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ORIGINAL RESEARCH

Clinical Value of Vascular Cancer Thrombus and Myometrial Invasion Combined with Tumor Markers in Predicting Sentinel Lymph Node Metastasis of Endometrial Cancer

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Purpose: To investigate the clinical value of vascular cancer thrombus and myometrial invasion combined with tumor markers (epididymal protein 4 (HE4), carbohydrate antigen 125 (CA125), carbohydrate antigen 153 (CA153), carbohydrate antigen 199 (CA199)) in predicting sentinel lymph node (SLN) metastasis of Endometrial Cancer (EC).

Methods: A retrospective study was conducted on 150 patients with EC during January 2022 to December 2024. Patients were divided into a metastatic group of 32 cases and a non-metastatic group of 118 cases. The clinical data and tumor markers [HE4, carcinoembryonic antigen (CEA), CA125, CA153, CA199 and alpha fetoprotein (AFP)] levels were collected. Logistic regression analysis was used to identify the influencing factors of metastasis. The predictive value was evaluated by Receiver operating characteristic curve (ROC). Principal component analysis (PCA) was performed to analyze the distribution characteristics.

Results: The incidence of vascular cancer thrombus (62.50%), the proportion of myometrial invasion ≥1/2 (90.63%) and serum levels of HE4, CA125, CA153 and CA199 in the metastatic group were significantly higher than those in the non-metastatic group ($P<0.05$). Vascular cancer thrombus, myometrial invasion ≥ 1/2, HE4, CA153, CA125 and CA199 were all influencing factors of SLN metastasis of EC ($P<0.05$). The AUC of the combined detection of vascular cancer thrombus, myometrial invasion, HE4, CA153, CA125 and CA199 was 0.904, with sensitivity and specificity of 85.59% and 84.38%, respectively. The combined detection has a high predictive value for SLN metastasis of EC. When the first principal component (PC1) was plotted against the second principal component (PC2), patients with SLN metastasis had significant disturbances in vascular cancer thrombus, myometrial invasion, HE4, CA153, CA125 and CA199. There were significant individual differences and dispersed distribution among EC groups, while patients without SLN metastasis could cluster well.

Conclusion: The combined detection of vascular cancer thrombus, myometrial invasion combined with HE4, CA153, CA125 and CA199 can effectively predict SLN metastasis of EC. But these influencing factors had great fluctuation and uncertainty in patients with SLN metastasis of EC, which may be related to the complexity and heterogeneity of the disease.

Keywords: vascular cancer emboli, myometrial invasion, tumor markers, endometrial cancer, sentinel lymph node metastasis

Introduction

Endometrial cancer (EC) is a malignant tumor originating from endometrial epithelial cells and is one of the three major malignant tumors related to the female reproductive system. EC is characterized by irregular vaginal bleeding as an early clinical symptom.[1] According to statistics, about 70%~75% of EC occurs in postmenopausal women, and its incidence rate has increased in recent years.[2] In China, with the change of lifestyle and the aging process of population, the incidence of EC continues to rise, which has become one of the important disease burden threatening women's health.[3] The tumor microenvironment also plays an important role in the occurrence of EC. Research has shown that factors such as metabolic microenvironment, inflammatory microenvironment, and immune microenvironment are all involved in the pathogenesis of EC. In addition, insulin receptors and hybrid receptors have a promoting effect on the growth of EC cells.[4]

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Lymph node metastasis (LNM) is the main pathway for tumor metastasis, and sentinel lymph nodes (SLN) are the earliest lymph nodes for tumor metastasis. Early diagnosis and treatment of SLN metastasis may play an important role in improving patient survival rates.[5] LNM is a key factor affecting the prognosis of patients with EC. Accurate assessment of lymph node status is essential for making individualized treatment plans, such as whether systematic lymph node dissection and adjuvant therapy are needed.[6] In addition, lymph node dissection is of great significance in the treatment of EC. It can not only accurately perform surgical pathological staging, clarify the severity of the disease, but also guide postoperative adjuvant therapy. However, lymph node dissection also carries certain risks and complications.[7] Therefore, accurate identification of patients with a high risk of SLN metastasis is of great significance for optimizing the clinical application of SLN metastasis and guiding surgical decision-making and subsequent treatment. However, there is a lack of efficient and reliable preoperative or intraoperative prediction models.

Vascular cancer thrombus refers to the invasion or accumulation of cancer cells in blood vessels or lymphatic vessels, forming clots that may cause blood clotting or thrombus formation. This phenomenon usually indicates that the tumor has infiltrated blood vessels and increased the risk of distant metastasis.[8] The presence of vascular cancer thrombus is an indicator of poor tumor prognosis, which means that cancer cells may spread to other organs or lymph nodes through blood vessels or lymphatic vessels. In breast cancer patients, vascular tumor thrombus invasion is considered to be an important predictor of ALN metastasis, and is associated with poor prognosis of patients.[9] Myometrial invasion refers to the invasion of cancer cells into the uterine muscle layer or the muscle layer of other organs, and the depth of infiltration is an independent risk factor affecting cancer prognosis.[10] In endometrial cancer, deep muscle infiltration is associated with an increased incidence of para-aortic LNM. As the depth of muscle infiltration increases, the 5-year survival rate of patients significantly decreases.[11]

Serum human epididymis protein 4 (HE4), carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), carbohydrate antigen 153 (CA153), carbohydrate antigen 199 (CA199), alpha fetoprotein (AFP), etc. are commonly used tumor markers in clinical tumor diagnosis. They have certain diagnostic and therapeutic value for various gynecological tumors such as ovarian cancer and cervical cancer.[12,13] Multiple studies have shown that serum HE4, CEA, CA125, CA153, CA199, and AFP have high diagnostic value for metastatic cancer.[14,15] Highly effective predictors are helpful for early identification of the risk of tumor metastasis, but the occurrence and development of tumors may be formed by the combined action of multiple factors. Although vascular cancer thrombus, muscular invasion, and tumor markers (such as HE4, CA153, CA125, etc.) have been shown to be associated with metastasis and poor prognosis in cancer patients, their predictive value may be relatively limited. Each factor or biomarker may only reflect a certain aspect or stage of tumor progression, so the accuracy of single factor prediction may not be high enough.[16] Combining multiple factors or biomarkers for detection can comprehensively consider information from multiple aspects, thereby improving the accuracy of prediction. This helps to more accurately determine the metastasis and prognosis of tumor patients, providing more reliable basis for clinical diagnosis and treatment.[17] However, there are relatively few studies on the combination of key pathological features and multiple tumor markers in predicting SLN metastasis of EC, and its comprehensive predictive value and feasibility of clinical application need to be further explored.

