Clinical Analysis of CA125, CA72-4, and Risk of Malignancy Index in Distinguishing Benign and Malignant Ovarian Masses

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Objective: To analyse CA125, CA72-4, and risk of malignancy index in distinguishing benign and malignant ovarian masses.
Methods: This was a retrospective study of patients with ovarian mass. Patients were divided into four groups according to the pathology results: group A included follicular cyst, corpus luteum cyst, and ovarian cyst; group B comprised chocolate ovarian cyst; group C included benign ovarian tumour; and group D involved malignant ovarian tumour. Serum CA125 and CA72-4 were measured. Risk of malignancy index was calculated by CA125 value, menopause status, and ultrasound status.
Results: A total of 249 patients were included. The median values of CA125 (178.7 U/mL), CA72-4 (6.05 U/mL), and risk of malignancy index (873.2) in group D patients were significantly higher than the normal cut-off value as well as in the other three groups. In group B, the median CA125 was higher than the cut-off value (51.15 U/mL), but CA72-4 and risk of malignancy index were normal. The sensitivities of CA125, CA72-4, and risk of malignancy index were 80.95%, 52.38%, and 73.81%, respectively; respective values for specificity were 70.97%, 79.29%, and 95.41%; for positive predictive value were 35.78%, 31.88%, and 75.61%; and for negative predictive value were 95.06%, 90%, and 94.97%.
Conclusions: Serum CA125 had the highest sensitivity and risk of malignancy index had the highest specificity. Combination of the three factors, CA125, CA72-4, and risk of malignancy index could be used to differentiate benign ovarian mass from ovarian cancer and increased the specificity to 98%. The positivity rates of the three factors increased in line with the clinical status of ovarian cancer, and could be used to better evaluate the risk of ovarian cancer, especially epithelial ovarian cancer.
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Introduction

Ovarian mass comprises several types of cysts and tumours, including benign tumour, malignant tumour, chocolate cyst, and ovarian cyst, of which ovarian tumour (benign and malignant) is the most common. Ovarian cancer is a malignant tumour of women, with a wide range of pathological (histological) types, and is the primary cause of death from the female reproductive tract[1]. According to global statistics, most ovarian cancers are diagnosed at an advanced stage, and less than 20% of women with advanced ovarian cancer (stages III and IV) are cured[2]. Less than 25% of ovarian cancers are identified at stage I[3], although the 5-year survival for early-stage ovarian cancer is more than 90%[4,5], and most patients can be cured by cytoreductive surgery with no need for chemotherapy or radiotherapy. Hence, early detection of ovarian cancer is very important, as are effective methods for diagnosis and evaluating preoperative risk. CA125 is a widely used tumour marker, but it has low sensitivity for early-stage ovarian cancer and its presence in some benign ovarian masses makes its specificity low. The specificity of CA72-4 is higher than that of CA125, but its sensitivity is lower, thus a combination of both tumour markers provides a better tool for diagnosis of ovarian cancer. Differentiation of ovarian masses is challenging preoperatively. According to Jacobs et al[6], increasing risk of malignancy index (RMI) score, CA125 level, menopausal status, and ultrasound status could be used to evaluate the risks for patients with ovarian tumours and to differentiate between benign and malignant ovarian masses. This study systematically

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analysed CA125, CA72-4, and RMI with the aim of differentiating ovarian cancer from benign ovarian mass. The study also analysed the relationship of these three factors with ovarian cancer status and pathological type for early detection of ovarian cancer.

Methods

Study Design and Population

This was a retrospective study of hospitalised patients presenting with ovarian mass in Jiamusi University First Affiliated Hospital, China from 1 December 2011 to 31 May 2013. All patients had newly diagnosed ovarian mass. Patients with polycystic ovary syndrome, previous oophorectomy, previous related treatment, such as surgery, radiotherapy, chemotherapy, or hormonal therapy, previous diagnosis of ovarian mass, and other related diseases or cancers were excluded. Patients were divided into four groups according to the postoperative pathology report: group A included follicular cyst, corpus luteum cyst, and ovarian cyst, group B comprised chocolate ovarian cyst; group C included benign ovarian tumour; and group D involved malignant ovarian tumour.

