Diagnostic Value of Combined Serum CEA, HE4, AFP, CA125, and CA199 Testing in Early-Stage Ovarian Cancer

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Abstract: Objective: To evaluate the diagnostic value of combined serum alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199), carbohydrate antigen 125 (CA125), and human epididymis protein 4 (HE4) in early-stage ovarian cancer. Methods: Subjects were recruited from January 2022 to December 2025 at the First Clinical Medical College of Three Gorges University (Yichang Central People's Hospital), including relevant patients and concurrent healthy examinees. Participants were stratified into three groups: early-stage ovarian cancer (n=75), benign ovarian tumors (n=71), and healthy examinees (n=70). All subjects underwent standardized testing for five serum tumor marker levels to evaluate their diagnostic utility in early-stage ovarian cancer. Results: (1) Significant differences existed in the levels of the five serum tumor markers among the three groups (P=0.000). All markers in the early-stage ovarian cancer group were significantly higher than those in the benign ovarian tumor group and the healthy examination group, far exceeding the normal range. The levels in the latter two groups decreased sequentially. (2) Serum tumor marker levels differed significantly among early-stage ovarian cancer patients at different stages (P=0.000). Marker levels in stage III–IV patients were significantly higher than those in stage I–II patients. (3) The diagnostic sensitivity and specificity of individual serum tumor marker tests were low. However, combined detection of all five markers achieved a sensitivity of 91.63% and specificity of 95.40%, significantly outperforming single-marker testing. Conclusions: (1) Serum levels of all five tumor markers were significantly higher in the early-stage ovarian cancer group compared to the other two groups. Marker levels also showed significant differences among patients with early-stage ovarian cancer at different stages. (2) Combined testing demonstrated far superior diagnostic performance compared to individual markers, indicating that combined testing reduces misdiagnosis and missed diagnosis, providing reliable evidence for the diagnosis and disease assessment of early-stage ovarian cancer.

***Keywords*:** serum markers; combined detection; diagnosis; early-stage ovarian cancer

1. Introduction

Ovarian cancer is a common malignant tumor of the female reproductive system. Its pathogenesis remains incompletely understood. The widely accepted continuous ovulation hypothesis suggests that the repair process following frequent ovarian ovulation may induce malignant transformation of epithelial cells. Additionally, reproductive history and genetic factors are closely associated with disease risk, with approximately 15% of ovarian epithelial cancers attributed to hereditary factors[1-4]. Ovarian cancer is termed the "silent killer" due to its insidious and nonspecific early symptoms. Most patients present only with mild abdominal discomfort, bloating, or frequent urination — symptoms easily overlooked — resulting in approximately 70% of patients being diagnosed at an advanced stage[5-8]. Clinical data[9] that ovarian cancer prognosis varies significantly across disease stages, making early, precise diagnosis crucial for improving patient outcomes and reducing mortality. Current clinical diagnosis primarily relies on imaging studies, tumor marker testing, and pathological examination. Among these, serum tumor marker testing has become an important tool for early screening andauxiliary diagnosis due to its convenience, non-invasiveness, and reproducibility[10-12]. Based on this, the present study aims to investigate the diagnostic value of combined serum CEA, HE4, AFP, CA125, and CA199 testing in early-stage ovarian cancer.

2. Subjects and Methods

2.1 Study Population

Subjects were recruited from January 2022 to December 2025 at the First Clinical Medical College of Three Gorges University (Yichang Central People's Hospital), comprising relevant patient cohorts and concurrent healthy examinees. Participants were stratified into three groups: early-stage ovarian cancer (n=75), benign ovarian tumors (n=71), and healthy examinees (n=70). The early-stage ovarian cancer group comprised patients aged 30–70 years (mean age: 51.44 ± 3.70

years), further classified by International Union of Gynecology and Obstetrics (FIGO) clinical staging into Stage I (n=9), Stage II (n=18), Stage III (n=35), and Stage IV (n=13). The ovarian benign tumor group ranged from 35 to 69 years old, with a mean age of (52.35 ± 3.41) years; the healthy examination group ranged from 36 to 72 years old, with a mean age of (51.16 ± 3.82) years. Clinical data comparisons among the three groups showed no statistically significant differences, indicating comparability.

2.2 Inclusion and Exclusion Criteria

Inclusion Criteria: (1) Clinical diagnosis meeting the criteria outlined in the Chinese Expert Consensus on Early Screening for Ovarian Cancer (2025 Edition)[13]; (2) Pathological confirmation of ovarian cancer; (3) Patients with early-stage ovarian cancer at initial diagnosis who had not received any prior radiotherapy, chemotherapy, targeted therapy, or immunotherapy, nor undergone ovarian-related surgical interventions; (4) Complete and traceable medical records.