In this study, we focused on EC patients treated in our hospital, aiming to analyze the early predictive value of combined detection of vascular cancer thrombus, myometrial invasion, and tumor markers for SLN metastasis of EC, in order to assist clinical diagnosis and treatment.

Materials and Methods

Clinical Materials

A total of 150 patients with EC diagnosed and treated in our hospital from January 2022 to December 2024 were retrospectively selected as the research objects. The sample size calculation was based on the results of the pilot experiment: Assuming that the incidence of SLN metastasis was about 20%, α=0.05, β=0.2, and the tolerance error was 5%. The minimum sample size was 138 calculated by PASS15.0 software, and 150 cases were finally included. According to the postoperative pathological results, patients were divided into a metastatic group of 32 cases and a non-metastatic group of 118 cases to determine whether SLN metastasis had occurred. The inclusion process was shown in Figure 1. Inclusion criteria: (1)

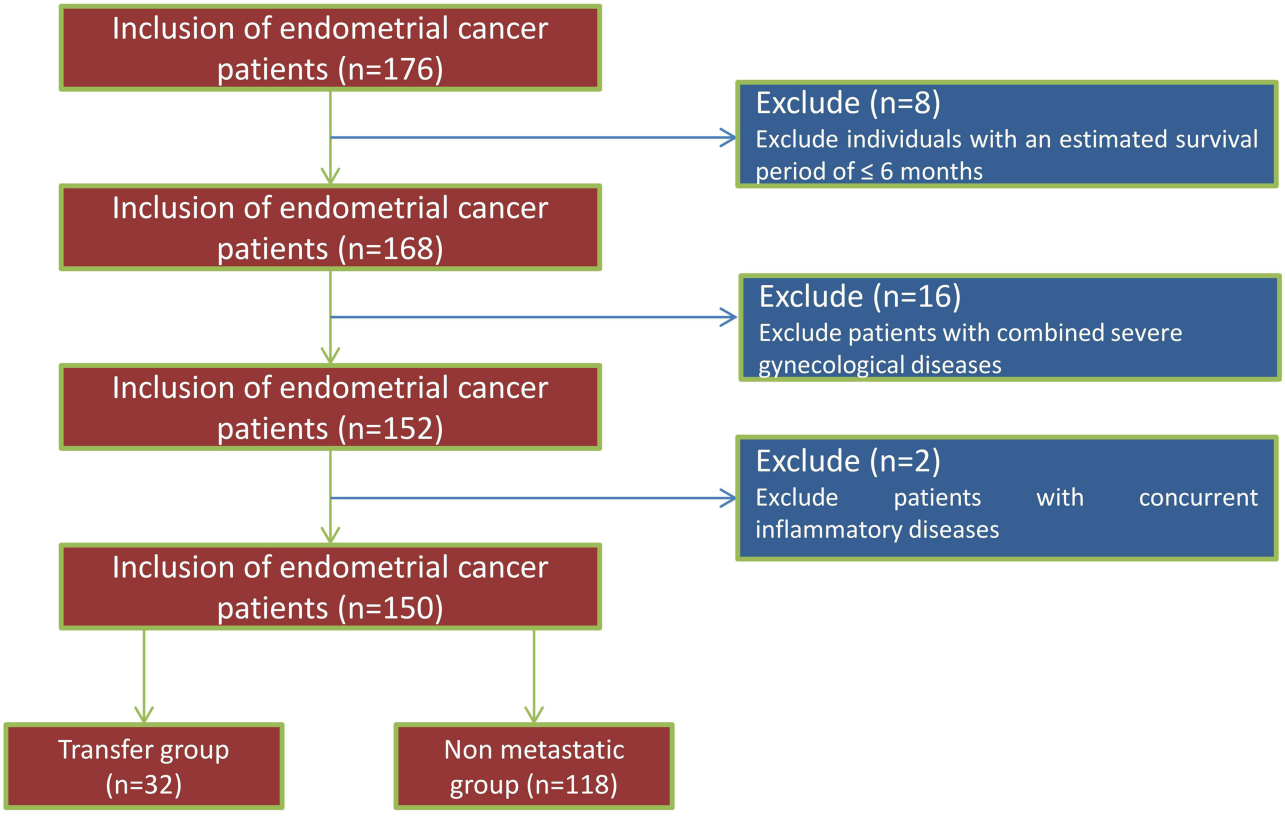

Figure 1 The inclusion process of the subjects.

All patients met the diagnostic and treatment criteria for EC,[18] and were diagnosed based on clinical symptoms, imaging, and pathological examinations; (2) Patients with complete clinical data (including preoperative tumor marker detection, intraoperative SLN localization, and postoperative pathological reports); (3) Patients without other malignant tumors; (4) All patients were first-time diagnoses; (5) Patients with expected survival period > 6 months. Exclusion criteria: (1) Patients with radiotherapy, chemotherapy, or immunosuppressive therapy history; (2) Patients merged renal or cardiac dysfunction; (3) Patients with combined blood system and endocrine system related diseases; (4) Patients with inflammatory diseases and myeloproliferative disorders; (5) Patients combine with other severe gynecological diseases. This study was ratified by the Ethics Committee of our hospital (approval number: 2025KY012) and was complied with the Declaration of Helsinki.