Methods and Determination of CA125, CA72-4, and Risk of Malignancy Index

Patients' venous blood samples (2 mL) were collected after 12 hours of fasting. The samples were collected without undergoing any anticoagulation procedures and were left for at least 30 minutes before centrifugation, and were then centrifuged for 20 minutes at 3000 rpm. After centrifugation, the serum was collected and stored in cryovials. In keeping with procedure, if there was any sediment present then re-centrifugation was done. Haemolysis and cell granules were not present. The serum was stored in a freezer at a temperature of 4°C to 5°C and assayed within 24 hours at the Nuclear Medicine Laboratory of the First Affiliated Hospital of Jiamusi University using ECLIA (electrochemiluminescence immunoassay) to check the serum levels of CA125 and CA72-4. The RMI was calculated according to patient's menopausal status, preoperative ultrasound status, and CA125 value as below:

$$RMI = M \times U \times serum\ CA125$$

M stands for menopausal status: 1 point for premenopausal patients or those having menstruation within 1 year of the blood collection; 3 points for postmenopausal patients, those who had had no menstruation for >1 year, those aged >50 years had had a hysterectomy, or those aged >55 years whose last menstruation was not known. U stands for ultrasound status: 1 point was given for the each

of the following criteria: multilocular cysts, solid areas, metastases, ascites, and bilateral lesions. Then we added up the scores and got the U value according to the following criteria: U = 0 if the score was 0; U = 1 if the score was 1; and U = 3 if the score was 2 to 5. Serum CA125 was measured in IU/mL[7]. The respective cut-off values for CA125, CA72-4, and RMI were 35 U/mL, 6 U/mL, and 200.

Statistical Analyses

The Statistical Package for the Social Sciences version 19.0 (IBM Corp., Armonk [NY], US) was used to analyse relevant data. Medians and interquartile ranges were used for determination of the four groups, and non-parametric tests were used to compare the three factors among the four groups. Analysis of the relationship of pathology type and clinical stage to the three factors was done for group D. Specificity, sensitivity, positive predictive value, negative predictive value, and area under curve of the three factors and the combination of the three factors were determined for group D. Kruskal-Wallis test was used to analyse the differences among the four groups. Mann-Whitney test and Wilcoxon W test were used for pairwise comparisons. A p value of <0.05 was considered statistically significant.

Results

A total of 249 patients were included in this study, including 27 in group A, 64 in group B, 116 in group C, and 42 in group D; their respective mean ± standard deviation age was 41.93 ± 13.23, 37.70 ± 6.99, 41.27 ± 12.20, and 53.62 ± 8.45 years. Group D patients' age ranged from 35 to 74 years, with 28 postmenopausal and 14 premenopausal patients. Among these four groups, group D patients were significantly older (p<0.05) and group B patients were significantly younger (p<0.05).

As shown in Table 1, the medians and interquartile ranges of all three markers in group A and group C patients were within the normal ranges. The median CA125 in group B patients (51.15 U/ml) was higher than the normal cut-off value, but those of CA72-4 (3.17 U/mL) and RMI (56.51) were within the normal ranges. In group D patients, their median CA125 (178.7 U/mL), CA72-4 (6.05 U/mL), and RMI (873.2) were all significantly higher than the normal range. Comparison of CA125, CA72-4, and RMI in the four groups showed statistical significance (p<0.05) [Table 2].

