other ovarian cancer.

Methods And Materials

Patients selected criteria

Patients aged over 18 years old and undergoing ovarian surgery in hospital between February 2019 and December 2020 were included in the cohort. None of these patients received any therapy before blood sample collection. Patients with sufficient material including age, disease history and menstrual condition were enrolled in this research. This study was approved by the Scientific and Research Ethics Committee of the International Peace Maternity and Child Health Hospital, and written informed consent was not deemed necessary due to the retrospective design.

BloodsamplecollectionandCA125, CA724 and HE4 measurement

Serum CA125, CA724 and HE4 and ultrasound data were obtained before patients received any therapy. Blood samples were collected within 6 hours of admission, and then centrifuged at 3000 rpm for 10 minutes. The serum and plasma samples were stored at -80℃. Electrochemiluminescence (ECL) technology for immunoassay analysis was used as per manufacturer's protocol (Roche Cobas e601module, Germany) to simultaneously measure the concentration of CA125, HE4 and CA724 in a single sample. All the cases were diagnosed by two experienced pathologists independently. The studied cohort was divided into training cohort and validate cohort randomly, in which training cohort was about 2/3 of the total number of cases, and validation cohort was 1/3 of the total number of cases.

Statistical analysis

Continuous variables were evaluated by nonparametric Kruskal-Wallis test, and the results were showed as Mean (25%, 75%). Chi-square test or Fisher's exact test was applied to calculate the categorical variables. Receiver operator characteristic (ROC) curve was used to evaluate the diagnostic efficiency of index, and the area under the curve (AUC) greater than 0.7 was considered to have good diagnostic performance. The logistic regression analysis was employed to assess the risk factors of HGSOC in cohort. The risk factors were incorporated into the nomogram to predict the hazard rate of HGSOC based on result of logistic regression model. A nomogram was formulated to provide visualized risk prediction using R software with the Hmisc, grid, lattice, Formula, ggplot2 and rms packages. Calibration curve was applied to evaluate the calibration ability and the discrimination was evaluated by C-index. ROMA calculation is based on the serum HE4 and CA125 as follows [6]:

ROMA1 as premenopausal status: Predictive Index (PI) = 12.0 + 2.38 × LN (HE4) + 0.0626 × LN (CA125)

ROMA2 as postmenopausal: Predictive Index (PI) =-8.09 + 1.04 × LN (HE4) + 0.732 × LN (CA125)

ROMA score (%) =$\left[\frac{\exp(PI)}{1+\exp(PI)} \times 100\right]$

All the analyses were conducted by R software (http://www.r-project.org/) and SPSS 22.0 statistical software. Differences were assumed statistically significant when $P < 0.05$.

Results

Patients' characteristics

Totally, 189 patients undergoing ovarian surgery were included in our cohort, which consisted of 58 HGSOCs, 33 borderline tumors, and 98 benign masses. Table 1 showed the clinical characteristics related to ovarian cancer of patients, including the age of patients and disease history.

Table 1
the clinical characteristics related to ovarian cancer of patients

Characteristics	HGSOC (n = 58)	Borderline malignancy (n = 33)	Benign ovaries (n = 98)	*P* value
Age (Mean ± SD)	52.9 ± 8.1	44.2 ± 12.0	40.0 ± 11.7	0.043
Medical comorbidities				
Hypertension	9	1	12	0.051
Type 2 diabetes	1	0	2	
Hypercholesterolemia	1	2	0	
Thyroid disorders	0	1	2	
Heart disease	3	0	3	

Ovarian diagnostic factors

The serum concentration of CA125, CA724 and HE4 were individually evaluated among samples collected from patients with HGSOC, borderline tumor and benign lesion. The mean expression of serum CA125, CA724 and HE4 in HGSOC were 652.0 ± 357.6, 23.3 ± 57.8 and 228.7 ± 197.6, respectively, which were significantly higher than those in borderline tumor and benign lesion ($P < 0.001$). The ROMA1 and ROMA2 scores were also calculated individually in the three groups. The ROMA1 scores in HGSOC, borderline tumor and benign lesion were 52.8 ± 7.6, 16.5 ± 3.1 and 9.1 ± 3.7, respectively ($P < 0.001$). The ROMA2 scores in HGSOC, borderline tumor and benign lesion were 62.3 ± 9.9, 32.3 ± 8.3 and 22.5 ± 6.3, respectively ($P < 0.001$). The results of these diagnostic factors were summarized in Fig. 1.

