Clinical value of combining transvaginal contrast-enhanced ultrasonography with serum human epididymisprotein-4 and the resistance index for early-stage epithelial ovarian cancer

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ABSTRACT

الأهداف: لزيادة دقة الكشف والتشخيص التفريقي لسرطان المبيض الظهاري المبكر (EOC) والتصوير السوناري المتباين عبر المهبل (TVCEUS) بالجمع بين مصل البربخ البشري 4 (HE4) ومؤشر المقاومة (RI).

الطريقة: هذه دراسة حالات مراقبة بأثر رجعي شملت 230 مريضاً يعانون من أورام المبيض في قسم أمراض النساء والتوليد، مستشفى تشونغنان، جامعة ووهان، ووهان، الصين بين يونيو 2008 وسبتمبر 2015. قبل العمل على 110 حاله تعاني من EOC (المجموعة أ) و 120 حاله مرضى يعانون من ورم المبيض الحميد (المجموعة ب) لاحظنا وحسبنا مقياس التشكل للموجات الصوتيه المتباينه للاورام الوعائية ومنحنى كثافة الوقت/ الشدة (TIC) والتعزيز التباييني للموجات فوق الصوتيه و كلا من HE4 و RI وتمت مقارنة النتائج مع نتائج التحليل التشريحي المرضي.

النتائج: كانت نتائج التشكل بالموجات فوق الصوتيه، ومعدل تعزيز كثافة الذروة (PI) مع معدل التعزيز (ER) مع مؤشرات TIC و HE4 اعلى في المجموعة ب مقارنة بالمجموعة أ وكان RI أقل من المجموعة ب. حُددت معدلات الكشف لجميع المؤشرات في المجموعات الحميدة والخبيثة و تمت مقارنتها مع النتائج النسيجية وكانت فروق معدل الكشف HE4 p=0.001، U p=0.001، PI p=0.001، ER p=0.001 RI p=0.001 وكانت جميعها ذات دلالة إحصائية (p<0.05).

الخاتمة: القيمة السريرية العالية من خلال كشف TVCEUSو HE4 و RI يمكن أن تزيد من حساسية التشخيص والتشخيص التفريقي لسرطان المبيض الظهاري المبكر.

Objectives: To increase accuracy of the detection and differential diagnosis of the early epithelial ovarian cancer (EOC) with transvaginal contrast-enhanced ultrasonography (TVCEUS) combining serum human epididymisprotein 4 (HE4), and resistance index (RI).

Methods: This retrospectively case-control study of 230 patients with ovarian tumors were reviewed at the Department of Gynecology and Obstetrics, Zhongnan Hospital, Wuhan University, Wuhan, China between June 2008 and September 2015. Before the operation of 110 cases with EOC (Group A) and 120 cases of patients with benign ovarian tumor (Group B), we observe and calculate both Groups' tumor vascular contrast-enhanced ultrasonography morphology scores (U), time-intensity curve (TIC) of contrast-enhanced ultrasonography, HE4, and RI. Results were compared with the histopathological analysis results.

Results: The ultrasonography morphology scores, peak intensity (PI) enhancement rate (ER) with the parameters of the TIC and HE4 are higher in Group A compared with patients in Group B and the RI was lower than Group B. The detection rates for all indexes in the benign and malignant groups and their comparisons to the histopathological results were determined. The detection rate differences for HE4 (p=0.001), RI (p=0.001), U (p=0.001), PI (p=0.001), and ER (p=0.001) were all statistically significant (p<0.05).

Conclusion: The high clinical value through combined TVCEUS, HE4, and RI detection can increase the sensitivity of the diagnosis and differential diagnosis of the early EOC.