Values of CA125 and RMI of group D were significantly higher than the other three groups. CA125

Table 1. Values of CA125, CA72-4, and risk of malignancy index among four groups of ovarian mass*

Group[†]	CA125 (U/mL)	CA72-4 (U/mL)	Risk of malignancy index
Group A	19.46 (11.53-25.51)	2.48 (1.01-5.08)	22.63 (13.07-30.51)
Group B	51.15 (31.91-84.20)	3.17 (1.73-6.12)	56.51 (39.64-101.86)
Group C	14.2 (9.90-23.83)	3.12 (1.43-5.89)	18.45 (11.70-35.55)
Group D	178.7 (53.02-838.33)	6.05 (2.24-22.46)	873.2 (187.32-4095.80)

* Values are shown as median (interquartile range)

† Group A = follicular cyst, corpus luteum cyst, and ovarian cyst; group B = chocolate ovarian cyst; group C = benign ovarian tumour; group D = malignant ovarian tumour

Table 2. Comparison of CA125, CA72-4, and risk of malignancy index among four groups of ovarian mass (using Kruskal-Wallis test)

Factor	Chi-square	Degrees of freedom	p Value
CA125	109.666	3	<0.001
CA72-4	12.135	3	0.01
Risk of malignancy index	109.177	3	<0.001

Table 3. Pairwise comparisons of CA125, CA72-4, and risk of malignancy index among four groups of ovarian mass*

Pairwise comparison*	CA125				CA72-4				Risk of malignancy index			
	Mann-Whitney U	Wilcoxon W	Z	p Value[†]	Mann-Whitney U	Wilcoxon W	Z	p Value[†]	Mann-Whitney U	Wilcoxon W	Z	p Value[†]
Groups A vs. D	109.50	487.50	-5.625	0.000	336.00	714.00	-2.840	0.005	65.00	443.00	-6.172	0.000
Groups B vs. D	696.00	2776.00	-4.186	0.000	950.50	3030.50	-2.542	0.011	389.00	2469.00	-6.169	0.000
Groups C vs. D	430.00	6646.00	-7.772	0.000	1621.50	7837.50	-2.901	0.004	301.00	6517.00	-8.299	0.000
Groups A vs. B	223.00	601.00	-5.569	0.000	675.00	1053.00	-1.642	0.101	273.00	651.00	-5.135	0.000
Groups B vs. C	971.00	7187.00	-7.996	0.000	3386.50	9602.50	-0.513	0.608	1379.00	7595.00	-6.732	0.000
Groups A vs. C	1288.00	7504.00	-1.130	0.26	1307.00	1685.00	-1.028	0.304	1439.50	7655.50	-0.317	0.752

* Group A = follicular cyst, corpus luteum cyst, and ovarian cyst; group B = chocolate ovarian cyst; group C = benign ovarian tumour; group D = malignant ovarian tumour

† $\alpha = 0.0083$; p values of <0.0083 (i.e. p of 0.05 / 6 pairs) had statistical significance

in group B was significantly higher than groups A and C, whereas values of CA72-4 in group D were significantly higher than groups A and C, but no significant differences were shown when compared with group B (i.e. p>0.0083). Comparisons of CA125, CA72-4, and RMI between groups A and C showed no statistical significance (Table 3).

The sensitivity (80.95%) and negative predictive value (95.06%) of CA125, as well as the specificity (95.41%), positive predictive value (75.61%) and diagnosis rate of RMI (95.98%) were highest in group D patients. The sensitivity of CA72-4 was lower for this group, but the specificity was higher than that of CA125. The combination

of the three factors decreased the sensitivity to 42.86%, but increased the specificity to 98.07%. The positive predictive value was higher with the three factors combined than for any single factor alone (81.82%; Table 4).

The area under the receiver operating characteristic curve (ROC AUC) value used to evaluate the relationship of sensitivity and specificity of CA125, CA72-4, and RMI in group D were 0.854, 0.657, and 0.911, respectively, in which the AUC of RMI was the greatest (Table 5 and Fig).

