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Serum CA125 in combination with ferritin improves diagnostic accuracy for epithelial ovarian cancer

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ABSTRACT

Introduction: CA125 has poor sensitivity and low specificity for detecting early ovarian cancer. Serum ferritin is elevated in many malignancies. We evaluated the performance of ferritin alone and in combination with CA125 as a diagnostic tool to detect epithelial ovarian cancer (EOC).

Methods: CA125 and ferritin were detected in the serum of 50 healthy control (HC), 50 women with benign gynaecological conditions and 124 women with EOC. The relationship between serum ferritin and CA125 and each of the clinicopathological parameters was assessed, and their diagnostic accuracy for discriminating ovarian cancer determined.

Results: Serum ferritin and CA125 were higher in patients with EOC compared to HCs and patients with benign conditions (both $p < 0.001$). There was no relationship between levels of ferritin and CA125. Both ferritin and CA125 discriminated HC from EOC ($p < 0.05$), but ferritin showed better diagnostic accuracy than CA125 ($p = 0.048$). Ferritin was superior to CA125 in discrimination early EOC ($p = 0.002$), but in advanced stages, CA125 was superior ($p = 0.026$). A combination of ferritin and CA125 marginally increases the diagnostic accuracy to discriminate EOC from HCs.

Conclusion: Ferritin discriminates between HCs and EOC patients, especially in early stage disease. The combination of serum ferritin and CA125 provides the higher diagnostic accuracy to screen for EOC. Serum ferritin could serve as an EOC biomarker to complement the standard CA125 test.

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Introduction

Ovarian cancer is the most serious gynaecological malignancy and the seventh leading cause of cancer-related death in women [1]. The starting symptoms of the disease are vague and may mimic other conditions, resulting in a delay in diagnosis. Accordingly, most patients with ovarian cancer are diagnosed in advanced metastatic stages (stage III/IV) [2,3]. Therefore, searching for novel biomarkers aimed at detecting ovarian cancer at its earliest stages is of great value.

Cancer antigen 125 (CA125) is the first and best known ovarian cancer serum marker [4]. It is a high-molecular-weight glycoprotein used to monitor epithelial ovarian cancer and to differentiate the diagnoses of pelvic masses [5,6]. Routine serum levels of CA125 can be used to monitor recurrence and to predict clinical course and response to chemotherapy in patients with ovarian cancer. It is now one of the most important prognostic biomarkers for ovarian cancer patients in the clinical setting. However, CA125 is not cancer-specific; it is also elevated in benign ovarian neoplasms and endometriosis, needing further investigation by imaging [7]. In addition, some ovarian cancers at early stage do not generate sufficient CA125 for diagnostic purposes [8]. Therefore, novel circulating biomarker(s) for the detection of ovarian cancer are urgently required.

Ferritin is a cytosolic protein; it is present in most tissues of the body and plays an important role in the storage of intracellular iron [9]. Measurement of serum ferritin provides the most useful indirect estimate of body iron stores and plays important roles in proliferation, immunosuppression, carcinogenesis and angiogenesis [10–14]. Serum ferritin is elevated in many malignancies, such as breast cancer, hepatocellular carcinoma, non-Hodgkin lymphoma and renal cell carcinoma [15–18]. Moreover, increased serum ferritin is associated with poor prognosis in patients with breast, pancreatic and gastric cancer [15,19,20]. These studies suggest that ferritin may serve as a notable biomarker of diagnosis and prognosis of various cancer types.

We conducted a case-control study to investigate the serum levels of ferritin and CA125 in patients with ovarian cancer and benign gynaecological conditions and healthy women, hypothesising that the two markers in combination would provide better discrimination for ovarian cancer.

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Material and methods

We recruited from patients presenting to the Department of Gynecology, the Affiliated Hospital of Qingdao University. Of these, 124 had histologically confirmed primary invasive epithelial ovarian cancer, and 70 had histologically confirmed benign ovarian neoplasms. All epithelial ovarian cancer patients were surgically staged according to the International Federation of Gynecology and Obstetrics (FIGO) criteria. Seventy healthy women attending the annual physical examination in our hospital were controls. All subjects had no family history of ovarian cancer and no diagnosis of cancer during follow-up. Patients gave a blood sample before any treatment, and those who underwent preoperative radiotherapy or/and chemotherapy were excluded. Clinical characteristics of the patients are given in Table 1. Written informed consent was obtained from all the donors, and the study was approved by the Affiliated Hospital of Qingdao University Ethics Committee.

