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Research on application value of combined detection of serum CA125, HE4 and TK1 in the diagnosis of ovarian cancer

Q.-P. XI[1], D.-H. PU[2], W.-N. LU[1]

[1]Department of Obstetrics and Gynecology, Changshu No. 1 People's Hospital, Changshu, Jiangsu Province, China
[2]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu Province, China

Qiuping Xi and Danhua Pu contributed equally to this wor

Abstract. – OBJECTIVE: To explore the diagnostic value of joint examination of cancer antigen 125 (CA125), thymidine kinase-1 (TK1) and human epididymis protein 4 (HE4) in the serum of patients with ovarian cancer.

PATIENTS AND METHODS: A total of 75 ovarian cancer specimens (ovarian cancer group), 40 benign ovarian specimens (benign group) and 35 ovarian specimens of healthy women (normal control group) were collected. The serum levels of HE4, CA125 and TK1 and the positive detection rates in the three groups were compared. Meanwhile, the sensitivity and specificity of the three tumor markers in the diagnosis of ovarian cancer in the three groups were compared.

RESULTS: The levels of HE4, CA125 and TK1 in the ovarian cancer group were significantly higher than those in the control group ($p<0.05$), and those in the ovarian cancer group were significantly higher than those in the benign group ($p<0.05$). The positive rates of CA125 as well as TK1 in the ovarian cancer group and the benign group were significantly higher than those in the control group ($p<0.05$), and those in the ovarian cancer group were significantly higher than those in the benign group ($p<0.05$). In the detection of an individual tumor marker, the sensitivity of CA125 was the highest, followed by HE4. The specificity of HE4 was the highest, followed by TK1. For the combination of two tumor markers, the sensitivity of CA125+HE4 ranked the first (92.18%), and the specificity of TK1+HE4 ranked the first (88.37%). The sensitivity and specificity of the joint detection of CA125+HE4+TK1 were 94.18% and 79.53%, respectively. The sensitivity of the joint detection of CA125+HE4+TK1 was significantly higher than that of the detection of a single tumor marker and that of joint detection of two tumor markers ($p<0.05$).

CONCLUSIONS: Combined detection of CA125, HE4 and TK1 can significantly improve the sensitivity in the diagnosis of ovarian cancer.

Key Words
Ovarian cancer, markers, CA125, TK1, HE4.

Introduction

Ovarian cancer represents a relatively common malignancy in the female reproductive system. It ranks third in female genital system malignant tumors, after cervical cancer and uterine cancer. For the past few years, the morbidity of ovarian cancer is increasing, but the mortality rate ranks first[1]. As the ovary is deep in the pelvic cavity, its early lesions are not easy to be found, and since there is no effective screening means, most patients are diagnosed with the late-stage ovarian cancer. Therefore, the early diagnosis and treatment of ovarian cancer are conducive to improving patient's survival rate.

At present, cancer antigen 125 (CA125) has been widely used in the diagnosis of various tumors, including the diagnosis of ovarian cancer. However, the sensitivity and specificity of single CA125 is relatively low for the medical diagnosis of ovarian cancer, and the false positive rate is relatively high. As a result, the joint detection of other tumor markers characterized by high sensitivity and specificity and CA125 is applied for the early diagnosis of ovarian cancer[2]. Thymidine kinase-1 (TK1) is a key enzyme in DNA synthesis and a kind of cell cycle-dependent biochemical index, which is closely related to cell division and proliferation. Accelerated division and proliferation of malignant tumor cells shorten the proliferation cycle, thus leading to

Corresponding Author: Weina Lu, MD; e-mail: 815967404@qq.com

abnormally increased TK1 concentration in a variety of tumors[3]. Human epididymis protein 4 (HE4) is a recently found cancer marker with high sensitivity as well as specificity for the diagnosis of ovarian cancer[4]. This study aimed to examine the concentrations of CA125, HE4 as well as TK1 in ovarian cancer patients and to explore the diagnostic value of the joint detection of these three tumor markers.