According to International Federation of Gynaecology and Obstetrics (FIGO), Spearman test of the

Table 4. Comparison of sensitivity, specificity, positive predictive value, and negative predictive value in ovarian cancer

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
CA125 (35 U/mL)	80.95 (34/42)	70.97 (154/215)	35.78 (34/95)	95.06 (154/162)	75.50 (188/249)
CA72-4 (6 U/mL)	52.38 (22/42)	79.29 (180/227)	31.88 (22/69)	90.00 (180/200)	81.12 (202/249)
Risk of malignancy index (200)	73.81 (31/42)	95.41 (154/218)	75.61 (31/41)	94.97 (154/219)	95.98 (239/249)
Combination of all markers	42.86 (18/42)	98.07 (203/207)	81.82 (18/22)	89.43 (203/227)	88.76 (211/249)

Table 5. ROC AUC comparisons of the three factors in ovarian cancer

Factor	ROC AUC	(95% Confidence interval)
CA125	0.854	0.787-0.921
CA72-4	0.657	0.556-0.759
Risk of malignancy index	0.911	0.854-0.968

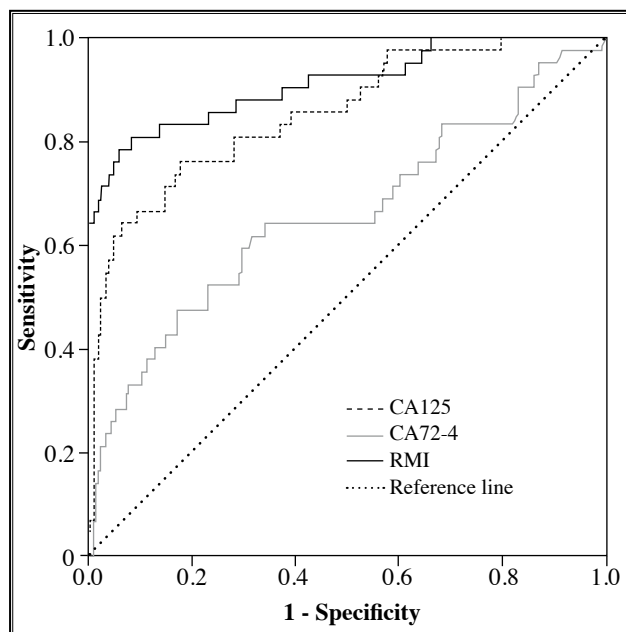Abbreviation: ROC AUC = area under the receiver operating characteristic curve

Figure. Receiver operating characteristic (ROC) curve analysis of CA125, CA72-4, and risk of malignancy index (RMI) for diagnosis of ovarian cancer[*]

[*] Diagonal segments are produced by ties

median of the three factors in different stages of ovarian cancer showed a p value of <0.01, with CA125, CA72-4, and RMI having positive relationships with FIGO clinical stage. According to FIGO clinical stage, the median, interquartile ranges, and positivity rates of CA125, CA72-4, and RMI all increased according to the development of the clinical stage (Tables 6 and 7).

According to the pathological type of ovarian cancer, the median CA125 in epithelial ovarian cancer was 210.00 U/mL, and that the value was highest in serous cystadenocarcinoma (422.05 U/mL). The median CA72-4 in epithelial ovarian cancer was 6.63 U/mL, and that the value was highest in clear cell carcinoma (20.99 U/mL). The median RMI of epithelial ovarian cancer was 786.87, and that the value was highest in serous cystadenocarcinoma (2051.40) and for clear cell carcinoma it was 305.10. The median values of CA125, CA72-4, and RMI in some types of non-epithelial ovarian cancer were higher than the normal range (Table 8).

Discussion

Ovarian cancer is the seventh most common cause of cancer death for woman worldwide[8]; according to the Global Burden of Disease Study in 2010, about 160,000 women died from ovarian cancer, up from 113,000 in 1990[9]. In the US, about 1.7% to 2.5% (1 in every 40 to 60) women have the possibility of developing ovarian cancer. The risk is greater among elderly women[10]. In 2010, a survey found that 21,880 women were diagnosed ovarian cancer, with 13,850 deaths[11]. The risk increases with age, and decreases as the number of pregnancies has increased[12]. The lifetime risk is approximately 1.6%, but the risk increases to 5% for women with a first-grade relative who has had ovarian cancer. Survival dramatically increases for women who are diagnosed at an early stage; however, about 70% of cases are diagnosed at stages III or IV, with greatly decreased 5-year survival rates[13].