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Ovarian tumors are common in the female reproductive system. Malignant ovarian tumors are one of 3 major tumor types that affect the female reproductive system with a high mortality rate.[1] A lack of effective screening programs has resulted in few early stage diagnoses of ovarian cancers (approximately 25% of patients).[2] In most such cases, the disease is surgically curable, and the 5-year survival rate for early stage (stages I or II) ovarian cancer is approximately 90%.[3] Ovarian cancer primarily comprises epithelial ovarian cancer (EOC). Therefore, the EOC diagnosis in its early stage has become key to its successful treatment. There are multiple techniques for the diagnosis of ovarian cancer, but the current qualitative methods are imperfect. Utilizing the existing diagnostic methods to improve the accuracy rates for the diagnosis of EOC and thereby provide early treatment is the key to improve the prognosis. Therefore, efforts to increase the quality of the early stage ovarian cancer diagnosis with the goal of improving patient prognoses will be clinically significant. Serum diagnostic methods for clinically screening early stage ovarian cancers involve the use of serum tumor markers. Carbohydrate antigen-125 (CA125) failed to have an ideal predictive power.[4] Of the 80% of ovarian cancer patients who are diagnosed with EOCs, approximately 50% have the early stage disease.[5] Moreover, the sensitivity and distinctness of CA125 is unacceptable for population screening to detect early-stage ovarian cancers.[6]

Human epididymisprotein 4 is detectable in the serum from patients with ovarian cancers and in ovarian cancer cell supernatant tissue.[7] It is a novel and specific biomarker for ovarian cancer. It is a promising marker for improving the sensitivity and specificity of detection, and it has been suggested as a tumor marker for the diagnosis of early stage ovarian cancers.[8] The RI reflects the intra-tumoral micro-vessel density and is a parameter that is commonly measured by ultrasonography. Contrast-enhanced ultrasonography (CEUS) has recently and gradually been applied to the diagnosis and identification of benign diseases of the kidney, pancreas, and breast, but there have been few reports regarding its diagnostic applications towards adnexal masses.[9] This study assesses the clinical value of a combined detection method that utilizes TVCEUS, HE4, and RI for early-stage EOCs.

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Methods. Two hundred thirty patients with ovarian tumors, ages 15-70 (average age of 50.5±8.7 years), from the Department of Gynecology and Obstetrics, Zhongnan Hospital, Wuhan University, Wuhan, China between June 2008 and September 2015, were selected, including 104 with EOCs, 6 with borderline ovarian epithelial tumors, and 120 with benign ovarian tumors.

The inclusion and exclusion criteria included the following: 1) no patients were pregnant or menstruating; 2) patients had no other malignant tumors; 3) patients had not received prior radiotherapy or chemotherapy; 4) patients exclusively had primary tumors; and 5) definitive post-surgical diagnoses were made by histopathological examination. The clinical stages were classified using the FIGO standards, and the histopathological classification was subject to the WHO standards. Because a borderline tumor features a high potential for malignancy, caution before, during and after surgery was essential. Therefore, such a tumor was classified into the malignant groups as previously described.[10] This study was conducted according to the principles of the Helsinki Declaration.

For medical examinations with the serum tumor marker human epididymisprotein 4 (HE4) >150 pmol/L was determined as positive.[11] The conventional transvaginal color Doppler ultrasonic examination was carried out, and the RI threshold was calculated as RI <0.6, which was determined as positive. The CEUS software (Q-Analysis, General Electric, Fairfield, USA) was used to determine the tumor morphology score, to observe vessel enhancement, and for the quantitative analysis of the CEUS time-intensity curve (TIC) parameter threshold. The ultrasonic diagnostic apparatus, which was manufactured by GEE9 (General Electric E9, Fairfield, USA) was used; the probe (C1-5; mechanical index, 0.11) frequency for the abdominal exam was 3.5-5 MHz, and the probe (C5-9; mechanical index, 0.07) frequency for the vaginal exam was 5-9 MHz. Sulfur hexafluoride (SF6; SonoVue, Bracco Corporate, Italy) was used as the contrast agent, with a mean microbubble diameter of 2.5 μm for the phospholipid microcyst. The RI was calculated using the blood vessel distribution inside and on the surface of the tumor by transvaginal color Doppler ultrasonography. A RI <0.6 was considered positive, and a RI >0.6 was considered negative. For the CEUS-based imaging examinations, the CEUS tumor vascular enhancement pattern was observed and compared to the pattern that was obtained by contrast-enhancement computer tomography (CT, Siemens, Munich, Germany) and magnetic resonance (MR, General Electric, Fairfield, USA). The scores for

