The mathematical models of serum HE4 and CA125 combined application to improve the pelvic tumor differential diagnosis rate

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Abstract. OBJECTIVE: The objective of this study was to explore the combined usage of human epididymis protein 4 (HE4) and CA 125 to help the diagnosis and differentiating malignant pelvic masses from benign diseases.
METHOD: This was a multicenter clinical study and nine hospitals had been involved and totally 779 patients with pelvic mass have been enrolled. The pathologic diagnosis confirmed that 288 patients suffered EOC. Serum levels of HE4 and CA125 were measured.
RESULTS: The distributions of HE4 and CA125 have been analyzed and the ROMA score have been calculated. Then three new calculation formulas have been compared and the best one have been used to evaluate its clinical significance. The specificity of this diagnosis model ( ) to prediction the risk of cancer and staging have reached over 75%.
CONCLUSION: The application of mathematical model in combined analysis multiple biomarkers could improve the clinical significance and proved more accurate laboratory information for clinical doctors.

Keywords: pelvic masses, epithelial ovarian cancer(EOC), Human epididymis protein 4 (HE4), CA125

1 Introduction

Human epididymis protein 4 (HE4) have been found in the early 1990s [1] and the clinical studies confirmed that was one of the promising new tumor biomarkers to help the diagnosing epithelial ovarian cancer (EOC) [2]. The further studies also showed that HE4 together with CA125 to calculating the risk of ovarian malignancy algorithm (ROMA) score could improve the diagnostic sensitivity and specificity [3]. ROMA also could improve the discriminative potential of HE4 in differential diagnosis of pelvic tumors [4]. Our previous multicenter study suggested that serum HE4 and CA125 were not only related with menopausal status, it also have an obvious age related variation, especially at 70 years point (the data under publishing). So in this study, the different mathematical model have been calculated and evaluated based on 70 years age point which could improve the risk stratification ability obviously.

2 Material and method

Nine hospitals have been involved in this study and 779 patients with pelvic mass have been enrolled. The gold standard pathologic diagnosis confirmed that 288 patients suffered EOC. Almont this group of patients, the age of 38 patients were over 70 years old. According to the principle of Statistics, there were 776 patients with pelvic mass have been involved in the mathematical model studies.

All blood samples were analyzed by Roche Elecsys Cobas 601 platform according the Standard operating procedures. If the result was over the upper limitation, the sample was diluted (1:20) and re-tested.

3 Results

The patient's clinical information had been showed as in Table 1. The pathological diagnostic results and stage for EOC are also listed.

Table 1a the pathological diagnostic results and stage(pre-menopausal)

Menopausal Status:	*Stage:*	*n*	*Mean*	*Std*	*max*	*q3*	*median*	*q1*	*min*
pre-menopausal	I	21	247.57	466.47	2090.0	165.53	86.12	48.950	31.05
pre-menopausal	II	14	351.67	461.77	1783.0	438.10	159.30	87.740	50.96
pre-menopausal	III	38	372.89	425.33	2030.0	623.20	167.20	75.830	32.09
pre-menopausal	IV	12	494.87	429.60	1341.0	886.85	349.65	150.515	46.34
pre-menopausal	unknown	28	139.81	217.14	1118.0	136.63	54.53	43.140	31.43
pre-menopausal	**all**	**113**	**302.17**	**407.42**	**2090.0**	**354.50**	**109.20**	**53.300**	**31.05**

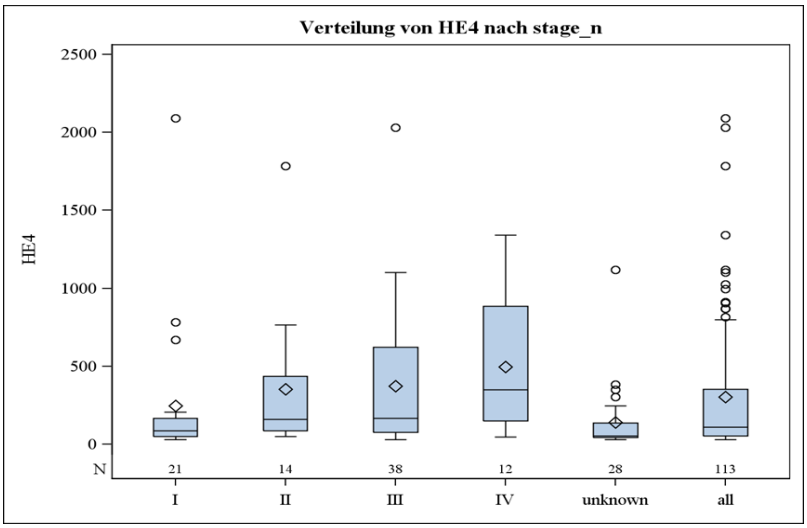

Fig. 1a The HE4 level in different cancer stage(pre-menopausal)