Logistic Regression model

In order to identify the risk factors related to HGSOC, we conducted logistic regression analysis to evaluate age, menopause, CA125, CA724, HE4, ROMA1 and ROMA2 based on training cohort. The results of logistic regression analyses of HGSOC risk were summarized in Table 2. The multivariable analyses results showed that age, CA125 and ROMA2 were related to the risk of HGSOC. Thus, we constructed a new index based on these three factors as follows:

Table 2
Univariable and Multivariable Logistic Regression Analysis of HGSOC risk on training cohort

	Univariable analysis			**Multivariable analysis**		
Variables	OR	95% CI	*P*	OR	95% CI	*P* value
Age	1.104	1.066–1.143	< 0.001	1.058	0.994–1.126	0.017
Menopause	5.062	2.608–9.829	< 0.001	2.496	0.582–10.702	0.218
CA125	1.002	1.001–1.003	0.002	0.998	0.996-1.000	0.033
HE4	1.029	1.016–1.042	< 0.001	1.086	0.936–1.259	0.276
CA724	1.084	1.035–1.134	0.001	1.021	0.974–1.070	0.393
ROMA1	1.062	1.041–1.083	< 0.001	0.855	0.608–1.203	0.369
ROMA2	1.056	1.039–1.072	< 0.001	1.054	1.009-1.100	0.017

Logit (P) =-9.048 + 0.052 × ROMA2 + 0.056 × age − 0.002 × CA125

Nomogram construction and validation

A nomogram for diagnosing HGSOC was constructed according to the results of multivariable logistic analyses (Fig. 2A). The predict value were determined based on the individual scores calculated using the nomogram. C-index of this nomogram was 0.88 (95% CI = 0.82–0.94) in the training cohort and 0.91 (95% CI = 0.86–0.96) in the validation cohort. High consistencies between the predicted and observed survival probability were showed in the calibration curves in both training (Fig. 2B) and validation cohort (Fig. 2C). The results of C-index and calibration curves indicated the nomogram has good discriminative and calibrating abilities.

ROC curve

Further, the efficiencies of CA125, CA724, HE4, ROMA1 and ROMA2 in distinguish HGSOC were compared by ROC curves. As showed in Fig. 3, HE4 had an AUC value of 0.886 (95% CI = 0.831–0.941), which was superior to CA125 (AUC = 0.732, 95% CI = 0.646–0.818), CA724 (AUC = 0.773, 95% CI = 0.699–0.847), ROMA1 (AUC = 0.876, 95% CI = 0.819–0.934) and ROMA2 (AUC = 0.835, 95% CI = 0.763–0.907). The

results showed that new index based on age, CA125and ROMA2 had a good diagnostic performance with an AUC value of 0.933 (95% CI = 0.896–0.969) ($P < 0.001$) in training cohort. The ROC results of validation cohort were showed in Supplemental table 1, and the AUC value of new index was 0.908 (95% CI = 0.855–0.962) ($P < 0.001$).

Specificity and sensitivity of the new index

Last, we evaluated the sensitivity and specificity of new algorithm based on the whole cohort. The sensitivity and specificity of new index were 89.66% and 82.44% in diagnosing HGSOC, and the positive predictive value and negative predictive value of new algorithm were 69.33% and 94.74%, respectively. As shown in Table 3, this new index has good consistency with pathological diagnosis, and the Kappa value was 0.665, indicating that the new index had a good level of agreement.