Blood was drawn into 10-mL serum tubes and centrifuged at 2200 rpm for 10 min. Ferritin levels were determined by ELISA (Laguna Scientific), CA 125 concentrations were measured in the Department of Clinical Laboratory Medicine, the People's Hospital of Jining using a commercially available Beckman DXI (Beckman Coulter, Inc., Brea, CA, USA) assay system. In clinical practice, the reference range for normal CA125 levels was defined as ≤35 U/mL.

Data with a Gaussian distribution are presented as the mean and standard deviation (SD). Data with a non-normal distribution are presented as median and range. Categorical variables were analysed by χ^2 test. Kruskal–Wallis tests were used as nonparametric methods for comparing ferritin and CA125 groups, and then, if there is a difference, a *post hoc* multiple comparison was made using Tukey's *post hoc* test. The relationship between serum ferritin and CA125 and each of the clinicopathological parameters was analysed using the Mann–Whitney U test. Spearman's rank correlation coefficients were used to assess the correlations between ferritin and CA125. Receiver operating characteristic (ROC) curves were obtained, and the area under the curve (AUC) was calculated with 95% confidence interval on ferritin and CA125. Statistical analysis was performed by SPSS.22 (SPSS GmbH Software, Munich). The level of significance was taken as $P < 0.05$.

Results

Clinical, demographic and laboratory data are shown in Table 1. The groups were matched for age, age at diagnosis, menopause status, volume of ascites, parity and body mass index. Both CA125 and ferritin were higher in ovarian cancer than in the other two groups, with no difference between those two other groups. Ferritin and CA125 failed to correlate significantly in benign ovarian diseases ($r = 0.095$, $p = 0.086$), healthy controls ($r = 0.13$, $p = 0.067$) and ovarian cancer ($r = 0.083$, $p = 0.094$).

Relationships between CA125 and ferritin levels and clinicopathological characteristics are demonstrated in Table 2. Elevated CA125 levels were associated with increased age and advanced FIGO stage, ascites, histological subtypes and lymph node metastasis. Undifferentiated, serous papillary and mixed types showed higher levels of CA125 than endometrioid, clear cell and mucinous subtypes. However, ferritin level was only associated with advanced FIGO stage and lymph node metastasis.

To evaluate the diagnostic accuracy of serum ferritin, CA125, and both markers for prediction of ovarian cancer among the different donor groups, we performed ROC analyses and then compared the area under the ROC curves (AUCs). For discriminating between healthy women and all stages of ovarian cancer (Figure 1(a)), the AUC of ferritin was significantly higher than that of CA125 ($p = 0.048$). Although the AUC of both markers combined was marginally superior to ferritin alone, this was not significant. For discriminating between healthy women and early stage (stages I/II) ovarian cancer (Figure 1(b), the AUC of ferritin was again significantly higher than that of CA125 ($p = 0.002$). Although the AUC of both markers combined was marginally superior to ferritin alone, this was not significant. For discriminating between healthy women and advanced stage (stages III/IV) ovarian cancer (Figure 1(c), the AUC of CA125 was significantly higher than that of ferritin ($p = 0.026$). Although the AUC of both markers combined was marginally superior to CA125 alone, this was not significant.

Discussion

Ovarian cancer is the main cause of gynaecological cancer-related death. Because of the lack of specific symptoms, more than 70% of patients with ovarian cancer was found and diagnosed at a later stage [21]. Therefore, early

Table 1. Clinical, demographic and laboratory data.