Pathological Detection and Evaluation Criteria

All patients underwent sentinel lymph node testing, general anesthesia, and lithotomy. Indocyanine green injection (tracer) was injected into the cervix at 3, 6, 9, and 12 o'clock respectively. The direction of lymphatic vessels was observed under laparoscopy and sentinel lymph nodes (SLN, the first lymph nodes to be successfully visualized) were searched. The SLN was cut out and sent for examination. Positive pathological test was considered as a metastatic group. The results were evaluated by at least two experts and the detection was followed by the evaluation criteria.[19]

Clinical Data Collection

The patient's electronic medical record system was used to collect clinical related data including age, body mass index (BMI), history of hypertension, history of diabetes, histological grading (G1, G2, G3), tumor diameter (<2 cm, ≥ 2 cm), vascular tumor thrombus, muscle invasion (<1/2, ≥ 1/2), etc.

Assessment of Vascular Cancer Thrombus

All patient tissue samples was obtained during the radical surgery for endometrial cancer, ensuring that the samples contained sufficient tumor tissue and surrounding normal tissue for subsequent analysis and evaluation. Immunohistochemistry (IHC) staining was used to determine the presence of vascular cancer thrombus in EC. Pre-processing steps such as fixation, dehydration, and embedding were performed on tissue samples to ensure they were suitable for IHC staining. Antibody markers CD31 and CD34 were selected for recognizing vascular endothelial cells, and D2-40 specifically label lymphatic endothelial cells for antigen repair, blocking, primary antibody incubation, secondary antibody incubation, and color development. The chromogenic reagent was 3,3-diaminobenzidine (DAB). After coloration, the staining results were observed under a microscope. If tumor cell embolus were observed in blood vessels or lymphatic vessels, it indicated the presence of vascular cancer emboli. The antibody markers used were described below. CD31 antibody (product number: ab28364, Abcam, United Kingdom) and CD34 antibody (product number: sc-7324, Santa Cruz Biotechnology, United States) were used to recognize vascular endothelial cells. D2-40 antibody (M3619, Dako, Denmark) was used to specifically label lymphatic endothelial cells.

Assessment of Muscle infiltration[20]

Transvaginal color Doppler ultrasound (TV-CDS) was used to evaluate the degree of myometrial invasion in EC. The patient needed to empty the bladder and took a lithotomy position to expose the uterus. Ultrasound equipment with color Doppler function, GE loqiq 5 color Doppler ultrasound diagnostic instrument, was used with a probe frequency of 5.0MHz. Firstly, a routine two-dimensional grayscale ultrasound scan was performed to observe the thickness, morphology, edge features of the endometrium, as well as the location, size, and internal echo of lesions in the uterine cavity. None myometrial invasion: clear and complete hypoechoic halo around the interface between endometrium and muscle layer; The muscle layer had uniform echoes and no abnormal echo areas; There was no significant change in blood flow signal distribution and resistance, or the blood flow signal was normal with the resistance index (RI) less than 0.85. Shallow infiltration (muscle infiltration $\leq 1/2$): The hypoechoic halo around the endometrium might be interrupted, or the endometrium might invade the muscle layer in a serrated manner; Uneven echo areas appeared within the muscle layer, but the depth of infiltration was less than half of the muscle layer thickness; There was an increase in color blood flow signals in the basal part of the endometrium, and the blood flow signals in the affected muscle layer were abundant and network like, with disordered vascular directions. RI value<0.5, but still higher than deep infiltration. Deep infiltration (muscle infiltration>1/2): The hypoechoic halo around the endometrium was blurred or even disappeared, and the boundary with the muscle layer was unclear; The thickness of the lesion edge from the serosa was less than half of the thickest part of the muscle layer; The muscle layer lesion area presented as linear or irregular uneven echoes, with infiltration depth greater than half of the muscle layer thickness; The blood flow signals in the affected muscle layer were very rich, and the vascular direction was more chaotic. RI value<0.3 indicated a decrease in blood flow resistance.

Serum Tumor Marker Detection

Before admission, 5mL of fasting venous blood was collected from the patient in the morning, and the supernatant was centrifuged. Enzyme-linked immunosorbent assay (ELISA) was used to detect the levels of serum HE4, CEA, CA125, CA153, CA199, and AFP. Steps: After the blood sample was left at room temperature or refrigerated, centrifuged for 20 minutes and the supernatant was collected for testing. EDTA or sodium citrate were used as anticoagulant, mixed for 10–20 minutes, centrifuged for about 20 minutes, and the supernatant was taken for detection. All reagents and samples were equilibrated to room temperature. Standard wells, test wells, and blank wells were set up. Diluted standard substance was added into the standard well, and the test sample was added into the test well. The target antibody was coated in a 96 well microplate to form a solid-phase carrier. Standard or sample was added to each well to bind the target antigen with the antibody on the solid-phase carrier. Horseradish peroxidase labeled antibody was added and incubated at 37°C for 60 minutes. The microplate was washed thoroughly to remove unbound components, and the plate was washed 5 times with detergent. A chromogenic substrate (such as TMB) was added to the well and colored under the action of the enzyme. Color reagents A and B were added, and the color was developed in the dark at 37°C for 15 minutes. At last, termination solution (sulfuric acid) was added to stop the color reaction. The absorbance (OD value) was measured using

an ELISA reader at a wavelength of 450 nm and the sample concentration was calculated. The concentration of the target protein in the sample was calculated based on the standard curve. For example, by fitting the standard curve equation with the concentration of the standard substance and the absorbance value, the sample concentration could be calculated. Detection kit for HE4 (item number: YM-EM8042), CA125 (item number: YM-PF0415), CA153 (item number: YM-PF0414), CA199 (item number: YM-PD9485), and AFP (item number: YM-SX0416) were all purchased from Shanghai Yuanmu Biotechnology Co., Ltd. The CEA detection kit (item number: IME00041) was purchased from Guangdong Gukang Biotechnology Co., Ltd.

Statistical Analysis

Statistical analysis was conducted using SPSS 24.0. The normality of continuous variables (HE4, CEA, CA125, CA153, CA199, AFP) was first verified by Shapiro–Wilk test. The normal distribution was expressed as $(\bar{x} \pm s)$, and the comparison between groups was analyzed by independent sample *t* test. Those who did not conform to the normal distribution were expressed as median (quartile), and the comparison between groups was analyzed by Mann–Whitney *U*-test. Enumeration data were expressed as [cases (%)], and comparison between groups was analyzed by chi-square test. Logistic regression model analysis of influencing factors on SLN metastasis in EC. The clinical value of ROC curve analysis in predicting SLN metastasis of EC based on influencing factors. Principal component analysis (PCA) was combined to analyze the distribution characteristics of various influencing factors. $P<0.05$ was considered statistically significant.