Table 3
The results of new index in diagnosis of HGSOC compared to pathological diagnosis

New index	Gold standard disease present	Gold standard disease absent	
Test positive	52	23	75
	True positive, TP	False positive, FP	Total test positive, TP + FP
Test negative	6	108	114
	False negative, FN	True negative, TN	Total test negative, FN + TN
	58	131	189
	Total diseased, TP + FN	Total normal, FP + TN	Total population
			95% C.I.
	Sensitivity = TP/(TP + FN)	89.66%	78.83%~96.11%
	Specificity = TN/(TN + FP)	82.44%	74.83%~88.53%
	PPV = TP/(TP + FP)	69.33%	57.62%~79.57%
	NPV = TN/(FN + TN)	94.74%	88.90%~98.04%

Supplemental table 1 ROC result of CA125, HE4, CA724, ROMA1 and ROMA2 in predicting probability for distinguishing patients with malignant HGSOC from non-HGSOC in validated cohort

Discussion

The mortality of HGSOC ranks first among four main types of ovarian carcinomas: serous, endometrioid, clear-cell, and mucinous carcinomas [7–9]. Although some screening strategies have been developed to monitor the risk of HGSOC, such as CA125 and ultrasound, the survival rate of HGSOC remains low due to late detection [10]. Most researchers have focused on improving the accuracy of early diagnosis of HGSOC. In the present study, we have identified and validated that new index based on age, CA125 and ROMA2 has a good diagnostic performance with accurate sensitivity and specificity. The AUC of the new algorithm based on age, CA125 and ROMA2 was 0.933 in training cohort and 0.908 in validation cohort. The sensitivity and specificity of the new algorithm based on age, CA125 and ROMA2 were 89.66% and 82.44% in diagnosing HGSOC. This new index could be a new auxiliary diagnostic indicator in distinguishing HGSOC from non-HGSOC.

CA125 serves as a tumor biomarker in diagnosing ovarian cancer for decades with low sensitivity and specificity. Being first described by Bast and colleagues, CA125 has been studied thoroughly in the screening, diagnosis and prognosis of women gynecologic carcinomas [11]. It still does not have acceptable accuracy in population-based screening method to distinguish ovarian cancer patients [12–14]. There two main approaches to improve the accuracy of CA125 in diagnosing ovarian cancer. One approach is to modify the cutoff value. However, this approach cannot achieve both sensitivity and specificity [15]. In our results, the AUC of the CA125 in diagnosing HGSOC was 0.732. Previous studies demonstrated that the level of CA125 in 95% of health population was under 37U/ml, and the patients with benign lesions with median CA125 level above 20U/ml [16–18]. Thus, simply increasing the cutoff value of CA125 will reduce false positives, but at the same time increase false negatives. The other approach is developing multiple biomarker panels containing CA125. Anderson et al. performed immunoassays to identify the serum levels of CA125, HE4, mesothelin, decoy receptor 3, B7-H4, and spondin-2 for diagnosing of ovarian cancer and indicated that serum levels of CA125, mesothelin and HE4 might help in diagnosing ovarian cancer [19]. Yurkovetsky et al. analyzed the levels of serum biomarkers in health individuals and ovarian cancer patients. They selected a panel of CA125, HE4, CEA and VCAM-1 for screening epithelial ovarian cancer [20]. Russell and colleagues developed a diagnostic model of four putative proteins including CA125 that has the potential to diagnose epithelial ovarian cancer before current diagnosis for 1–2 years [21]. However, this clinical trial only included 49 epithelial ovarian cancer cases and 31 health controls, and the algorithm based on four putative proteins only classified 64% type II ovarian cancer at 1 year and 28% cases at 2 years.