the CEUS-based enhancement of the tumor vessels were then determined. The quantitative analysis was performed for the TIC parameters when U >7. For the calculation of the TIC parameters, positivity was determined at peak intensity (PI) ≤48.65 dB and enhancement rate (ER) = (PI-AI/PT-AT) >1.56 .

Statistical analyses of the results were conducted using SPSS version 19.0 (SPSS, Statistical Product and Service Solutions Inc., Chicago, IL, USA) to perform t-tests for equal variance, rank sum test for no equal variance and calculate Kappa, 95% confidence interval (CI) and area under the curve (AUC). Consistency with the histopathological results was confirmed as follows: Kappa <0.4, a poor result; ≤0.4 Kappa <0.75, a good result; Kappa ≥0.75, an excellent result. When the AUC decrease between 0.5 and 1.0, the result was considered good. A $p<0.05$ was determined to be statistically significant for all analyses.

Results. The detection rates for all indexes in the benign and malignant groups and their comparisons to the histopathological results were determined. The detection rate differences for HE4 ($p=0.001$), RI ($p=0.001$), ultrasonography morphology scores (U) ($p=0.001$), PI ($p=0.001$), and ER ($p=0.001$) were all statistically significant ($p<0.05$) (**Table 1**). The CEUS-based enhancement of the tumor blood vessel morphology was highly consistent with the CECT/CEMR. The Kappa values for HE4, RI, and U exceeded 0.60 and were highly consistent with the histopathological results. The AUC for HE4 was equal to 0.872 and higher than that of RI, U, PI, and ER, which was highly consistent with the pathological results (**Table 2**).

Table 1 - Quantitative analysis of the TIC parameters for TVCEUS, HE4, RI, and U in benign and malignant groups.

TIC parameters	Benign group (n=120)	Malignant group (n=110)	*P*-value
AT (s)	13.53 ± 2.92	13.53 ± 2.29	0.999
AI (dB)	-66.65 ± 1.91	-66.50 ± 1.59	0.520
PI (dB)	-46.80 ± 2.60	-42.19 ± 6.46	0.001
ER (dB/s)	1.67 ± 0.51	2.28 ± 0.72	0.001
HE4	41.31 ± 7.64	516.61 ± 27.12	0.001
RI	0.62 ± 0.12	0.39 ± 0.08	0.001
U	4.17 ± 1.09	9.53 ± 1.65	0.001

TIC - time-intensity curve, CEUS - contrast-enhanced ultrasonography, HE4 - human epididymis protein 4, RI - resistance index, AT - arrival time, AI - arrival intensity, PI - peak intensity, ER - enhancement rate, U - vascular contrast-enhanced ultrasonography morphology score

Table 2 - Comparison of detection rates of the HE4, RI, U, and TVCEUS parameters with histopathological examination results.