Table 1b . the pathological diagnostic results and stage(post-menopausal)

Menopausal Status:	*Stage:*	*n*	*Mean*	*Std*	*max*	*q3*	*median*	*q1*	*min*
post-menopausal	I	12	163.67	117.46	488.5	191.00	126.20	97.460	39.98
post-menopausal	II	23	432.30	522.64	1702.0	763.20	116.30	66.490	37.08
post-menopausal	III	83	713.03	800.48	4603.0	985.40	419.00	192.600	34.44
post-menopausal	IV	23	903.76	1039.56	3844.0	1393.00	434.20	281.200	43.90
post-menopausal	unknown	33	454.30	577.62	2084.0	450.70	174.80	75.310	41.90
post-menopausal	**all**	**174**	**614.18**	**760.04**	**4603.0**	**878.00**	**305.10**	**110.100**	**34.44**

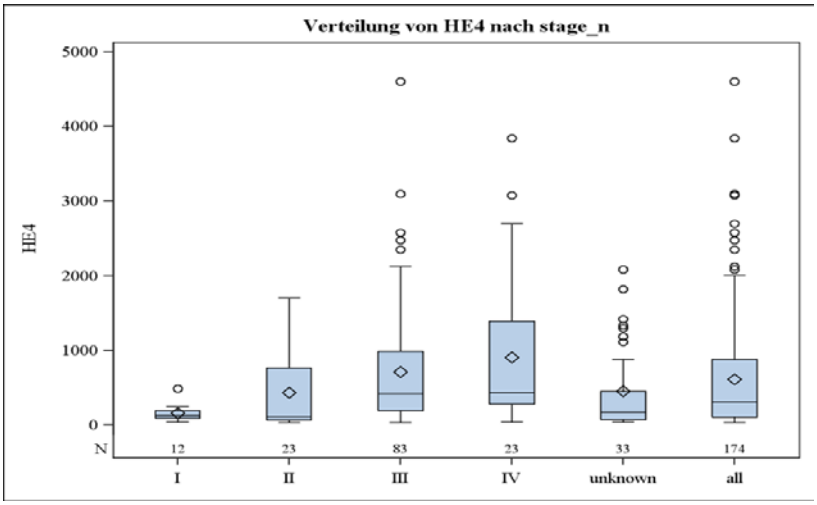

Fig. 1b The HE4 level in different cancer stage(post-menopausal)

According to the previous studies, the ROMA score have been calculated and the data showed as table 2a and 2b. This data suggested that ROMA could be slightly improving the clinical significance of HE4.

Table 2a. The ROC of ROMA score and HE4, CA125(pre-menopausal)

Items	ROC	SE	P	The 95% confidence interval	
				Upper limit	Lower limit
HE4	0.835	0.031	0.000	0.774	0.896
CA125	0.829	0.030	0.000	0.769	0.888
ROMA	0.837	0.031	0.000	0.775	0.898

Table 2b The ROC of ROMA score and HE4, CA125(post-menopausal)

Items	ROC	SE	P	The 95% confidence interval	
				Upper limit	Lower limit
HE4	0.892	0.019	0.000	0.854	0.929
CA125	0.906	0.017	0.000	0.872	0.940
ROMA	0.917	0.017	0.000	0.884	0.950

For exploring the further improvement of HE4 and CA125, According to the original literatures and previous analysis, the HE4 and CA125 value have been converted, the conversion rules was as follow:

- **Rule one:** To convert HE4 and CA125 original value by natural log transformation;
- **Rule two:** To convert HE4 and CA125 original value by reciprocal transformation;
- **Rule three:** To convert HE4 original value by reciprocal transformation and CA125 measured values by the square root transformation.

Predictive index calculated as the following formula:

$$pr = \frac{\exp(PI)}{1+\exp(PI)} \times 100$$

Rule one

When the patient's age was over 70 years old, the mathematical model was as following:

$$PI = -7.6208 + 0.9334 \times \ln(CH4) + 0.7123 \times \ln(CA125)$$

When the patient's age was under 70 years old, the mathematical model was as following:

$$PI = -8.1983 + 0.9816 \times \ln(CH4) + 0.7778 \times \ln(CA125)$$

Rule two

When the patient's age was over 70 years old, the mathematical model was as following:

$$PI = 2.2889 - 157.4 \times (1/CH4) - 29.3367 \times (1/CA125)$$

When the patient's age was under 70 years old, the mathematical model was as following:

$$PI = 2.0843 - 127.4 \times (1/CH4) - 28.2478 \times (1/CA125)$$

Rule three

When the patient's age was over 70 years old, the mathematical model was as following:

$$PI = 2.7726 - 122.3 \times (1/CH4) - 11.5367 \times \left(1/\sqrt{CA125}\right)$$

When the patient's age was under 70 years old, the mathematical model was as following:

$$PI = 2.6963 - 99.6507 \times (1/CH4) - 12.2809 \times \left(1/\sqrt{CA125}\right)$$

The area of ROC for different mathematic rule had been compared. The resulted showed in figure 2.