Patients and Methods

Patients

Seventy-five ovarian cancer patients who were diagnosed and surgical treated in Changshu No. 1 People's Hospital or The First Affiliated Hospital of Nanjing Medical University from June 2014 to December 2016 were collected. All patients were diagnosed by postoperative pathology. Among all the 75 ovarian cancer patients, 37 patients had serous cystadenocarcinoma, 9 patients had mucinous cystadenocarcinoma, 5 patients had ovarian germ cell tumors, 19 patients had endometrial carcinoma and 5 patients had clear cell carcinoma. According to the surgical-pathological staging formulated by the International Federation of Gynecology and Obstetrics (FIGO), 28 patients were at Stage I-II (the early stage) and 47 patients were at Stage III-IV (the late stage). In addition, 40 patients with benign ovarian lesions who were admitted to Changshu No. 1 People's Hospital were included as the benign group, and 36 healthy women were selected as the normal control group. Women in the normal control group did not suffer from coronary heart disease, diabetes mellitus, hypertension and other diseases and had no abnormalities in the liver and kidney function tests. For all the patients in the ovarian cancer group, they received no preoperative radiotherapy, chemotherapy or other treatments before diagnosis and treatment in our hospital. Patients with the history of other system cancers were excluded. This study was approved by the Ethics Committee of Changshu No. 1 People's Hospital and The First Affiliated Hospital of Nanjing Medical University. Signed written informed consents were obtained from the patients and/or guardians.

Specimen Collection

When the specimens were collected, the subjects were in the non-menstrual period. 5 mL fasting elbow vein of the above mentioned study objects was collected and placed in a red test tube. After standing for 60 min, the cells were centrifuged at 3000 r/min for 15 min at 4°C and the supernatant was added to the sterile Eppendorf (EP) tube (Hamburg, Germany) and stored in the refrigerator at -20°C to be tested. The content of serum CA125 was measured by chemiluminescence. CA125 antibody kits were provided by Derivative Product Company (DPC, Cambridge, MA, USA). The content of serum HE4 was examined by ELISA assay. HE4 kits were provided by Shanghai Li Min Industrial Co., Ltd. (Shanghai, China). The content of serum TK1 was detected by immuno-enhanced chemiluminescence (immuno-ECL), and the detection instrument cis-1 chemiluminescence digital imager and kits were provided by Sino-Swed Tong Kang Bio-Tech Co., Ltd. (Shenzhen, China).

Positive Judgment

The normal value range of serum CA125 is 0-35 U/mL and CA125>35 U/mL represents that the result is positive; the normal value range of serum TK1 is 0-2 pmol/L, and TK1>2 pmol/L represents that the result is positive; the normal value range of serum HE4 is 0-150 pmol/L, HE4>150 pmol/L represents the result is positive.

Statistical Analysis

The data were processed by Statistical Product and Service Solutions (SPSS, Version X; IBM, Armonk, NY, USA) 19.0, and the measurement data were expressed as ($\bar{x} \pm s$). The covariance analysis was used in the comparisons among three groups. The *t*-test was applied to detect the comparison between two groups. The count data were described as percentage and detected by χ^2 test. α=0.05 was set as the test standard.

Results

Comparisons of the Levels of CA125, HE4 and TK1 in the Ovarian Cancer Group, the Begin Group and the Control Group

The mean levels of serum HE4 in the ovarian cancer group, the benign legion group and the control group were (288.63±135.67) pmol/L, (75.18±45.36) pmol/L and (57.57±18.28) pmol/L, respectively; the mean levels of serum CA125 were (775.38±142.28) U/mL, (24.28±12.88) U/mL and (11.85±7.68) U/mL, respectively; the

Table I. Comparisons of the levels of CA125, HE4 and TK1 in the ovarian cancer group, the begin group and the control group.

Group	N	HE4 (pmol/L)	CA125 (U/mL)	TK1 (pmol/L)
Ovarian Cancer Group	75	288.63±135.67	775.38±142.28	3.15 ± 1.06
Benign Group	40	75.18±45.36	24.28±12.88	0.45 ± 0.13
Control Group	35	57.57±18.28	11.85±7.68	0.40 ± 0.10

Table II. The positive rates of CA125, TK1 and HE4 in the ovarian cancer group, the benign group and the control group.