Results

Comparison of Clinical Data Between Two Groups

There was no significant difference in age, BMI level, history of hypertension, history of diabetes, histological grade and proportion of tumor diameter between the metastatic group and the non-metastatic group ($P>0.05$). The incidence of vascular cancer thrombus in the metastatic group (62.50%) was significantly higher than that in the non-metastatic group (23.73%), and the difference was 38.77 percentage points ($\chi^2=17.390$, $P<0.001$). The proportion of myometrial invasion ≥1/2 in the metastatic group (90.63%) was 2.32 times higher than that in the non-metastatic group (38.98%) ($\chi^2=26.854$, $P<0.001$) (Table 1). This result quantified the enrichment of vascular cancer thrombus and deep myometrial invasion in the metastatic group for the first time in a large sample, and provides a pathological basis for subsequent combined analysis.

Table 1 Comparison of Clinical Data Between Two Groups $(\bar{x} \pm s)$

Groups	Metastatic Group (n=32)	Non-Metastatic Group (n=118)	t/χ^2	*P*
Age (year)	59.36±12.49	60.42±13.39	0.403	0.688
BMI (kg/m^2)	24.80±3.13	25.12±3.42	0.478	0.634
History of hypertension (%)	9 (28.13)	30 (25.42)	0.096	0.757
History of diabetes (%)	3 (9.38)	13 (11.02)	0.071	0.790
Histological grade (%)			0.536	0.765
Grade G1	9 (28.13)	27 (22.88)		
Grade G2	20 (62.50)	76 (64.41)		
Grade G3	3 (9.38)	15 (12.71)		
Tumor diameter (cm)			3.805	0.051
<2	2 (6.25)	25 (21.19)		
≥2	30 (93.75)	93 (78.81)		
Vascular cancer thrombus (%)	20 (62.50)	28 (23.73)	17.390	<0.001
Myometrial invasion (%)			26.854	<0.001
<1/2	3 (9.38)	72 (61.02)		
≥1/2	29 (90.63)	46 (38.98)		

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Table 2 Comparison of Tumor Marker Levels Between Two Groups ($\bar{x} \pm s$)

Groups	Metastatic Group (n=32)	Non-Metastatic Group (n=118)	*t*	*P*
HE4 (pmol/L)	84.69±17.26	50.38±15.25	10.970	<0.001
CEA (μg/L)	1.82±0.61	1.52±0.43	3.180	0.002
CA153 (U/mL)	8.69±1.46	5.58±1.36	11.295	<0.001
CA125 (U/mL)	41.36±17.53	16.42±4.00	14.259	<0.001
CA199 (U/mL)	27.39±6.95	14.22±4.62	12.719	<0.001
AFP (μg/L)	2.71±0.72	2.53±0.84	1.106	0.270

Abbreviations: HE4, Human Epididymis Protein 4; CA125, Carbohydrate Antigen 125; CA153, Carbohydrate Antigen 153; CA199, Carbohydrate Antigen 199; AFP, Alpha-fetoprotein.

Comparison of Tumor Marker Levels Between Two Groups

Compared with the non-metastatic group, the serum levels of HE4, CEA, CA125, CA153, and CA199 of patients in the metastatic group were increased ($P<0.05$). The increase of CA199 in the metastatic group (92.6%) was significantly higher than that of other markers, suggesting that it may be more closely related to the progression of metastasis. There was no significant statistical difference in serum AFP levels between the two groups of patients ($P>0.05$, Table 2).

Multivariate Logistic Regression Analysis of SLN Metastasis in EC

Take whether SLN metastasis occurred as the independent variable (0=no metastasis, 1=metastasis), take relevant factors as the dependent variable, and values were assigned. Among them, vascular cancer thrombus 0=No, 1=Yes, myometrial invasion <1/2=0, ≥ 1/2=1. Continuous variables such as HE4, CEA, CA125, CA153 and CA199 were not assigned. Logistic regression analysis confirmed that vascular cancer thrombus, myometrial invasion ≥ 1/2, HE4, CA153, CA125 and CA199 were all influencing factors of SLN metastasis in EC ($P<0.05$, Table 3 and Figure 2). Among them, CA199 had the highest OR value (13.599), which was the strongest independent predictor of SLN metastasis of EC. Its weight was higher than that of myometrial invasion, which provided a priority basis for biomarker combination.

ROC Curve Analysis of the Predictive Value of Vascular Cancer Thrombus, Myometrial Invasion Combined with Tumor Markers for SLN Metastasis in EC

ROC analysis showed that HE4 had the highest AUC (0.859), followed by CA199 (0.804). The AUC of combined detection (0.904) was significantly higher than that of HE4 ($Z=2.13$, $P=0.033$) and CA199 ($Z=2.47$, $P=0.013$). The sensitivity (85.59%), specificity (84.38%) and Youden index (0.700) of combined detection were better than all single indicators. The Youden index was 16.5%, higher than that of HE4 (0.601) (Table 4 and Figure 3). Through statistical verification (Z test), the superiority of the combined model was proved, and the clinical pain point of insufficient prediction efficiency of single index was solved.

Table 3 Multivariate Logistic Regression Analysis of SLN Metastasis in EC

Indicators	*B* Value	Standard Error	*Wald* Value	*P* Value	*OR* Value	95% CI
Vascular cancer thrombus	1.953	0.603	10.490	<0.001	7.050	2.162~22.989
Myometrial invasion≥1/2	2.507	0.796	9.919	0.001	12.268	2.578~58.382
HE4	1.153	0.362	10.145	<0.001	3.168	1.557~6.443
CEA	2.746	1.627	2.848	0.092	15.583	0.642~378.379
CA153	0.940	0.325	8.365	0.012	2.560	1.354~4.840
CA125	1.473	0.543	8.576	0.010	4.362	1.627~11.693
CA199	2.610	0.813	10.316	<0.001	13.599	2.766~66.866

Abbreviations: OR, Odds Ratio; CI, Confidence Interval; HE4, Human Epididymis Protein 4; CA125, Carbohydrate Antigen 125; CA153, Carbohydrate Antigen 153; CA199, Carbohydrate Antigen 199; AFP, Alpha-fetoprotein.

