Clinica Chimica Acta 440 (2015) 57–63

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Development of a multimarker assay for differential diagnosis of benign and malignant pelvic masses

Xinliang Chen [a,1], Hui Zhou [b,1], Rui Chen [c], Jian He [a], Ying Wang [a], Lisi Huang [a], Longqiaozi Sun [a], Chaohui Duan [a], Xiaohong Luo [a], Haiyan Yan [a,*]

[a] Department of Clinical Laboratory, Sun Yat-Sen Memorial Hospital, Guangzhou, Guangdong 510080, PR China
[b] Department of Gynaecology, Sun Yat-Sen Memorial Hospital, Guangzhou, Guangdong 510080, PR China
[c] Department of Respiratory Medicine, Sun Yat-Sen Memorial Hospital, Guangzhou, Guangdong 510080, PR China

ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form 27 October 2014
Accepted 13 November 2014
Available online 15 November 2014

Keywords:
Pelvic mass
Ovarian cancer
Uterine cancer
Tumor markers
HE4
ROMA

ABSTRACT

Background: HE4, a novel tumor marker for detecting ovarian cancer, has been recently applied to clinical practice. However, the comprehensive evaluation of HE4 combined with other markers is still missing. We evaluated an optimal mode of HE4 employment for differential diagnosis of benign and malignant pelvic masses.
Methods: Serum HE4, CA125, CA153, CA199, CA211 and CA724 were measured from 232 patients with pelvic messes (100 malignant masses, 132 benign diseases), and the risk of ovarian malignancy algorithm (ROMA) was also calculated. Receiver operating characteristic curves (ROC), the area under the curve (AUC), sensitivity and specificity were estimated.
Results: The combination of HE4 and CA125 (AUC of 0.963, sensitivity of 96.6%, specificity of 65.7%) provided the best differential power in diagnosing ovarian cancer. ROMA performed better in the diagnosis of pelvic masses (AUC of 0.917, sensitivity of 82.0%, specificity of 78.8%) and uterine cancer (AUC of 0.838, sensitivity of 82.0%, specificity of 60.0%) compared with applying HE4 and CA125 individually.
Conclusion: The optimal cut-off values (CA125: 93.2 U/ml, HE4: 87.6 pmol/l, ROMA: 18.1% for pre- and 31.5% for postmenopausal women), simultaneous use of CA125 and HE4 complemented by ROMA showed better performance than the traditional detection modes for differential diagnosis of ovarian cancer. We also observed that ROMA added more accuracy for differentiating the benign and malignant pelvic masses and auxiliary diagnosis of uterine cancer.

1. Introduction

Pelvic masses are often detected in pelvic examinations among females with ovarian cancer and uterine cancer, both of which are the most lethal types of cancer. However, due to non-specific early symptoms and lack of reliable screening tools, ovarian cancer often reaches an advanced stage (FIGO III–IV) when it is detected and its 5-year survival rate is only 20%–30% [1]. Thus, a diagnosis at early stage is critical, which requires a high sensitivity and specificity [2].

The screening and diagnosis of ovarian cancer are based on physical examination, semeiology, tumor markers, imaging and proteomics, etc. In the last few decades, several tumor markers have been evaluated in patients with ovarian cancer, among which, only CA125 correlates closely with ovarian cancer [3]. Since CA125 is also upregulated in many other tumors and therefore results in the reduction of sensitivity and specificity, currently attention has been moved onto a novel marker with higher specificity: human epididymis-specific protein 4(HE4).

HE4 is a glycoprotein overexpressed in epithelial ovarian cancer, especially serous and endometrioid adenocarcinoma [4]. HE4 is associated with cancer cell adhesion, migration and tumor growth [5] and serves as a valuable prognostic factor for the overall survival in patients with epithelial ovarian cancer and also an early indicator of the recurrence of ovarian cancer [3,6–9]. Previous reports showed that HE4 used in conjunction with CA 125 improves specificity for ovarian cancer [10]. The risk of ovarian malignancy algorithm (ROMA), a simple index based on CA125 and HE4, is helpful to improve the accuracy in differentiating benignancy from malignancy [4,11,12]. Besides, HE4 combined with CA125 has been reported as a new tool for preoperative evaluation and postoperative surveillance of endometrial cancer patients [13,14].