Variables	Histopathological types		Kappa (95% confidence interval)	AUC
	Benign (n=120)	Malignant (n=110)		
HE4 (pmol/L)			0.608 (0.505-0.711)	0.872
≤150	97 (80.8)	22 (20.0)		
>150	23 (19.2)	88 (80.0)		
RI			0.564 (0.458-0.671)	0.747
≤0.6	25 (20.8)	85 (77.3)		
>0.6	95 (79.2)	25 (22.7)		
U			0.520 (0.410-0.630)	0.757
≤7	94 (78.3)	29 (26.4)		
>7	26 (21.7)	81 (73.6)		
CEUS parameters				
PI			0.518 (0.408-0.628)	0.803
≤50	100 (83.3)	35 (31.8)		
>50	20 (16.7)	75 (68.2)		
ER			0.537 (0.428-0.646)	0.746
≤1.56	98 (81.7)	31 (28.2)		
>1.56	22 (18.3)	79 (71.8)		

HE4 - human epididymis protein 4, RI - resistance index, CEUS - contrast-enhanced ultrasonography, U - vascular contrast-enhanced ultrasonography morphology score, PI - peak intensity, ER - enhancement rate, CI - Confidence Interval, AUC - area under curve

The detection rates of all combined indexes (all positive were positive, all negative were negative, combined positive and negative were positive) in the benign and malignant groups and their comparisons with histopathological results were also determined. For the examination of the HE4 and RI or U combinations, the Kappa value exceeded 0.55, with histopathological consistency and clinical values exceeding those from the single examinations with RI, U, PI, and ER. The AUC values for HE4+RI+U was 0.887 and HE4+RI+U+PI+ER was 0.888, with pathological consistency and clinical values exceeding those from the single examinations with RI, HE4, U, PI, and ER (**Table 3**).

Diagnostic efficiency comparisons were made for all indexes of the single and combined examinations of the benign and malignant groups. The sensitivity and accuracy results for HE4 were highest in the single examinations; its specificity was also high. The specificity for PI was highest, but its sensitivity and accuracy were low. For the combined examinations with HE4 and RI or U, or PI, or ER, the sensitivity was higher than the four indexes in the single examination;

but the specificity decreased with the rise in sensitivity (**Table 4**).

Predictive values for epithelial ovarian cancers of different stages and tissue types with different indexes were evaluated. The sensitivities of HE4, RI, U, PI and ER for stage III and IV EOCs were higher than those for stage I and II EOCs. The sensitivities of HE4, RI, and ER for serous cystadenocarcinoma (SCCa) were higher than those for mucinous cystadenocarcinoma (MCCa). The sensitivity of PI for MCCa was higher compared with SCCa. The combined examinations with CEUS, HE4, and RI increased the sensitivity for stage I and II EOCs, serous cystadenocarcinomas (**Table 5**). (Due to poorly differentiated adenocarcinoma [PDACa], endometrial carcinoma [ECa], clear cell carcinoma [CCCa] have less sample size, no statistical significance).

Table 3 - Comparison of detection rates of combined indexes to histopathological examination results.

Variables	Pathological pattern		Kappa (95% confidence interval)	AUC
	Benign (n=120)	Malignant (n=110)		
HE4+RI			0.610 (0.509-0.712)	0.885
(-)	90 (75.0)	15 (13.6)		
(+)	30 (25.0)	95 (86.4)		
HE4+RI			0.559 (0.453-0.664)	0.864
(-)	85 (70.8)	16 (14.5)		
(+)	35 (29.2)	94 (85.5)		
RI+U			0.557 (0.449-0.664)	0.755
(-)	92 (76.7)	23 (20.9)		
(+)	28 (23.3)	87 (79.1)		
HE4+RI+U			0.578 (0.476-0.694)	0.887
(-)	81 (67.5)	10 (9.1)		
(+)	39 (32.5)	100 (90.9)		
HE4+RI+U+PI+ER			0.545 (0.443-0.647)	0.888
(-)	75 (62.5)	8 (7.3)		
(+)	45 (37.5)	102 (92.7)		

HE4 - human epididymis protein 4, RI - resistance index, CEUS - contrast-enhanced ultrasonography, U - vascular contrast-enhanced ultrasonography morphology score, PI - peak intensity, ER - enhancement rate, CI - Confidence Interval, AUC - area under curve

Discussion. Since the EOC is located deep within the pelvic cavity and due to its early-stage EOC patients do not experience clinical symptoms, the EOC is not

Table 4 - Comparison of detection rates of combined indexes with histopathological examination results (%).