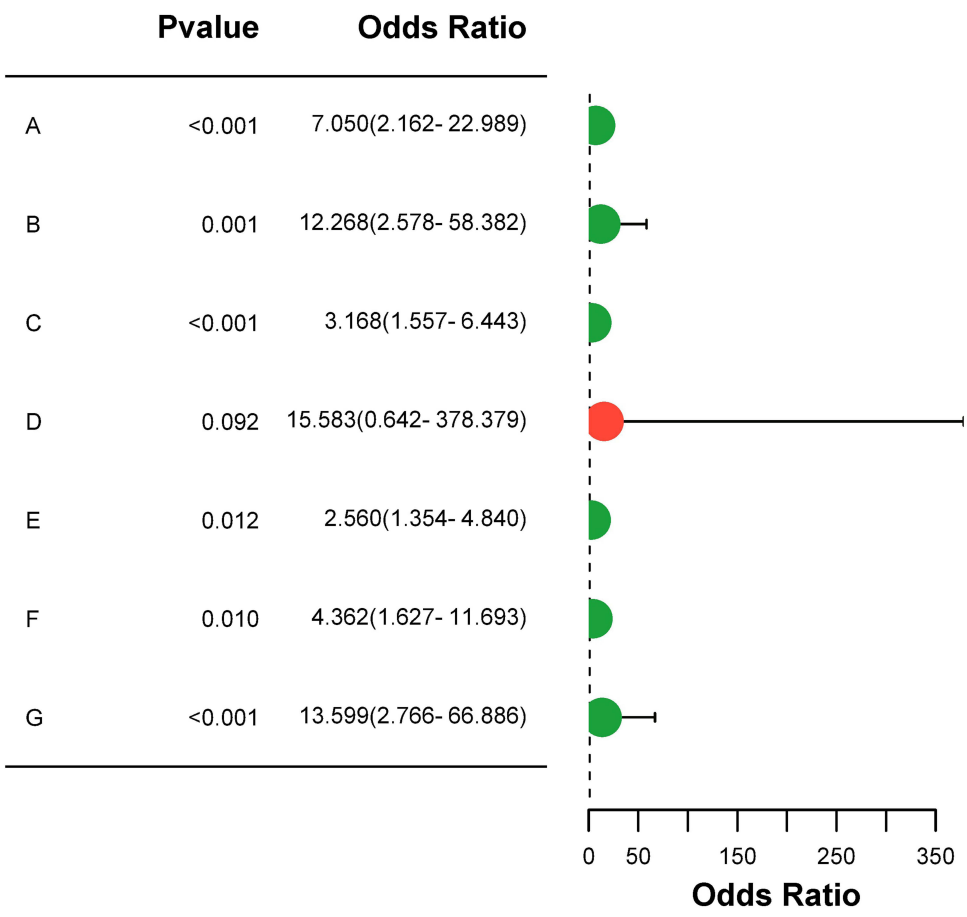

Figure 2 Forest plot analysis of influencing factors on SLN metastasis in EC. **(A)** Vascular cancer thrombus; (**B**) Myometrial invasion; (**C**) HE4; (**D**) CEA; (**E**) CA153; (**F**) CA125; (**G**) CA199.
Abbreviations: OR, Odds Ratio; CI, Confidence Interval.

PCA Graph Analysis

PCA model using vascular cancer thrombus, myometrial invasion, HE4, CA153, CA125, CA199 was established. The first principal component (PC1) was plotted against the second principal component (PC2). PC1 mainly reflected the levels of tumor markers (HE4, CA125, CA199 load > 0.7), and PC2 mainly reflected the pathological characteristics (vascular cancer thrombus, myometrial invasion load > 0.6). The distribution of scores on PC1 and PC2 in the metastatic group was significantly larger than that in the non-metastatic group (P<0.001). It is confirmed from multiple dimensions that the pathology-molecular characteristics of metastatic patients are more disordered, which provides visual evidence

Table 4 ROC Curve Analysis of the Predictive Value of Vascular Cancer Thrombus, Myometrial Invasion Combined with Tumor Markers for SLN Metastasis in EC

Indicators	AUC	Sensitivity	Specificity	Youden's Index	Cut Off Value	*P* Value	95% CI
Vascular cancer thrombus	0.694	76.27	62.50	0.388	——	<0.05	0.600–0.787
Myometrial invasion	0.758	61.02	90.63	0.516	——	<0.05	0.691–0.826
HE4	0.859	78.81	81.25	0.601	71.87pmol/L	<0.05	0.785–0.934
CA153	0.691	83.42	53.22	0.366	7.06U/mL	<0.05	0.568–0.814
CA125	0.785	98.31	68.75	0.671	32.74U/mL	<0.05	0.656–0.913
CA199	0.804	88.98	68.78	0.577	27.09 U/mL	<0.05	0.698–0.910
Combined detection	0.904	85.59	84.38	0.700	——	<0.05	0.839–0.968

