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Value of serum human epididymis secretory protein 4 as a marker for differential diagnosis of malignant and benign gynecological diseases of patients in southern China

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ABSTRACT

Background: This study investigated the clinical value of HE4 in distinguishing malignant and benign gynecological diseases of patients in southern China.
Methods: Preoperative serum CA125 and HE4 concentrations were tested in samples of women with malignant or benign gynecological diseases using fully automated methods (Abbott ARCHITECT) and validated cutoff values.
Results: For the discrimination of ovarian cancer from benign gynecological diseases, in premenopausal women, the sensitivity and specificity were 89.8% and 67.5% for CA125, 68.5% and 97.8% for HE4, and 88.9% and 78.6% for ROMA, whereas in postmenopausal women, the sensitivity and specificity were 86.6% and 88.9% for CA125, 57.3% and 100% for HE4, and 85.4% and 94.4% for ROMA. For the discrimination of endometrial cancer from benign gynecological diseases, in premenopausal women, the sensitivity and specificity were 20.3% and 67.5% for CA125, 56.8% and 97.8% for HE4, and 74.3% and 78.6% for ROMA, whereas in postmenopausal women, the sensitivity and specificity were 17.8% and 88.9% for CA125, 31.5% and 100% for HE4, and 32.9% and 94.4% for ROMA.
Conclusions: We showed that HE4 had better specificity than CA125 in discriminating ovarian cancer, and endometrial cancer from benign gynecological diseases in southern China population.

1. Introduction

Malignant gynecological diseases, especially ovarian cancer and endometrial cancer, are the most frequent cause of death from gynecological cancer. Proper treatment regimens of these malignant gynecological diseases requires a complete specialized knowledge of the probable differential diagnosis. The prognosis of ovarian cancer is relied on the disease stage, with a 5-y survival rate of about 85% for patients with stage I. But only 30% for patients with advanced stage (FIGO stages III–IV). Moreover, the improved clinical therapy of ovarian cancer is dependent on the diagnosis in early stage [1]. Unfortunately, there is a lack of a biomarker for precancerous lesions, reliable screening tests, and the early symptoms are vague resulting in delayed diagnosis and early widespread dissemination and high-grade malignancy for ovarian cancer patients [2]. Therefore, additional diagnostic factor with better sensitivity and specificity are urgently needed.

At present, ultrasound is usually used to identify pelvic masses. However, ultrasound has difficulties in discriminating benign or malignant. Although the specificity is enhanced by combining Doppler ultrasound with a morphology index, the performance varies from operators [3]. Meanwhile, according to a recent study, long term survival of ovarian cancer patients is better when these patients are remedied in specialized cancer centers, by gynecologists with expertise in gynecologic oncology [4]. More accurate determination of the pathological property of the diseases attribute to avoiding unnecessary anxiety and overtreatment, which is also able to spare medical resources in China.

Carbohydrate antigen 125 (CA125) is the most widely used biomarker for evaluating high-risk population of ovarian cancer. However, CA125 serum concentrations exhibits a sensitivity of <60% for patients with early stage disease, and specificity is also limited, because it could be elevated in a series of benign conditions such as pelvic inflammatory, endometriosis, pancreatitis, heart failure and fibroids [5–8]. Currently, several new biomarkers have been studies in order to search promising markers that can distinguish benign diseases from malignant

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gynecological diseases. Human epididymis secretary protein 4 (HE4) is seem to be one of the most promising biomarkers.

HE4 is an N-glycosylated protein which encoded by a gene located in chromosome 20q12-13.1, which is primarily expressed in the reproductive and respiratory tracts, and then secreted into the extracellular environment. Expression of HE4 is low in normal tissue, and is frequently overexpressed in the serum of patients with ovarian cancer. The exactly function of HE4 in tumorigenesis has not yet to be understood, while several studies revealed that HE4 is associated with natural immunity and fibrosis [9]. Recently, several groups demonstrated that HE4 served as a biomarker as well as CA125 for the detection of ovarian cancer [10,11]. Yu et al. reported higher sensitivity and specificity of diagnostic performance for HE4 than CA125 [12]. In addition, Moore et al. proposed the clinical utility of risk of ovarian malignancy algorithm (ROMA) which is a qualitative serum test that combines the results of CA125, HE4 and menopausal status to a numerical score to predict the probability of risk of ovarian cancer [13]. ROMA has been shown to better differentiate ovarian cancer from benign pelvic mass, even in early stage disease [14–16]. However, most of the published data are based on Europe and the United States population, there is little study was conducted to evaluate the diagnostic value of CA125, HE4, and ROMA for ovarian cancer in Chinese population, especially for southern Chinese. In this study, we were aimed to investigated the role of HE4 and ROMA as a predicting the risk of ovarian cancer in southern China population. At the same time, we reported that HE4 may play as a predicting biomarker for differentiating endometrial cancer from pelvic masses.

2. Materials and methods

2.1. Patient population and study design

From Sept. 2012 to April 2014, 1189 women aged from 12 to 82 y old received treatment in the First Affiliated Hospital of Sun Yat-sen University were enrolled in the study. A total of 1064 of who were diagnosed with gynecologic diseases by ultrasound, CT scan, PET-CT scan or MRI, while other 125 were healthy female controls. Patients with a past or concomitant history of malignant diseases and those with previous bilateral oophorectomy were excluded from the study. All patients' medical records were retrospectively reviewed and disease stages, pathological diagnosis were verified by 2 different pathologists.