Group	Positive rate of HE4 (%)	Positive rate of CA125 (%)	Positive rate of TK1 (%)
Ovarian Cancer Group	72 (54/75)	81.33 (61/75)	50.67 (38/75)
Benign Group	0	**10 (4/40)**	7.5 (3/40)
Control Group	0	0	2.78 (1/36)

mean levels of serum TK1 were (3.15±1.06) pmol/L, (0.45 ± 0.13) pmol/L and (0.40 ± 0.10) pmol/L, respectively. Pairwise comparisons of the results were conducted, which showed that the differences in the mean levels of serum HE4, CA125 and TK1 in the ovarian cancer group were statistically significant ($p<0.05$) as compared to those in the benign group as well as the control group. The differences in the levels of the three markers in the benign group as well as the control group were not statistically significant ($p>0.05$) (Table I).

The Positive rates of CA125, TK1 and HE4 in the Ovarian Cancer Group, the Benign Group and the Control Group

The positive rates of HE4, CA125 and TK1 in the ovarian cancer group were 72% (54/75), 81.33% (61/75), and 58.67% (44/75), respectively. The positive rate was measured by χ^2-test, and the results showed that there was no statistical significance between the positive rate of HE4 and that of CA125 ($\chi^2=1.826$, $p=0.177$); the difference between the positive rate of HE4 and that of TK1 was statistically significant ($\chi^2=7.196$, $p=0.007$); the difference between the positive rate of CA125 and that of TK1 was statistically significant ($\chi^2=15.716$, $p=0.000$). The positive rate of HE4 in the benign group and control group was 0%. The positive rates of CA125 and TK1 in the benign group were 10% and 7.5%, respectively. The positive rate of HE4 in the benign group was significantly different from that of CA125 and TK1, and the differences were statistically significant ($p<0.0001$), indicating that the false positive rates of serum CA125 and TK1 were significantly higher than that of HE4 in the benign group (Table II).

Sensitivity and Specificity of the Setection of a Single Tumorì Marker (CA125, TK1 or HE4) and the Joint Detection of Three Tumor Markers to the Dagnosis of Ovarian Cancer

We compared the diagnostic sensitivity (%) as well as specificity (%) of HE4, CA125, TK1, HE4+CA125, HE4+TK1, CA125+TK1 and HE4+CA125+TK1 of ovarian cancer. The sensitivity of the joint detection of HE4+CA125 and HE4+CA125+TK1 was 92.18% and 94.18%, respectively, and the specificity was 82.53% and 79.53%, respectively. After the statistical analysis, we compared the combined detection of tumor markers and the detection of a single tumor marker, which showed that the difference was statistically significant. In addition, the combined detection improved the sensitivity of detection, but the combined detection did not reduce the specificity (Table III).

Table III. Sensitivity and specificity of CA125, TK1 and HE4.

Tumor biomarker	Sensitivity (%)	Specificity (%)
HE4	73.53	100
CA125	88.24	82.64
TK1	63.14	83.86
HE4+CA125	92.18	82.52
HE4+TK1	88.68	88.37
CA125+TK1	90.23	82.55
HE4+CA125+TK1	94.18	79.53

Table IV. Comparisons of CA125, TK1 and HE4 in different histological types of ovarian cancer.

Histological Type	N	HE4 (pmol/L)	CA125 (U/mL)	TK1 (pmol/L)
Serous Carcinoma	37	475.91±214.62	592.56±210.53	3.02±1.22
Mucinous Carcinoma	9	168.87±97.74	216.85±149.15	2.89±1.05
Endometrioid Carcinoma	19	485.50±33.56	415.09±107.33	2.97±1.24
Clear Cell Carcinoma	5	112.54±36.44	56.34±25.12	2.76±0.93
Germ Cell Tumor	5	163.00±30.38	49.51±24.36	3.12±1.32

Comparisons of the Mean Levels of Serum HE4, CA125 and TK1 in Patients with Ovarian Cancer in Different Histological Types

The mean levels of serum HE4 in patients with serous and endometrial carcinoma were (475.91±214.62) pmol/L and (485.50±33.56) pmol/L, respectively, which were significantly higher than those in patients with mucinous carcinoma, clear cell carcinoma and germ cell tumor (the comparison of the mean value of the two samples was detected by *t*-test; $p<0.05$); the mean level of serum CA125 in patients with serous carcinoma [(592.56±210.53) U/mL] was significantly higher than that in patients with other histological types ($p<0.05$). There was no difference of the mean level of serum TK1 in patients with different histological types ($p>0.05$) (Table IV).