	Healthy controls ($n = 70$)	Benign ovarian disease ($n = 70$)	Ovarian cancer ($n = 124$)	*P* value
Age (years)	57 (15)	57 (15)	59 (13)	0.346
Age at diagnosis (years)	Not applicable	54 (12)	57 (10)	0.085
Menopause status (pre/post)	43/27	37/33	73/51	0.273
The volume of ascites (cm^3)	Not applicable	370 (25–2680)	486 (15–2800)	0.142
Parity (yes/no)	32/38	36/34	73/51	0.283
Body mass index (kg/m^2)	25 (17–35)	25 (20–34)	25 (17–43)	0.378
CA125 (U/mL)	30 (4–75)	37 (5–109)	234 (9–2463)	<0.001
Ferritin (μg/L)	9 (0.85–64)	10 (0.9–60)	66 (10–399)	<0.001

Notes: Data are mean (SD) or median (range). *P* value by ANOVA, Kruskal-Wallis or Chi-squared testing.

Table 2. Association between serum CA125 and ferritin levels and clinicopathological characteristics of ovarian cancer patients.

Clinicopathological parameters	Number of patients	CA125 (U/mL)	*P* value	Ferritin (μg/L)	*P* value
Age (years)			0.038		0.23
≥60	83	946 (127)		136 (24)	
<60	41	655 (85)		146 (26)	
Grading			0.164		0.296
G1 + G2	30	727 (89)		148 (26)	
G3	94	847 (93)		15 (30)	
FIGO stage			<0.0001		<0.001
Early stages (I–II)	49	570 (66)		197 (47)	
Late stages (III–IV)	75	1327 (235)		100 (10)	
Lymph node metastasis			<0.0001		<0.001
Yes	51	1280 (155)		177 (35)	
No	73	518.3 (45)		115 (28)	
Presence of ascites			0.042		0.148
Yes	54	905 (103)		146 (21)	
No	70	695 (78)		135 (19)	
Histologic type			0.013		0.252
Serous papillary	72	921 (121)		946 (127)	
Endometrioid	11	710 (75)		148 (22)	
Clear cell	10	670 (57)		133 (22)	
Mucinous	9	579 (45)		110 (19)	
Undifferentiated	10	1086 (193)		165 (30)	
Mixed	12	874 (72)		151 (28)	

Note: Data are mean (standard deviation).

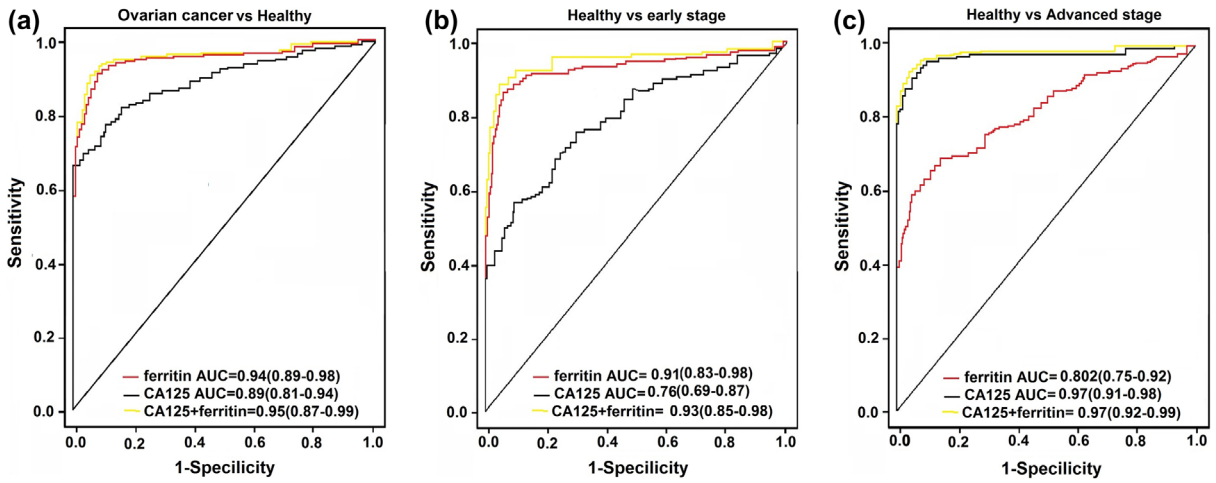

Figure 1. ROC analysis of ferritin and CA125. (a) ROC curves of healthy controls and ovarian cancer patients at all stages; (b) ROC curves of healthy controls vs. stages I/II; (c) ROC curves of healthy controls vs. stages III/IV.

diagnosis is the key to successful treatment of ovarian cancer. Although CA125 is the most widely used serum biomarker for monitoring ovarian cancer, its use in screening or early detection of ovarian cancer usually leads to high false-positive rates because of its low specificity and sensitivity [22]. In addition, serum CA125 may also be increased in patients with benign gynaecological disorders and pathology of organs from epithelial cells [23]. Therefore, we require new biomarkers with high specificity and high sensitivity to improve the treatment of ovarian cancer.