Besides CA125, HE4 and CA724 were employed as routine serum biomarkers for the screening of ovarian carcinoma. HE4 is expressed in normal ovarian tissues at a low level and amplified in ovarian cancer, which could help to diagnose ovarian cancer [22, 23]. CA724 is glycoprotein that increases in various cancers, such as gastric, colon, breast and ovarian cancer. Because it is not affected by the menstrual cycle and pregnancy, CA724 has advantages than CA125 in diagnosing ovarian cancer [24, 25]. Some studies tried to screen ovarian carcinoma by combination of CA125, HE4 and CA724. Anastasi et al. analyzed the serum concentrations of CA125, HE4 and CA724 to discriminate ovarian cancer from ovarian endometrioma. They suggested that CA125, HE4, and CA724 are all increased in patients with epithelial ovarian carcinoma, while CA125 is elevated in patients with ovarian endometrioma [26]. The

panel of these biomarkers yielded a sensitivity of 90% and a specificity of 70% in the diagnosis of epithelial ovarian carcinoma.

In order to improve sensitivity and specificity simultaneously, diagnostic algorithms are used in diagnosing ovarian cancer. First introduced by Jacobs et al, the Risk of Malignancy Index (RMI) was used for evaluating the probability of malignancies in pelvic mass [27]. In multiple studies, RMI showed compatible diagnostic performance with moderate sensitivity and specificity in distinguishing epithelial ovarian carcinoma from other lesions [28, 29]. Further, the ROMA algorithm was developed to assess the risk of epithelial ovarian malignancies [6]. But the sensitivity of ROMA was lower in premenopausal women than that in postmenopausal women. In the study cohort, we recalculated the diagnostic efficiencies of CA125, CA724, HE4, ROMA1 and ROMA2 by logistic regression model and found a new algorithm based on CA125, age, and ROMA2 has highest diagnostic performance in distinguish HGSOC from non-HGSOC lesions. Here, the sensitivity and specificity of new index were 89.66% and 82.44% in diagnosing HGSOC. This optimized the ROMA algorithm, making it more sensitive and specific in diagnosing ovarian cancer. What is more, we constructed a nomogram based on the risk factors identified by logistic regression analysis, leading to a more convenient way to diagnose ovarian cancer in clinical practice. However, it should be noted that this study was performed in single-center cohort and the number of cases was relatively small. Future studies should be performed to validate the applicability of this model.

Conclusions

In summary, the diagnostic performance of the new panel based on CA125, age and ROMA2 was better than that of CA125, CA724, HE4, ROMA1 and ROMA2.A nomogram was calculated to predict the risk rate of HGSOC in women with pelvic mass. Our study provides a valuable reference for clinical tumor prevention, and also provides a new theoretical basis of tumor markers in HGSOC diagnosis.

Abbreviations

HGSOC, High-grade serous ovarian cancer; ROC, Receiver-Operator Curves; AUC, Area Under the Curve; 95% CI, 95% confidence intervals; CA125, Carbohydrate antigen 125; CA724: Carbohydrate antigen 724; HE4, Human epididymis 4; ROMA, Risk of Ovarian Malignancy Algorithm; ECL, Electrochemiluminescence; RMI, Risk of Malignancy Index.

Declarations

Acknowledgements

We are very grateful to all laboratory staffs for their support during data collection.

Author contributions

DYH: Project development and manuscript writing. JQ: Project administration and interpreted the data; FY: Data visualized analysis. BW: Data collection; WH: Supervision; JW: Data collection; HOY: Project development and Manuscript revised. The authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical approval and consent to participate

This study was approved by the Scientific and Research Ethics Committee of The International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University School of Medicine and written informed consent was not deemed necessary due to the retrospective design.

Consent for publication

This manuscript, including tables and figures, has not been published elsewhere and is not under consideration by another journal. All authors agreed to publish the manuscript.

Availability of data and materials

The data of this study are available from the authors, but restrictions apply to the availability of these data, which are not publicly available. However, data are available from the authors upon reasonable request.

Funding

None.