The study protocol was approved by the Human Research Ethics Committees of the First Affiliated Hospital of Sun Yat-sen University according to Helsinki conventions, and all subjects enrolled in this study signed informed consent. Five milliliters blood samples were collected the day of surgery before anesthetized in the morning between 7:00 a.m. and 10:00 a.m. All the samples were centrifuged and frozen at −80 °C until tested by the ARCHITECT CA125 II assay and ARCHITECT HE4 assay (Abbott Diagnostics, Abbott Park, IL) according to the manufacturer's instructions.

The cutoff value of CA125 is 35 U/ml, and the cutoff value of HE4 is 70 pmol/l for premenopausal, and 140 pmol/l for postmenopausal women, respectively, according to the manufacturer's instructions. Three indexes, serum CA125 value, serum HE4 value and menopausal status, were used to calculate the risk of ovarian malignancy algorithm (ROMA) predictive index (PI) as the following equations [13]:

For premenopausal women $PI = -12.0 + 2.38 \times LN(HE4) + 0.0626 \times LN(CA125)$; for postmenopausal women $PI = -8.09 + 1.04 \times LN(HE4) + 0.732 \times LN(CA125)$.

LN means the natural logarithm. ROMA value is calculated from the PI as follows: ROMA value $= \exp^{(PI)} / [1 + \exp^{(PI)}] \times 100$. Cutoff values of ROMA were ≥7.4 for premenopausal women and ≥25.3 for postmenopausal women, respectively, according to the manufacturers' instructions [17].

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were then calculated based on the diagnosis confirmed by the pathologist.

2.2. Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were then calculated based on the diagnosis confirmed by the pathologist. Tumor marker concentrations in normal healthy controls and patients were compared using the Wilcoxon-Mann-Whitney two sample test and the Kruskal-Wallis test. Receiver operating characteristic plots (ROC) analyses and area under the curve (AUC) value were utilized in evaluating diagnostic power of tumor markers. The association between tumor marker concentrations and clinicopathological parameters were analyzed by ANOVA. In all the analyses, the concentration of statistical significance was set at $P < 0.05$ and all P values were calculated by two-sided significance tests. All data analyses were performed using SPSS ver 13.0.

3. Results

We assigned ovarian cancer and endometrial cancer as malignant diseases, and others as benign diseases. Seven-hundred twenty-seven out of these 1064 (68.3%) patients had benign diseases, 190 (17.9%) had ovarian cancer (aged 18–82 y, median 50 y), and 147 (13.8%) had endometrial cancer (aged 24–79 y, median 51 y). 190 specimens of ovarian cancer included different kinds of pathological types: serous (n = 80), mucinous (n = 42), endometrioid (n = 40), clear cell (n = 21), undifferentiated (n = 7). 727 benign diseases specimens included 212 (29.1%) simple epithelial ovarian cysts (aged 13–79 y, median 38 y), 182 (25.0%) endometriosis (aged 18–52 y, median 33.5 y), 229 (31.6%) uterine leiomyoma (aged 19–79 y, median 43 y), and 104 (14.3%) teratoma (aged 12–78 y, median 30 y). In addition, another 125 healthy women were enrolled as control group (aged 22–76 y, median 33 y). Table 1 show the distribution of disease and healthy normal control.

Of the 1064 women with gynecological diseases, 76.0% (819 out of 1064) were premenopausal, and 24.0% (245 out of 1064) were postmenopausal. While most women with benign diseases were premenopausal (n = 637 vs. 90). However, there is no significant difference of the distribution between premenopausal and postmenopausal women in malignant disease group (n = 182 vs. 155).

Serum HE4 concentrations detected in patients with gynecological diseases, and healthy controls, demonstrated that serum HE4 concentrations were significantly elevated in ovarian cancer and endometrial cancer (Fig. 1). The median concentrations of serum CA125 and HE4 in women with ovarian cancer, endometrial cancer, simple epithelial ovarian cysts, endometriosis, uterine leiomyoma, teratoma, and healthy controls were shown in Table 2. In healthy control group, serum HE4 median concentrations were significantly higher in postmenopausal women than in premenopausal (47 vs. 33.7 pmol/l, $P < 0.0001$). But

Table 1
Histological types, patient age, and distribution of disease for menopause status.

	Premenopausal		Postmenopausal	
	Number	Median age (range)	Number	Median age (range)
Normal control	118	33 (22–50)	7	64 (55–76)
Simple epithelial ovarian cysts	170	34 (13–51)	42	56.5 (52–79)
Endometriosis	176	33 (18–50)	6	52 (51–53)
Uterine leiomyoma	196	42 (19–51)	33	56 (52–79)
Teratoma	95	29 (12–49)	9	54 (52–78)
Ovarian cancer	108	44.5 (18–51)	82	59.5 (52–82)
Serous	37		43	
Mucinous	31		11	
Endometrioid	23		17	
Clear cell	12		9	
Undifferentiated	5		2	
Endometrial cancer	74	45 (24–51)	73	58 (52–79)

Age was shown as median (range).