Table 6. Values and positivity of CA125, CA72-4, and RMI in ovarian cancer by FIGO stage

FIGO stage	CA125		CA72-4		RMI	
	Median (interquartile range)	Positivity	Median (interquartile range)	Positivity	Median (interquartile range)	Positivity
I (n=10)	24.26 (18.35-231.03)	4 (40%)	2.02 (1.09-5.02)	2 (20%)	59.40 (19.81-693.75)	5 (50%)
II (n=10)	77.85 (59.20-428.83)	9 (90%)	6.05 (2.48-19.41)	6 (60%)	577.26 (187.32-1434.52)	7 (70%)
III and IV (n=20)[*]	609.50 (155.50-2186.00)	20 (100%)	13.41 (4.84-42.19)	13 (65%)	3313.50 (720.78-8896.50)	19 (95%)
Unstaged (n=2)[†]	-	1/2	-	0/2	-	1/2
Total (n=42)	178.7 (53.02-838.33)	34 (80.95%)	6.05 (2.24-22.46)	21 (50.00%)	873.2 (187.32-4095.80)	32 (76.19%)

Abbreviations: FIGO = International Federation of Gynaecology and Obstetrics; RMI = risk of malignancy index

[*] Since there are only two cases of stage IV ovarian cancer, we combined stages III and IV, which is considered to be 'late stage'

[†] Two cases of ovarian cancer were unstaged, and were not put into test analysis in this comparison

Table 7. Relativity of CA125, CA72-4, and RMI and ovarian cancer by FIGO stage

	Spearman Rs[*]	p Value
CA125 (n=40)	0.629	<0.001
CA72-4 (n=40)	0.472	0.001
RMI (n=40)	0.629	<0.001

[*] When the confidence level (one-sided) was 0.01, the relativity was significant

FIGO = International Federation of Gynaecology and Obstetrics; RMI = risk of malignancy index

Effective methods for diagnosis and evaluating preoperative risk, such as tumour markers, imaging, and consideration of risk factors are important. CA125 is a widely used tumour marker, but it has low sensitivity in the early stages of ovarian cancer and its presence in some women with benign ovarian masses reduces its specificity.

It is recommend that women older than 30 years should have a physical examination every year and, if ultrasound shows a mass, they should have their serum tumour markers checked and undergo further investigation to evaluate the risk of developing ovarian cancer. CA125 and CA72-4 are widely used in clinical practice. Jacobs et al[6] proposed an evaluation tool for ovarian cancer in the form of the RMI, which uses menopausal status, ultrasound status, and CA125 level to calculate the risk of cancer in a patient with an ovarian mass.

This research systematically analysed CA125, CA72-4, RMI, and a combination of the three factors in order to distinguish ovarian cancer from benign ovarian mass, as well as to analyse the relationship of the three factors with ovarian cancer status and pathological type for early detection of ovarian cancer. The serum level of CA125 in chocolate ovarian cyst patients (group B) was higher than the normal range, while CA72-4 and RMI levels were within the normal range. The CA125 range could be used to differentiate ovarian chocolate cyst (31.91-84.20 U/mL) from ovarian cancer (53.02-838.33 U/mL), with their respective medians being 51.15 U/mL and 178.7 U/mL (p<0.01). From this study, for patients with an ovarian mass presenting with high CA125, a combination of CA72-4 and RMI could be used; if they were both within the normal range, it was highly likely that the ovarian mass was a chocolate ovarian cyst, and ovarian cancer could be excluded and evaluated as low risk (p<0.01).