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Figures

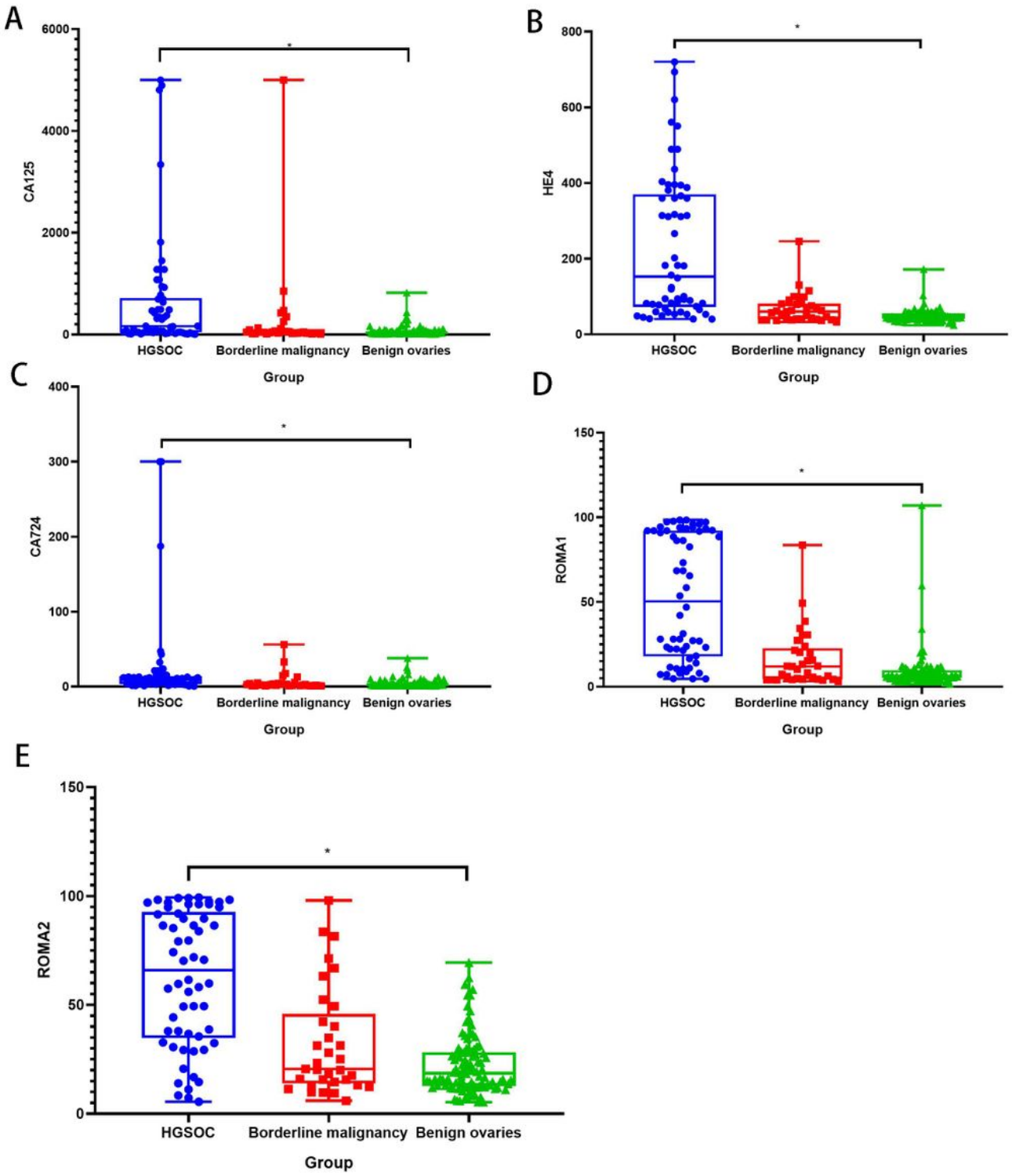

Figure 1

The serum levels of CA125, HE4 and CA724 and the ROMA1 and ROMA2 scores of the whole cohort