2. Materials and methods

2.1. Patients, and clinical pathology information

A total of 232 female patients diagnosed with pelvic masses were scheduled to have surgeries between March 2012 and March 2014 in

* Corresponding author at: Department of Clinical Laboratory, Sun Yat-Sen Memorial Hospital, 107 Yaniiangxi Road, Guangzhou, Guangdong 510080, PR China.
E-mail address: 327444677@qq.com (H. Yan).
[1] These authors contributed to the work equally and should be regarded as co-first authors.

http://dx.doi.org/10.1016/j.cca.2014.11.013
0009-8981/

our hospital. All pathologic tissue specimens were confirmed by histopathological evaluation postoperatively. Patients with preoperatively-known relapse of a previous cancer or with an active cancer other than ovarian cancer were excluded. All of the patients provided informed consents. The cases were divided into the following four groups according to the pathological types.

From this, 232 patients, 60 patients were pathologically confirmed postoperatively with ovarian cancer, including the following histological subtypes: serous (n = 27), mucinous (n = 6), endometrioid (n = 11), clear cell (n = 7), undifferentiated (n = 9) (aged 23–81 y, median 53 y). Seventy patients were diagnosed with benign ovarian diseases including ovarian chocolate cysts (n = 11), simple cyst (n = 5), ovarian endometriosis cysts (n = 23), mature teratoma (n = 20), mucinous cystadenoma (n = 5), serous cystadenoma (n = 6) (age 17–77 y, median 33 y). Forty patients were diagnosed with uterine cancer in the following histological subtypes: leiomyosarcoma (n = 3), endometrial cancer (n = 37), (age 33–81 y, median 51 y). Sixty-two patients had benign uterine diseases including hysteromyoma (n = 43), endometrial polyp (n = 10), endometrial hyperplasia (n = 5), and endometrioma (n = 4) (age 24–61 y, median 41 y) (Table 1).

2.2. Methods

Serum samples were collected preoperatively from our patients. Three milliliters of fasting blood samples were collected in the morning. All samples were centrifuged at 2000 × *g* for 10 min. The serum without hemolysis and lipemia was separated to be stored at −20 °C until measurement.

Following the standard operating procedure (SOP) provided by the manufacturer, serum HE4, CA125, CA153, CA199, CA211 and CA724 were quantitatively measured by electrochemiluminescence immunoassay analyzer (ECLIA, Roche E170). The reference values of each index are HE4 <140 pmol/l, CA125 <35 U/ml, CA153 <25 U/ml, CA199 <34 U/ml, CA211 <3.3 ng/ml and CA724 <7 U/ml. Cases with marker levels above threshold levels were considered to have a positive result. Three clinical routine CDMs (combined detection modes) were used to compare with HE4. These 3 modes include CDM1 (CA125 + CA153), CDM2 (CA125 + CA153 + CA724) and CDM3 (CA125 + CA153 + CA724 + CA211). The test was defined as positive if one of the markers was positive in combined detection, and negative if all of the markers were negative.

Serum HE4, CA125 levels and menopausal status were used to calculate ROMA. Menopause was considered when there was suspension of menstrual bleeding for at least 12 months [15]. A predictive index for ovarian cancer was calculated using the following formulae established by Moore et al. [16]: premenopausal PI = $-12 + 2.38 \times \ln(\text{HE4}) + 0.0626 \times \ln(\text{CA125})$, postmenopausal PI = $-8.09 + 1.04 \times \ln(\text{HE4}) + 0.732 \times \ln(\text{CA125})$. The ROMA value (predictive value) was calculated using the following formula: ROMA (%) = $e^{PI} / (1 + e^{PI}) \times 100$.

According to the indications of the HE4 manufacturer, indexes of at least 11.4% and 29.9% indicate a high risk of ovarian cancer in both pre- and postmenopausal women.