We can evaluate the risk of cancer for patients with ovarian mass using this method. According to the RMI, preoperative evaluation can assign women into high-risk and low-risk groups, with RMI of >200 being high risk for ovarian cancer when CA125 and CA72-4 were higher than the normal range. For ovarian cancer patients, RMI specificity was 95.41%, the positive predictive value was the highest at 75.61%, and the diagnostic rate being the highest at 95.98%. CA125 sensitivity was the highest at 80.95%, with negative predictive value also being the highest at 95.06%. Regarding CA72-4, its sensitivity was lower than that of CA125, yet its specificity being higher. The combination of the three factors could increase the

Table 8. Values and positivity of CA125, CA72-4, and risk of malignancy index according to ovarian cancer pathology

Histological classification	CA125	
	Median (interquartile range)	Positivity
Epithelial ovarian cancer		
Serous cystadenocarcinoma (n=24)	422.05 (77.85-1901.50)	21 (87.5%)
Mucinous cystadenocarcinoma (n=3)*	56.74	2 (66.7%)
Endometrioid adenocarcinoma (n=1)*	64.34	1 (100%)
Clear cell carcinoma (n=7)	120.20 (26.81-295.50)	5 (71.4%)
Overall (n=35)	**210.00 (57.56-1000.33)**	**29 (82.9%)**
Sex cord (stromal)		
Granular cell tumour (n=1)*	10.98	-
Thecoma (n=2)*	483.75	2/2
Fibroma (n=1)*	1066.00	1/1
Overall (n=4)	**167.50**	**3/4**
Germ cell		
Malignant teratoma (n=1)*	17.55	-
Metastatic ovarian cancer (n=2)*	138.67	2/2

* Interquartile ranges were not stated in view of too few cases for analysis

specificity to 98.07% and the positive predictive value to 81.82%, while the sensitivity was decreased.

Using the normal range to ascertain the ROC AUC of CA125, CA72-4, and RMI in ovarian cancer, the ROCs were 0.854, 0.657, and 0.911, respectively. The ROC AUC of the RMI was the greatest, with the highest diagnosis rate meaning that RMI is the most effective value for evaluating the risk of ovarian cancer. This information will help to decide such factors as the tests needed, whether to move the patient to a specialised hospital, the best treatment plan, and the best time for treatment, thus obtaining the best treatment outcome and prognosis.

This study used Spearman's correlation to analyse the relationship between FIGO clinical stage and the three factors, and the results were statistically significant. The correlation was positive for CA125, CA72-4, and RMI and increased with the development of each clinical stage. Therefore, using the three factors, we can evaluate the risk of ovarian cancer, the prognosis, and the 5-year survival rate. This method enables risk evaluation and appropriate treatment of patients with ovarian mass.

In conclusion, in a comparison of CA125, CA72-4, and RMI, RMI was the better tool to distinguish benign and malignant ovarian mass, and can help in evaluating the risk of cancer for patients with ovarian mass. The combination of CA125, CA72-4, and RMI can be used to better evaluate the risk of ovarian cancer, especially epithelial ovarian cancer, for early detection and prognosis.

Declaration

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CA72-4		Risk of malignancy index	
Median (interquartile range)	**Positivity**	**Median (interquartile range)**	**Positivity**
7.74 (4.71-24.91)	15 (62.5%)	2051.40 (423.90-7508.25)	20 (83.3%)
2.49	-	170.22	1 (33.3%)
0.20	-	193.02	-
20.99 (1.87-44.76)	4 (57.1%)	305.10 (120.20-2034.90)	5 (71.4%)
6.63 (2.45-25.39)	**19 (54.3%)**	**786.87 (205.09-4698.60)**	**26 (74.3%)**
2.64	-	32.94	-
16.71	-	4353.75	1/2
5.01	-	3198.00	1/1
2.64	**-**	**1507.50**	**2/4**
1.95	-	17.55	-
6.02	1/2	1248.03	2/2

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