A: CA125; B: HE4; C: CA724; D: ROMA1; E. ROMA2

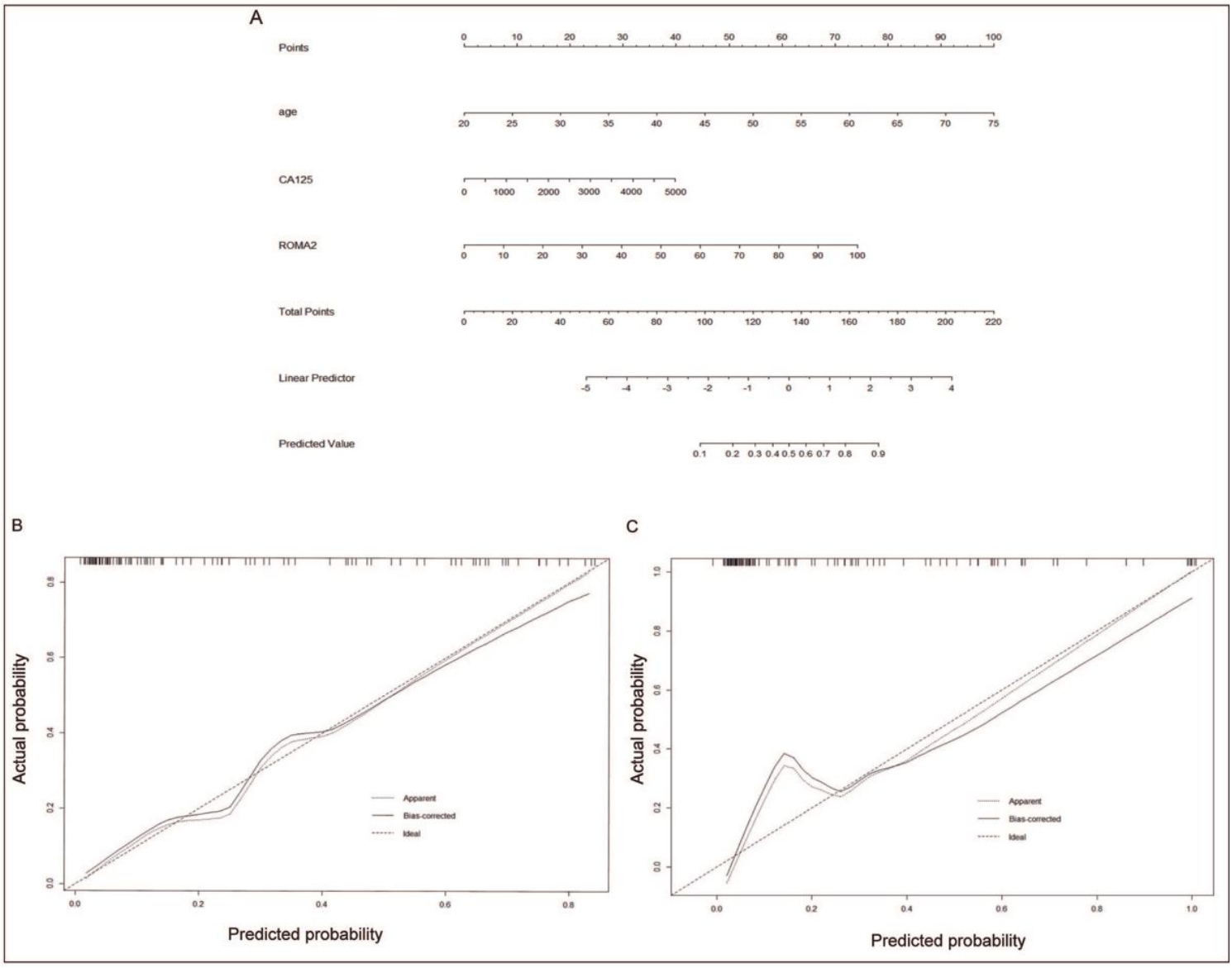

Figure 2

Nomogram Analysis for diagnosing HGSOC

A: nomogram was constructed to predict the risk of HGSOC; B: Calibration curves of nomogram for in training cohort; C.Calibration curves of nomogram in the validation cohort