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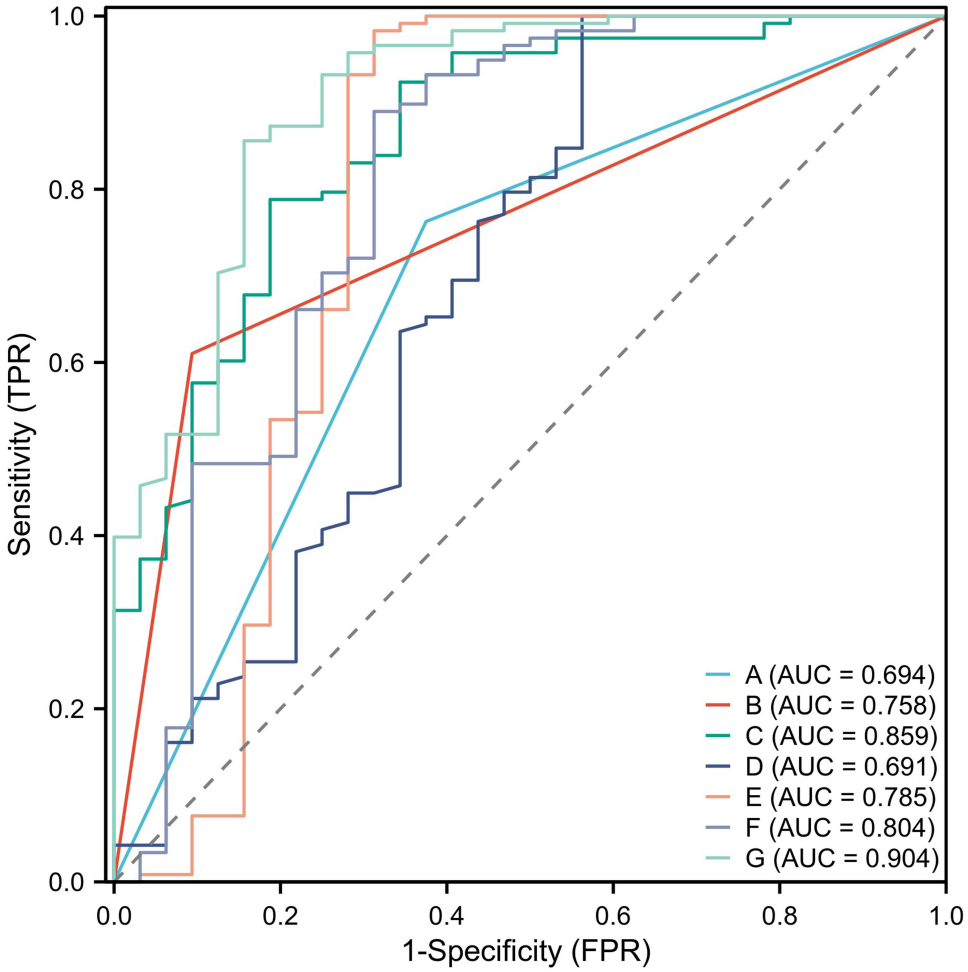

Figure 3 ROC curve analysis of the predictive value of vascular cancer thrombus, muscle infiltration combined with tumor markers for SLN metastasis in EC. (**A**) Vascular cancer thrombus; (**B**) Myometrial invasion; (**C**) HE4; (**D**) CEA; (**E**) CA153; (**F**) CA125; (**G**) Combined detection.
Abbreviations: AUC, Area Under the Curve; HE4, Human Epididymis Protein 4; CA125, Carbohydrate Antigen 125; CA153, Carbohydrate Antigen 153; CA199: Carbohydrate Antigen 199.

for the biological rationality of combined detection and is an important supplement to traditional univariate analysis (Figure 4).

Discussion

Endometrial cancer (EC) is a malignant tumor of the female reproductive system with an incidence rate second only to cervical cancer. Surgical resection, including hysterectomy, pelvic and abdominal lymph node dissection, etc were main treatment methods for EC. However, studies have found that lymph node dissection cannot improve the survival rate of patients and may increase the risk of postoperative complications such as lower limb pain and bladder fistula.[21] Therefore, the identification of efficient and reliable predictors of SLN metastasis is of great practical significance for guiding the clinical application of SLN biopsy, individualized surgical decision-making and postoperative adjuvant therapy. This study focused on the predictive value of combined detection of vascular cancer thrombus, depth of myometrial invasion and serum tumor markers for SLN metastasis, aiming to provide a more accurate risk assessment tool for clinical practice.

SLN are the main lymphatic drainage sites for malignant tumors, the first stop lymph nodes for tumor metastasis, and also the earliest site of metastasis.[22] This study analyzed the related factors of SLN metastasis in EC and found that the proportions of vascular cancer emboli (62.50% vs 23.73%, $P<0.001$) and depth of myometrial invasion (≥1/2) (90.63% vs 38.98%, $P<0.001$) in the metastatic group were significantly higher than those in the non-metastatic group.