Exclusion Criteria: (1) Concurrent malignancies in other systems or acute/chronic systemic infectious diseases; (2) Severe underlying conditions such as cardiac, pulmonary, cerebral, hepatic, or renal insufficiency; (3) Comorbidities with complications that may affect tumor marker measurement results; (4) Incomplete medical records.

2.3 Research Methods

All participants underwent venous blood collection between 7:00–8:00 AM after fasting. Blood was drawn using disposable sterile vacuum tubes. After routine disinfection of the puncture site, 5 mL of peripheral venous blood was collected. Following collection, samples were centrifuged using a high-speed centrifuge with strictly controlled parameters: 3000 rpm, 15 minutes, and a centrifugal radius of 10 cm. After centrifugation, serum samples were stored sealed in a -20°C freezer until testing. Serum tumor marker testing employed the Roche Cobas E601 electrochemiluminescence analyzer with its corresponding reagents and calibrators. All assays utilized the electrochemiluminescence method, adhering strictly to standardized procedures outlined in the instrument and reagent manuals. The specific markers tested in this round include AFP, CEA, CA199, CA125, and HE4. The normal reference ranges for each marker strictly adhere to the reagent instructions and clinical standards, as follows: AFP: 0–5 IU/mL; CEA: 0–5 ng/mL; CA199: 0–27 U/mL;CA125: 0–35 U/mL; HE4: 0–140 pmol/L. All specimens underwent single testing. If abnormal results were detected, specimens were re-collected for retesting, with the retest results serving as the final data.

2.4 Observation Indicators

(1) Serum levels of five tumor markers in the three study groups

(2) Serum tumor marker levels in patients with early-stage ovarian cancer at different stages

(3) Diagnostic value of individual and combined detection of five serum tumor markers in early-stage ovarian cancer

2.5 Statistical Methods

Data analysis was performed using SPSS 26.0 software. For normally distributed quantitative data, results were presented as mean ± standard deviation ($\bar{x}$±s) and assessed for significant differences using t-tests. Comparisons among the three groups were conducted using one-way analysis of variance (ANOVA), with pairwise comparisons performed using the Holm-Sidak test. A P value < 0.05 was considered statistically significant.

3. Results

3.1 Serum Tumor Marker Levels in the Three Groups

Statistically significant differences were observed in the levels of all five serum tumor markers among the three groups (P=0.000). The early-stage ovarian cancer group exhibited significantly higher levels than both the benign ovarian tumor group and the healthy control group, with values far exceeding the normal range. Levels decreased progressively in the latter two groups.

Table 1. Serum tumor marker levels in the three groups ($\bar{x}$±s)

Group	Number of Cases	CEA (ng·mL⁻¹)	HE4 (pmol·L⁻¹)	AFP (ng·mL⁻¹)	CA125 (U·mL⁻¹)	CA199 (U·mL⁻¹)
Early-stage ovarian cancer group	75	34.09±4.86	354.60±43.72	204.67±48.36	326.69±33.45	263.40±52.64
Benign ovarian tumor group	71	6.72±0.91	68.56±7.21	2.65±0.53	43.67±4.62	40.18±4.68
Health Examination Group	70	3.18±0.55	39.06±4.32	2.01±0.24	17.84±4.51	14.98±2.33
F value		1568.410	2523.260	1246.570	4320.720	1465.650
P-value		0.000	0.000	0.000	0.000	0.000

3.2 Levels of Five Serum Tumor Markers in Early-Stage Ovarian Cancer Patients at Different Stages

Significant differences were observed in the levels of five serum tumor markers among early-stage ovarian cancer patients at different stages (P=0.000), with stage III–IV patients exhibiting significantly higher levels across all markers compared to stage I–II patients.

Table 2. Levels of five serum tumour markers in patients with early-stage ovarian cancer at different stages ($\bar{x}\pm s$)

Category	Number of Cases	CEA (ng·mL⁻¹)	HE4 (pmol·L⁻¹)	AFP (ng·mL⁻¹)	CA125 (U·mL⁻¹)	CA199 (U·mL⁻¹)
Stages I–II	27	26.11±3.84	233.48±31.22	160.54±13.71	197.60±14.56	213.45±16.37
Stage III–IV	48	39.67±4.45	410.56±42.60	234.29±23.40	323.11±29.74	390.78±25.63
t-value		13.286	18.098	14.968	20.544	32.377
P-value		0.000	0.000	0.000	0.000	0.000

3.3 Diagnostic Value of Five Serum Tumor Markers in Early-Stage Ovarian Cancer: Individual and Combined Testing

Results showed that the sensitivity and specificity of individual serum tumor marker detection were low, whereas combined detection achieved a sensitivity of 91.63% and specificity of 95.40%, significantly outperforming single-marker detection. This indicates that combined detection enhances diagnostic efficacy for early-stage ovarian cancer.