Table 1
Patient age, menopause status and tumor characteristics.

Tumor characteristics	Premenopausal	Postmenopausal	All (%)
Benign ovarian diseases			
Median age (range)	31 (24–47)	57 (49–77)	34 (24–77)
Simple	3	2	5 (7.1)
Endometriosis	21	2	23 (32.9)
Mature teratoma	15	5	20 (28.6)
Serous	2	4	6 (8.6)
Mucinous	2	3	5 (7.1)
Chocolate cysts	5	6	11 (15.7)
Total	48	22	70 (30.2)
Ovarian cancer			
Median age (range)	45 (23–51)	57 (49–81)	53 (23–81)
Serous	5	22	27 (45.0)
Mucinous	3	3	6 (10)
Endometrioid	5	6	11 (18.3)
Clear cell	3	4	7 (11.7)
Undifferentiated	4	5	9 (15.0)
Total	20	40	60 (25.9)
Benign uterine diseases			
Median age (range)	39 (24–49)	54 (50–61)	41 (24–61)
Hysteromyoma	39	4	43 (69.4)
Endometrial polyp	9	1	10 (16.1)
Endometrial hyperplasia	4	1	5 (8.1)
Endometrioma	4	0	4 (6.4)
Total	56	6	62 (26.7)
Uterine cancer			
Median age (range)	46 (33–50)	56 (50–81)	51 (33–81)
Leiomyosarcoma	3	0	3 (7.5)
Endometrial	16	21	37 (92.5)
Total	19	21	40 (17.2)
Total	153	89	232

2.3. Statistics

All calculations were performed with SPSS17.0 statistical software. The Wilcoxon–Mann–Whitney test and the Kruskal–Wallis test were used to compare biomarker distributions across two and more than two subgroups of patients, respectively; The χ^2 test was used to evaluate the qualitative data (the frequency of the positive or negative specimens); sensitivity and specificity for CA125, HE4, ROMA, and combined detection modes were calculated. The predicted probabilities for each mode were used to construct receiver operating characteristic (ROC) curves. The area under the curve (AUC) values and the optimal cut-off values were calculated. A $p < 0.05$ was considered statistically significant.

3. Results

3.1. The evaluation of serum tumor markers in patients with either ovarian cancer or uterine cancer

Median levels of different tumor markers in pre- and postmenopausal women are shown in Table 2. The median values of HE4, CA125, ROMA and positive rates are significantly higher among the patients with ovarian cancer compared with the benign cases both in pre- and postmenopausal women ($p < 0.05$). The median values of CA153, CA724, CA211 and positive rates for patients with ovarian cancer are significantly higher than patients with benign ovarian diseases ($p < 0.05$) except CA199 ($p > 0.05$). The median values of CA125, HE4 and ROMA for postmenopausal women were not significantly different from premenopausal women in the ovarian cancer group ($p > 0.05$). The HE4 and ROMA are statistically higher in patients with uterine cancer than in patients with benign uterine diseases ($p < 0.05$). 35.0% of the patients with uterine cancer had elevated values of CA125, and only 20.0% had elevated values of HE4.

3.2. The characterization of CA125 and HE4 in different ovarian cancer types

We evaluated the levels of CA125 and HE4 in various ovarian cancer types. We found that the median HE4 for 46 patients in late stages (stages III + IV) are significantly higher than 14 patients in early stages (stages I + II) ($p < 0.05$) (Figs. 1, 2). The classification of different stages is based on the criteria of the International Federation of Gynecology and Obstetrics (FIGO) [17]. The levels of CA125 and HE4 in ovarian cancer of various pathological types (Table 3) are significantly different ($p < 0.05$). In particular, the median values of CA125 and HE4 are higher in the serous of ovarian cancer compared with the clear cell and mucinous types ($p < 0.05$); the positive rates of HE4 for the latter 2 types are only 57.1% and 16.7%, respectively. Besides, the median value of CA125 is also higher in endometriosis and chocolate cyst, compared