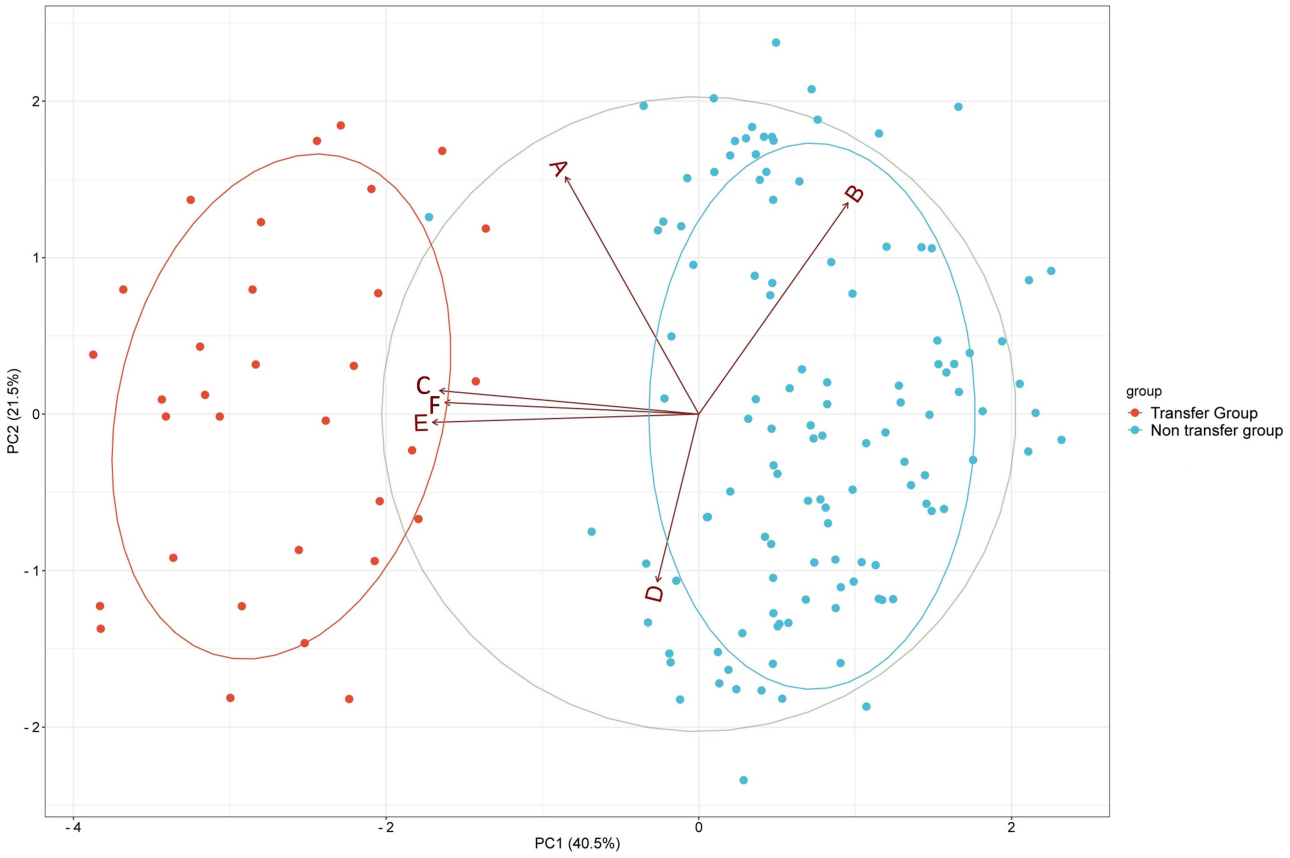

Figure 4 Analysis of two sets of PCA graphs. (**A**) Vascular cancer thrombus; (**B**) Myometrial invasion; (**C**) HE4; (**D**) CA153; (**E**) CA125; (**F**) CA199.
Abbreviations: PC1, The first principal component; PC2, The second principal component.

Multivariate Logistic regression analysis further confirmed that, vascular cancer thrombus (OR=7.050), depth of myometrial invasion (OR=12.268), serum HE4 (OR=3.168), CA153 (OR=2.560), CA125 (OR=4.362), CA199 (OR=13.599) were all independent influencing factors for SLN metastasis (P<0.05). These findings support the important role of vascular cancer thrombus, depth of myometrial invasion, and specific tumor markers in predicting SLN metastasis of EC. Vascular cancer thrombus refers to the presence of cancer thrombus in the blood vessels, which usually means that tumor cells have invaded blood vessels or lymphatic vessels and may metastasize to other parts with the flow of blood or lymphatic fluid.[23] During the formation of vascular cancer thrombus, cancer cells detach from the primary lesion and aggregate in blood vessels or lymphatic vessels, making it easier for these aggregated cancer cells to spread through the lymphatic system to sentinel lymph nodes.[24] In addition, tumor induced lymphangiogenesis and changes in lymphatic endothelial cells can also promote cancer cell metastasis. Relevant data shows that the expression of lymphatic endothelial markers such as LYVE-1 and Prox-1 in tumors may be associated with lymphatic metastasis. The changes in these biomarkers may affect the permeability of lymphatic vessels and the migration ability of cancer cells.[25] In addition, the formation of vascular cancer thrombus may also alter the local microenvironment, such as increasing vascular permeability, promoting inflammatory response, etc., providing more favorable conditions for the growth and metastasis of cancer cells.[26] Therefore, in the treatment of EC, it is necessary to pay attention to both vascular cancer thrombus and SLN metastasis in order to develop more precise treatment plans. In a study of gastric cancer, it was found that compared to patients without vascular cancer thrombus, patients with vascular cancer thrombus had a higher postoperative distant metastasis rate and lower survival rate. It might be because tumor cells can spread through blood vessels, leading to rapid tumor growth and exacerbating the deterioration of the disease.[27] As the depth of muscle infiltration increases, tumor cells are more likely to enter the lymphatic system through lymphatic vessels, leading to SLN metastasis. In addition, cancer tissues with deep muscle infiltration may cause changes in the local microenvironment,

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such as inflammatory response, immune suppression, etc., providing more favorable conditions for the growth and metastasis of tumor cells, thereby increasing the risk of SLN metastasis.[28] Research has found that when the disease progresses and the tumor invades the deep muscle layer or the cervical stroma, LNM is more likely to occur. Patients with LNM have a 5-year survival rate of less than 50%, which seriously affects their quality of life.[29,30]

Partial cancer cell antigens may shed during tumor progression or distant metastasis, leading to a significant increase in cancer cell antigens in serum.[31] CA153, CA125, and HE4 are all relevant biomarkers for diagnosing malignant tumors. In the study of breast cancer, it was found that the expression level of CA153 was significantly increased in patients with LNM of breast cancer. CA153 is an independent influencing factor for predicting LNM, which is beneficial for early prediction of LNM in patients.[32] CA125 is a commonly used serum tumor marker in clinical practice. Research has found that serum CA125 levels in patients with EC are significantly elevated, and the level of CA125 increases with the progression of the disease.[33] HE4 is a whey acidic protein that can be expressed in tissue epithelium, including the female reproductive tract, and the expression level of HE4 is significantly elevated in various cancers. A study suggests that HE4 has higher sensitivity and specificity than CA125 in the detection of EC, and its expression is associated with LNM status, survival rate, and recurrence rate.[34,35] A study has found that CA125 and HE4 are significantly upregulated in EC, and patients in the poor prognosis group have much higher levels of CA125 and HE4 than those in the good prognosis group. The combined detection is beneficial for the detection of diseases and prognosis.[36] CA199 and CEA are two commonly used tumor markers, which have important value in the diagnosis and prognostic evaluation of various cancers. Research has shown[37] that high levels of CEA are associated with LNM in ovarian cancer patients, especially in early-stage patients, where CEA detection helps identify patients who may have more advanced disease. In colorectal cancer patients,[38] CA199 levels are significantly correlated with LNM, and CA199 positive patients have a shorter survival period. In patients with pancreatic cancer,[39] elevated levels of CA199 usually indicate that LNM or metastasis in other parts may have occurred. Based on the above results, it is believed that serum tumor marker levels better reflect the role of tumor markers in cancer progression, which may play an important role in predicting disease progression. The predictive value of a single tumor biomarker or pathological feature may be limited, while combining multiple biomarkers or features for detection can significantly improve the accuracy of prediction. In this study, ROC curves showed that the combined detection of vascular cancer thrombus, myometrial invasion, HE4, CA153, CA125, and CA199 had high predictive value for SLN metastasis in EC. Therefore, for patients predicted as high-risk by the ROC curve, more aggressive treatment measures can be taken, such as expanding the scope of surgery, strengthening chemotherapy or radiotherapy, etc., to reduce the risk of recurrence and metastasis.