Comparisons of the Mean Levels of Serum HE4, CA125 and TK1 in Patients With Ovarian Cancer at Different Clinical Stages

The difference in the mean level of serum HE4 between patients with ovarian cancer at Stage I-II [(213.46±98.32) pmol/L] and patients [(268.32±84.79) pmol/L] at Stage III-IV was not statistically significant (the comparison of the mean value of the two samples was detected by *t*-test; $p>0.05$). The differences in the mean level of serum CA125 [(588.35±143.29) U/mL] and TK1 [(3.68±1.25) pmol/L] in patients with ovarian cancer at Stage III-IV with the mean level of serum CA125 [(162.37±78.32) U/mL] and TK1 [(2.13±0.65) pmol/L] at Stage I-II were statistically significant ($p<0.05$) (Table V).

Discussion

Tumor markers can predict the presence as well as growth of cancer cells in the body. The same type of tumor can have a variety of tumor markers, and different tumors or the same tumor in different histological types can have the same or different tumor markers[5,6]. The detection of serum tumor markers is easy to be operated with small traumas, and tumor markers belong to objective indexes, so it has been widely used in the screening of various tumors, the early diagnosis, the evaluation of therapeutic effects and the monitoring of recurrence.

At present, CA125 is a widely recognized serum cancer marker for the diagnosis of ovarian cancer, which is of practical clinical significance for chemosensitivity, tumor recurrence and differential diagnosis of other pelvic benign tumors[7,8]. At present, serum CA125 has been used in the screening, diagnosis, curative effect assessment as well as prognosis monitoring of ovarian cancer. At the same time, CA125 has been found in practice to be more sensitive to the diagnosis of ovarian cancer, especially in the diagnosis of advanced ovarian cancer. However, the specificity of CA125 is poor[9]. HE4 is a recently found tumor marker with high sensitivity and specificity to ovarian cancer. The activation of HE4 genes may be used for targeted gene therapy in ovarian cancer[10,11]. Thymidine kinase (TK) is a key enzyme in the synthesis, including two isozymes cytoplasmic thymidine kinase (TK1) and mitochondrial thymidine kinase (TK2). TK1 is closely related to cell proliferation and division. The concentration of TK1 in tumor tissues or peripheral blood of patients cannot only reflect the growth and proliferation condition of gastric cancer[12],

Table V. Comparisons of CA125, TK1 and HE4 at different clinical stages of ovarian cancer.

Clinical Stage	HE4 (pmol/L)	CA125 (U/mL)	TK1 (pmol/L)
I-II	213.46±98.32	162.37±78.32	2.13±0.65
III-IV	268.32±84.79	588.35±143.29	3.68±1.25
p-valve	>0.05	<0.05	<0.05

bladder cancer[13], breast cancer[14] and so on, but also receives much concern as an important index of tumor recurrence or efficacy evaluation.

If we diagnose early ovarian cancer only by the detection of one serum tumor marker alone, the sensitivity and specificity are not ideal[8]. At present, it is believed that the joint detection of various serum tumor markers in the early diagnosis of ovarian cancer can make up for the deficiencies of the detection of one tumor marker alone[15,16]. In this work, we compared and analyzed the sensitivity as well as specificity of the detection of a single tumor marker and the combined detection of any two tumor markers or three tumor markers (HE4, CAl25 or/and TK1) for the diagnosis of ovarian cancer, and found that the sensitivity of the combined detection of three tumor markers was the highest (94.18%), much higher than that of the detection of a single tumor marker (HE4, CA125 or TK1); the specificity of the detection of HE4 was 100%, but the sensitivity was low, so it was necessary to combine with other tumor markers to detect the cancer. In this study, the sensitivity and specificity of CA125 were 88.24% and 82.64%, respectively, and those of TK1 were 63.14% and 83.86%, respectively. The sensitivity as well as specificity of the joint detection of three tumor markers were higher than those of the joint detection of any two tumor markers, and there was no significant difference in the specificity between the former detection and the latter detection, suggesting that the joint detection of the three tumor markers will not significantly reduce the specificity and the expansion of the sample size may increase the specificity to the diagnosis of ovarian cancer.

Conclusions

The experimental results showed that the joint detection of the three tumor markers CA125, TK1 and HE4 could significantly increase the sensitivity in the diagnosis of ovarian cancer, as well as facilitate the screening of early ovarian cancer so as to give timely treatments.

Conflict of Interest

The authors declared no conflict of interest.

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