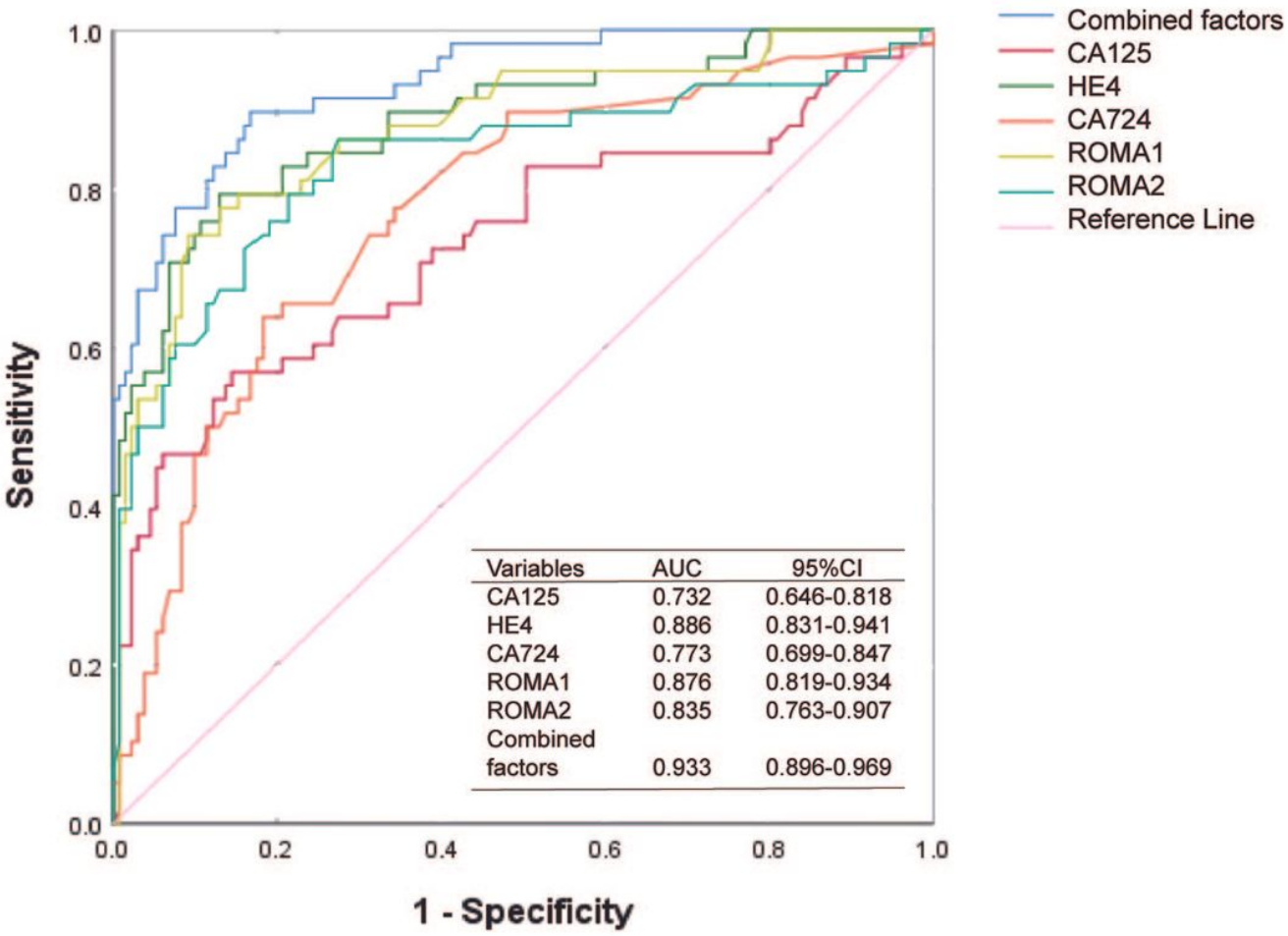

Figure 3

The ROC curves of CA125, HE4, CA724, ROMA1, ROMA2 and combined factors in the training cohort