In this study, ROC analysis confirmed that the combined detection of vascular cancer embolus, depth of myometrial invasion and HE4, CA153, CA125, CA199 could significantly improve the prediction performance, with an AUC of 0.904, sensitivity and specificity of 85.59% and 84.38%, respectively. This result is a direct response to the study objective, showing that the combined prediction of multiple factors is superior to the single indicator. The high AUC values of the combined model indicate that it has important potential for clinical application, especially in decision support for SLN biopsy. For patients predicted to be at low risk, it can enhance the confidence of the surgeon to avoid unnecessary extension of lymph node dissection, thereby reducing the risk of surgical complications. However, for patients predicted to be at high risk (the sensitivity of combined detection in this study was 85.59%), it is strongly suggested that SLN biopsy should be strictly performed or even more extensive lymph node evaluation should be considered, and postoperative adjuvant therapy may need to be strengthened to improve the prognosis.

PCA, as an effective dimensionality reduction method, was able to integrate the information contained in multiple variables (vascular cancer thrombus, muscle infiltration, HE4, CA153, CA125, CA199) into a few principal components in this study. Plotting PC1 against PC2 can visually present the distribution characteristics and internal structure of data. Vascular cancer thrombus and muscular invasion, as traditional pathological features, are closely related to SLN metastasis. The occurrence of disorder indicates that these factors undergo complex pathophysiological changes during disease progression, which may alter the body's microenvironment and metabolic status by affecting the invasion and metastasis pathways of tumor cells. This is further reflected in the abnormal fluctuations of related tumor markers (such as HE4, CA153, CA125, CA199) levels. This disorder suggests that they may jointly participate in promoting SLN metastasis and interact to form a complex network, increasing the malignancy of the disease and the difficulty of clinical

management. The results presented by the PCA model revealed the complex relationship between SLN metastasis and multiple factors in EC, providing important theoretical basis and data support for further research on the pathogenesis, metastasis prediction, and personalized treatment of this disease in the future. At the same time, individual differences of patients and heterogeneity of diseases should be fully considered in clinical practice to develop more precise and effective diagnosis and treatment strategies.

In recent years, the research of circulating miRNA as an early diagnostic tool for EC has attracted much attention. MiRNA plays a key role in the occurrence and development of EC by regulating the expression of tumor-related genes. Its characteristics of high stability and convenient detection make it a potential early diagnostic marker.[40] For instance,it has been confirmed that miR-125 can be used as a biomarker for differential diagnosis in breast cancer. Although its role in EC needs further verification, it provides a new direction for early screening of tumors.[41] In addition, the association between nutritional supplements and cancer prevention in women has become increasingly clear. Myo-inositol, a B vitamin analogue, not only plays a role in ovulation induction and menopause management, but also has antioxidant and metabolic properties that may help reduce the risk of EC and other tumors.[42,43] The application of compounds such as frankincense and betaine in improving breast density also provides a cross-disease reference for tumor prevention.[44] This study focuses on the prediction of SLN metastasis, and early diagnostic tools such as miRNA and nutritional intervention strategies can complement the combined prediction model of this study. The former helps early identification of the disease, and the latter provides ideas for prevention, so as to improve the diagnosis and treatment system of EC together. At the same time, the safety of SLN biopsy has been verified in gynecological tumors, such as the high accuracy of sentinel lymph node biopsy after vulvar cancer,[45] which also supports the clinical significance of optimizing the lymph node treatment plan by accurately predicting the risk of metastasis in this study. With the improvement of cancer survival rate, fertility protection and tumor survival management have become important issues. The experience of tumor fertility counseling and fertility preservation of breast cancer patients shows that the fertility needs of patients with malignant tumors should be paid attention to.[46] For young patients with EC, the combined prediction model in this study can create conditions for fertility preservation by avoiding the impact of excessive lymph node dissection on ovarian function, which is also consistent with the current emphasis on individualized diagnosis and treatment and humanistic care.

In general, based on the results of this study, we confirmed that vascular cancer thrombus, myometrial invasion depth (≥1/2) and elevated serum levels of HE4, CA153, CA125 and CA199 were important predictors of SLN metastasis of EC, and their combined detection model (AUC=0.904) showed excellent predictive performance. This model is expected to be useful in clinical practice, especially in guiding SLN biopsy strategies (avoiding dissection vs Must evaluate) and adjuvant treatment decision-making, thereby promoting the diagnosis and treatment of EC to the direction of more precise and individualized, and ultimately serving the fundamental goal of improving the quality of life and prognosis of patients.

Our study has certain limitations. Vascular cancer thrombus is the result of postoperative pathology, and its acquisition depends on surgical specimens. The lack of timeliness before or during operation may limit its direct application in preoperative diagnosis and treatment decision-making. This characteristic suggests that future research can combine preoperative indicators (such as the depth of myometrial invasion assessed by transvaginal ultrasound and the dynamic changes of serum tumor markers) and intraoperative indicators (such as frozen section pathology of SLN and the imaging characteristics of indocyanine green-labeled lymph nodes) to construct a more timely combined prediction model, in order to enhance the preoperative prediction ability of SLN metastasis and provide earlier references for the individualized formulation of surgical plans. Nevertheless, this study has confirmed that the combined detection of vascular cancer thrombus, myometrial invasion, and tumor markers has a high predictive value, providing a basis for understanding the multi-factor mechanism of SLN metastasis and laying a foundation for subsequent studies integrating preoperative / intraoperative indicators.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of First Affiliated Hospital of Bengbu Medical University and was complied with the Declaration of Helsinki.

Patient Consent for Publication

Informed consent was obtained from participants for the participation in the study and all methods were carried out in accordance with relevant guidelines and regulations.

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Disclosure

The authors declare that they have no competing interests.

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