There were 38 patient's age were over 70 years old and 20 cases have been diagnosed as epithelial ovarian cancer(figure 2a).

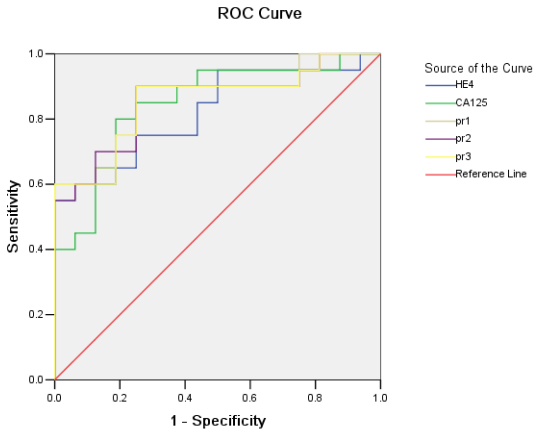

Fig. 2a The area of ROC of different tumor marker and Pr

There were 741 patient's age were under 70 years old and 268 cases have been diagnosed as epithelial ovarian cancer(figure 2b).

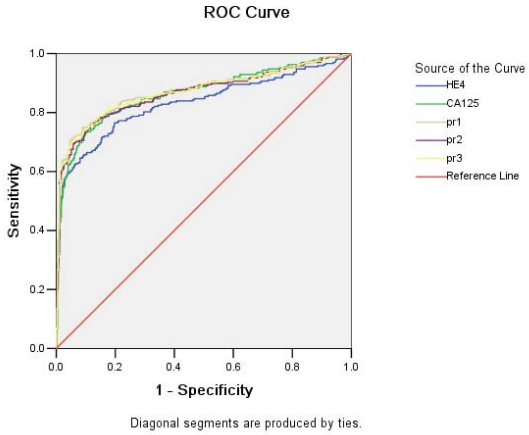

Fig. 2b The area of ROC of different tumor marker and Pr

From the above analysis, the logarithmic transformation (rule one) is better than any other two models, the following risk stratification analysis have been on the basis of rule one.

Risk stratification: According to the specificity 75% and the sensitivity of the highest predictive value as the cut-off point. In the age of more than 70 years old patient, the PR≥ 36.14 3486% is considered high risk. For the low risk, the sensitivity was 90%; the positive predictive value was 81.82%. In the age of < 70 years old patient, the PR ≥ 21.409722% is considered as high risk. For the low risk, the sensitivity was 84.3%; the positive predictive value was 65.70%.

Prediction index risk stratification and tumor staging: The data specificity reached 75% for the intersection point, then will be≥ 36.143486% as the age of more than 70 women with epithelial ovarian cancer high risk, and low risk; the≥ 21.409722% as age < 70 women with epithelial ovarian cancer high risk, and low risk of epithelial ovarian cancer patients of stage summary.

Table 3. Prediction index risk stratification and tumor staging

	≥70 years old					＜70 years old				
	high risk		low risk		total	high risk		low risk		total
stage	n	%	n	%	n	n	%	n	%	n
I	1	100.00	0	0.00	1	26	81.25	6	18.75	32
II	3	100.00	0	0.00	3	29	85.29	5	14.71	34
III	9	81.82	2	18.18	11	100	90.91	10	9.09	110
IV	2	100.00	0	0.00	2	32	96.97	1	3.03	33
Unknown	3	100.00	0	0.00	3	40	65.57	21	34.43	61
Benign	4	25.00	12	75.00	282	117	24.89	353	75.11	470

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4 Discussion

The results indicated that combined measuring the serum level of HE4 and CA125 was helpful for improving their clinical significance. As previous reports suggested that ROMA score could improving the clinical application value of HE4 [5], but our results showed that ROMA score is only slightly better than single tumor marker. We also found that HE4 and CA125 both have an age related variation in female. Our previous data indicated that this variation was not only based on menopausal status, but also have an obvious change at the age of 70 years. So we have analyzed the data based on this age point.

Three calculating rules had been evaluated and the best one had been used to risk stratification of EOC and tumor staging. It is illustrated that our new diagnostic model could both improving the diagnosis value of HE4 and helping the staging of cancer by using the blood of patients.

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