Ferritin is an iron storage protein found in all organisms, including bacteria, mammals and plants [24]. The role of ferritin in cancer is not yet fully understood [15–18]. For example, elevated risk of gastric cancer is associated with a decrease in serum levels of ferritin [25], whilst in patients with involuntary weight loss, a ferritin above 100 μg/L could rule-out colon cancer, but not gastric or rectal cancer [26]. The average cytoplasmic ferritin levels in renal cell carcinoma are higher than in normal renal parenchyma [27], and serum ferritin can be used to assess staging and postoperative recurrence and to predict survival of patients with renal cancer [27]. However, the reason for the increase of ferritin in renal cell carcinoma is unclear.

We examined the clinical usefulness of ferritin in diagnosis of patients with ovarian cancer, finding that serum levels were significantly higher in patients with ovarian cancer compared to those with benign ovarian diseases and healthy controls. Ferritin level was associated with advanced FIGO stage and lymph node metastasis, suggesting that ferritin could be used as a prognostic marker in ovarian cancer. In addition, the serum levels of ferritin were significantly higher in stage I/II than that of in stage III/IV. Using ROC analysis, we showed that ferritin discriminated between normal and ovarian cancer patients, especially in early stage disease.

CA125 can be used in the diagnosis and monitoring of ovarian cancer, but the sensitivity of CA125 to identify early stage disease is limited as a screening tool [28,29]. We found that the sensitivity of CA125 to identify the late stages (III/IV) was better than that in early stages (I/II) of ovarian cancer. These results suggest a potential use of serum CA125 values for diagnosis of ovarian cancer in late stages of ovarian cancer.

Given that CA125 alone may not provide sufficient sensitivity or specificity for diagnosing late stages of ovarian cancer, developing a feasible multivariable model is needed. Our results suggest that serum ferritin may provide the additional sensitivity and specificity needed to improve the diagnostic and prognostic value of the CA125 test. The combination of serum ferritin and CA125 values improved the diagnostic accuracy to screen for ovarian cancer, especially for early detection. In conclusion, our results provide convincing evidence to support the correlation between serum ferritin levels and ovarian cancers.

This study has a few limitations. Patients with cancers other than ovarian cancer were excluded from the analyses, and therefore, we could not determine the specificity of the serum biomarkers for ovarian cancer among other cancer types. In addition, the study principally included the small numbers of cases for histologic type analyses, which may be insufficient for determining the associations between histologic type and serum CA125 and ferritin levels. Thus, a large number of cases should be included in the future. Finally, increased ferritin may reflect an oncologic acute phase response and/or the anaemia of cancer [30–33], and as we were unable to report inflammatory markers (CRP, ESR) and further iron studies we cannot speculate on any roles for these aetiologies.

This work represents an advance in biomedical science because it shows that ferritin is able to discriminate cancer patients, especially in early stage disease. Serum ferritin could serve as a valuable ovarian cancer biomarker to complement the standard CA125 test, so may represent a novel diagnostic marker for ovarian cancer.

Summary table

What is known about this subject
- Serum biomarker is needed for early diagnosis of ovarian cancer.
- Serum ferritin is elevated in many malignancies.
- Whether serum ferritin in combination with CA125 could increase the diagnostic accuracy for ovarian cancer is unknown.

What this study adds
- Serum ferritin and CA125 are high in patients with ovarian cancer.
- Ferritin is a better discriminator of early ovarian cancer, CA125 of advanced ovarian cancer.
- The parallel use of both markers failed to provide significant additional discriminatory power.

Disclosure statement

No potential conflict of interest was reported by the authors.

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