Examination	Sensitivity (n=120)	Specificity (n=110)	Accuracy
HE4	80.0 (71.0-86.8)	80.8 (72.4-87.2)	80.4
RI	77.3 (68.1-84.5)	79.2 (70.6-85.8)	78.2
U	73.6 (64.2-81.4)	78.3 (69.7-85.1)	76.1
PI	68.2 (58.5-76.6)	83.3(75.2-89.3)	76.1
ER	71.8(62.3-79.8)	81.7(73.3-87.9)	77.0
HE4+RI	86.4 (78.2-91.9)	75.0 (66.1-82.3)	80.4
HE4+U	85.5 (77.2-91.2)	70.8 (61.7-78.6)	77.8
RI+U	79.1 (70.1-86.0)	76.7 (67.9-83.7)	77.8
HE4+RI+U	91.0 (83.5-95.3)	67.5 (58.3-75.6)	78.7
HE4+RI+U+PI+ER	92.7 (85.7-96.6)	62.5 (53.2-71.0)	77.0

HE4 - human epididymis protein 4, TIC - time-intensity curve, RI - resistance index, CEUS - contrast-enhanced ultrasonography, PI - peak intensity, ER - enhancement rate

Table 5 - Predictions of combinations of HE4, RI, U, PI and ER for different stages and histopathological types.

Examination	Stages		Histopathlogical types				
	I-II (n=48)	III-IV (n=62)	SCCa (n=59)	MCCa (n=31)	PDACa (n=10)	ECa (n=7)	CCCa (n=3)
HE4	31 (64.6)	57 (91.9)	52 (88.1)	26 (80.6)	5 (50.0)	3 (42.9)	2 (66.7)
RI	29 (60.4)	56 (90.3)	49 (83.1)	23 (74.2)	7 (70.0)	4 (57.1)	2 (66.7)
U	26 (54.2)	55 (88.7)	43 (72.9)	24 (77.4)	6 (60.0)	4 (57.1)	3 (100.0)
PI	24 (50.0)	51 (82.3)	41 (69.5)	26 (83.9)	3 (30.0)	2 (28.6)	1 (33.3)
ER	23 (47.9)	56 (90.3)	8 (81.3)	21 (67.7)	6 (60.0)	2 (28.6)	1 (33.3)
HE4+RI	35 (72.9)	60 (96.7)	54 (91.5)	27 (87.1)	7 (70.0)	4 (57.1)	2 (66.7)
HE4+U	36 (75.0)	58 (93.5)	52 (88.1)	27 (87.1)	7 (70.0)	5 (71.4)	3 (100.0)
RI+U	34 (70.8)	53 (85.5)	49 (84.7)	24 (83.9)	7 (70.0)	4 (57.1)	3 (100.0)
HE4+RI+U	40 (83.3)	60 (96.8)	54 (91.5)	29 (93.5)	8 (80.0)	5 (71.4)	3 (100.0)
HE4+RI+U+PI+ER	42 (87.5)	60 (96.8)	55 (93.2)	30 (96.8)	8 (80.0)	5 (71.4)	3 (100.0)

HE4 - human epididymis protein 4, RI - resistance index, U - vascular contrast-enhanced ultrasonography morphology score, PI - peak intensity, ER - enhancement rate. SCCa - serous cystadenocarcinoma, MCCa - mucinous cystadenocarcinoma,PDACa - poorly differentiated adenocarcinoma, ECa - endometrial carcinoma, CCCa - clear cell carcinoma

easily detectable. Ultimately, 7 of every 10 patients progress to end-stage disease.[12] Epithelial ovarian cancer treatments are based around a combination of surgery and chemotherapy.[2] The post-treatment 5-year survival rate was over 90% for patients with stage I malignant ovarian tumors and was approximately 70% for the patients with stage II tumors; these numbers are clearly higher than those for patients with stage III and IV tumors (<30%).[13] Therefore, an early diagnosis of EOC is vital. The realization of an early diagnosis remains a major challenge for gynecologists and other physicians.