Table 3. Diagnostic value of individual and combined testing for five serum tumour markers in early-stage ovarian cancer

Item	Sensitivity	Specificity	PositivePredictiveValue	NegativePredictiveValue
CEA	58.11	82.20	58.72	80.31
HE4	59.62	85.50	66.43	80.15
AFP	59.77	81.00	58.92	80.23
CA125	54.10	82.00	59.47	78.56
CA199	60.62	84.60	65.31	80.42
5-markerpanel	91.63	95.40	92.81	94.68

4. Discussion

In recent years, the incidence of ovarian cancer in China has been rising annually, with approximately 52,000 new cases reported each year and a trend toward younger age at diagnosis[14].CA125 remains the most widely used ovarian cancer biomarker in clinical practice, exhibiting sensitivity exceeding 80% for epithelial ovarian cancer. However, its positivity rate in early-stage ovarian cancer is only 50%-60%, and it is easily influenced by benign conditions such as endometriosis and pelvic inflammatory disease, resulting in low specificity. Therefore, CA125 alone cannot meet the diagnostic demands for early-stage detection[15-17].HE4, as a novel ovarian cancer biomarker, demonstrates significantly higher specificity than CA125, particularly in early-stage ovarian cancer. Its specificity reaches 95%-98% in patients with stage I/II ovarian cancer and exhibits minimal interference from benign conditions, thereby compensating for the limitations of CA125..Furthermore, although CEA, AFP, and CA199 are not specific to ovarian cancer, they may also exhibit abnormal expression during the development of the disease. However, relying on a single tumor marker has limitations in sensitivity or specificity, making it difficult to achieve precise screening and diagnosis of early-stage ovarian cancer. Therefore, this study selected three groups of subjects to clarify the application value of five serum tumor markers in early-stage ovarian cancer.

Research findings indicate that levels of all five markers in the early-stage ovarian cancer group were significantly higher than those in the benign ovarian tumor group and the healthy screening group, far exceeding normal reference ranges. This correlates closely with the pathogenesis of ovarian cancer. Tumor cells abnormally synthesize and release these markers during proliferation and differentiation, leading to markedly elevated serum levels. In contrast, marker synthesis in benign tumors and healthy individuals remains within normal physiological ranges. These results align with the conclusions of Zhang Caihong's study[20], which demonstrated that serum tumor marker levels in ovarian cancer patients are significantly higher than in those with benign conditions, serving as an important reference for distinguishing malignant from benign lesions, which indicated that multiple serum tumor marker levels in ovarian cancer patients were significantly higher than in those with benign diseases, serving as an important reference for distinguishing benign from malignant lesions. Comparing marker levels among early-stage ovarian cancer patients at different stages revealed that all indicators were significantly

higher in stage III–IV patients than in stage I–II patients. This suggests a positive correlation between the levels of the five serum tumor markers and the severity of ovarian cancer, potentially aiding clinical disease staging assessment.This finding aligns with clinical patterns: as ovarian cancer progresses through stages, increased tumor burden and expanded infiltration enhance tumor cell secretion of markers, leading to elevated serum levels[21-24].This also corroborates the conclusion from Xu Hengye's study[25] that "tumor marker levels reflect the progression of ovarian cancer," providing reliable laboratory evidence for early disease assessment.Diagnostic efficacy analysis revealed that the sensitivity and specificity of individual marker detection were relatively low. However, combined detection achieved a sensitivity of 91.63% and specificity of 95.40%, significantly outperforming single-marker detection. This outcome highlights the advantages of multi-marker combined detection.Individual markers have inherent limitations: CA125 is susceptible to interference from benign gynecological conditions; HE4 exhibits high specificity but insufficient sensitivity; CEA, AFP, and CA199 lack specificity for ovarian cancer. Combined testing achieves complementary advantages, reducing the probability of missed or misdiagnosis[26-29]. Compared with studies by Maiorano and Xing Wenting, their AI model demonstrates slightly higher diagnostic efficacy but relies on multicenter big data and complex algorithms, making it difficult to implement in primary healthcare settings. In contrast, the five-marker combined detection used in this study is operationally simple, cost-effective, and better suited to practical clinical needs at the grassroots level.

In summary, the combined detection of serum CEA, HE4, AFP, CA125, and CA199 effectively enhances the diagnostic accuracy of early-stage ovarian cancer while aiding in disease staging assessment. Its simplicity and practicality make it a valuable laboratory tool for early ovarian cancer screening, diagnosis, and disease evaluation, warranting clinical implementation.However, this study has certain limitations. The subjects were sourced from a single center with a limited sample size, and the study did not analyze differences in biomarker levels among patients with different pathological types of ovarian cancer. Future research could expand the sample size and conduct multicenter studies to further refine the conclusions.

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