The advantages of CEUS are its safety (no allergic reactions), real-time performance, and low cost (no CT/MRI inspection fees, relatively low inspection fees). Moreover, examinations can be conducted in the hospital for numerous projects, and it can be used as a screening tool. As imaging technology has developed, it has become important to increase the sensitivity of the captured signal so the images ensure a fully observable tissue perfusion throughout the phase changes. Contrast-enhanced ultrasonographyis particularly advantageous when CT\MRI-based imaging cannot produce a high time resolution of the whole tumor. Furthermore, its operability and real-time performance are its major advantages. Contrast-enhanced ultrasonography is also known as acoustic contrast. It reflects blood perfusion in tissues, which increases the accuracy of the ultrasound-based diagnosis.[14] The CEUS-based enhancement of the tumor's microvasculature results in an improved tumor detection rate. The quantitative analysis of the parameters for the ovarian tumor TVCEUS time intensity curves, PI values, and ER values showed statistically significant differences between the benign and malignant groups.[15] Fleischer et al reported a similar result, but Testa et al has no report during the study.[16-18] The analysis indicated that malignant lesions were associated with large differences in arrival times, which suggests that individual differences may affect the arrival time. When a single tumor U was used, the sensitivity values for the tumor TVCEUS TIC parameters and the PI and ER detection of the benign and malignant tumors were 73.6%, 68.2%, and 71.8%, respectively, and the specificity values were 78.3%, 83.3%, and 81.7%, respectively; the sensitivity was not high.

Abnormal new blood vessel growth is the pathological basis for color Doppler ultrasound blood flow imaging.[19] Per the theory of tumor blood vessel formation, a reduced blood flow resistance may be an early sign of the ovarian cancer lesion.[20] Therefore, a low resistance to blood flow may indicate a malignant tumor. There are no uniform, worldwide diagnostic criteria for RI. Multiple scholars believe that a RI <0.6 is the threshold for an ovarian cancer diagnosis that would reduce the false positivity rate;[21] RI <0.6 was determined to be positive for this study. In the ultrasound's diagnostic sensitivity of the early stage ovarian cancer group, the sensitivity was low at 77.3%. In this study, HE4: 150 pmol/L was the critical value, and the HE4 sensitivity was 80%, specificity was 80.8%, and accuracy was 80.4%. This was consistent with data reported by Shah et al,[22] who indicated that if the HE4 ovarian cancer susceptibility was sufficiently enough, it would result in a misdiagnosis. Additionally, the HE4 levels in endometrial cancer were increased,[23] which showed that the use of HE4 as the sole diagnostic indicator of early ovarian cancer has limitations.

The results show that each single method has limitations for assessing ovarian cancer because each is prone to a misdiagnosis and failure to detect the early stage disease. When U, HE4, and RI were combined for detection, the sensitivity was 91%, and when the U, PI, ER and HE4 were combined, the RI joint detection sensitivity was 92.7%.[24] The combination improves the detection accuracy and decreases the false positivity rate.[25] Our retrospective study is the first to increase the sensitivity of the early detection and differential diagnosis of EOC with TVCEUS combined with HE4 and RI.

Study limitations. The small sample size (n=230) may pose limitations to an accurate statistical evaluation of the results. Moreover, we did not compare the diagnostic results of the general imaging tools with those of the TVCEUS/HE4 and RI combination, which could distinguish the benign and malignant ovarian tumors.

In conclusion, combined detection can improve accuracy, decrease the false positivity rate, determine a differential diagnosis at an earlier time and prolong the survival time by 5 years. It has a broad practical and clinical value. However, further studies using a larger sample size and diverse ethnic populations are necessary to fully confirm our findings.

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