RESEARCH ARTICLE

HE4 as a Serum Biomarker for ROMA Prediction and Prognosis of Epithelial Ovarian Cancer

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Abstract

Background and Purpose: Human epididymis protein 4 (HE4) has been suggested to be a novel biomarker of epithelial ovarian cancer (EOC). The present study aimed to evaluate and compare HE4 with the commonly used marker, carbohydrate antigen 125 (CA125), in prediction and therapy-monitoring of EOC. Patients and Methods: Serum HE4 concentrations from 123 ovarian cancer patients and 174 controls were measured by Roche electrochemiluminescent immunoassay (ECLIA). Risk of ovarian malignancy algorithm (ROMA) values were calculated and assessed. In addition, the prospects of HE4 detection for therapy-monitoring were evaluated in EOC patients. Results: The ROMA score could classify patients into high- and low-risk groups with malignancy. Indeed, lower serum HE4 was significantly associated with successful surgical therapy. Specifically, 38 patients with EOC exhibited a greater decline of HE4 compared with CA125. In contrast, elevation of HE4 better predicted recurrence (of 46, 11 patients developed recurrence, and with it increased HE4 serum concentrations) and a poor prognosis than CA125. Conclusions: This study suggests that serum HE4 levels are closely associated with outcome of surgical therapy and disease prognosis in Chinese EOC patients.

Keywords: Ovarian cancer - biomarker - human epididymis protein 4 - CA125 - prognosis

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Introduction

Ovarian cancer is a common malignant disease and represents the primary cause of death for gynecological cancers (Ferlay et al., 2010). The American Cancer Society estimated that 22,430 women will be diagnosed with ovarian cancer in 2012, with 15,280 deaths (Siegel et al., 2012). Overall, the 5-year survival rate associated with ovarian cancer is less than 30% (Andersen et al., 2008). Therefore, it is crucial to establish more sensitive and specific diagnostic tests and algorithms for the prediction of ovarian cancer at an early stage (Anton et al., 2012; Macuks et al., 2012). Moreover, optimization of treatment strategies and therapy-monitoring policies may have a substantial impact on patient survival.

In the last two decades, significant effort has been made to find new serum markers for the early diagnosis of ovarian cancer, especially for its dominant subtype, epithelial ovarian cancer (EOC) (Lokshin, 2012; Su et al., 2013). Currently, carbohydrate antigen 125 (CA125) is the established biomarker for detecting ovarian cancer recurrence and monitoring therapeutic response. However, this glycoprotein is not expressed in up to 20% of ovarian cancer patients and can be elevated in various benign conditions (Rosen et al., 2012; Maggino et al., 2013). Human epididymis protein 4 (HE4), also known as WAP-type four disulfide core 2 (WFDC2), has been shown to be overexpressed in EOC. It has been found in the serum of patients with EOC, and is mainly expressed in serous and endometrioid cancers EOCs (Devan et al., 2013; Hellstrom et al., 2003; Bouchard et al., 2006; Drapkin et al., 2005; Mokhtar et al., 2013). Our previous study suggested that the detection of HE4 could be helpful for the diagnosis of ovarian cancer, especially in postmenopausal patients (Lu et al., 2009). In a recent study, use of HE4 as a single biomarker or in combination with CA125 indicated the highest sensitivity, especially in early stage ovarian cancers (Moore et al., 2008; Ferraro et al., 2013). Moreover, detection of both CA125 and HE4 has been implemented in some clinical trial centers to discriminate patients with benign and malignant ovarian tumors preoperatively by calculating the risk of ovarian malignancy algorithm (ROMA) score (Moore et al., 2009; Moore et al., 2010; Ruggeri et al., 2011). This algorithm has been approved by the Food and Drug Administration (FDA). However, although there is increasing evidence that HE4 is a valuable biomarker for ovarian malignancies (Lu et al., 2010; Park et al., 2012; Speeckaert et al., 2013), the exploration of the usefulness of this glycoprotein in the assessment of treatment response is still ongoing. In this study, the combinatory ROMA algorithm for EOC were evaluated in regard to their ability to differentiate

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Table 1. ROMA Analysis for Malignant Prediction in EOC Patients

Groups	Pathological diagnosis		Cut-off value	Sensitivity	Specificity	AUC (95%CI)
	Malignant	Benign				
Premenopausal						
High risk	48	16	12.2	88.9%	70.4%	0.884 (0.813 -0.954)
Low risk	6	38				
Postmenopausal						
High risk	63	3	25.8	91.3%	80.0%	0.938 (0.887 -0.988)
Low risk	6	12				

low- versus high-risk pelvic masses initially suspected to be of ovarian origin. We also investigated the predictive significance of serum HE4 levels in the treatment response of 46 patients with primary ovarian cancer, compared with CA125.

Materials and Methods

Patient population and study design

From June 2010 to March 2013, 123 patients with EOC including premenopausal (n = 54) and postmenopausal patients (n = 69), and 69 patients with benign adnexal lesions consisting of 54 premenopausal and 15 postmenopausal cohorts were enrolled in this study. The patients' medical records were retrospectively reviewed to collect diagnostic information including disease stages and histologic types. In addition, 105 healthy controls without adnexal masses including 70 premenopausal women and 35 postmenopausal women participated in the study. All subjects, enrolled in this study at the Fudan University Shanghai Cancer Center (Shanghai, China), gave written informed consent. The Institute Ethical Committee approved the study protocol according to the guidelines of Helsinki conventions.

HE4 and CA125 assays

Serum samples for CA125 and HE4 analysis were obtained by venous puncture, centrifuged at × 2000 g for 10 min and stored at -70°C until use. HE4 concentrations in sera of all subjects were quantified on the Cobas e601 analyzer with Elecsys HE4 kits (Roche, Mannheim, Germany). This assay utilizes an electrochemiluminescent immunoassay (ECLIA) principle for quantitative detection of HE4 antigen in human serum. CA125 levels of the same subjects were measured using the Elecsys CA125 II kits (Roche, Mannheim, Germany). This assay also uses the ECLIA method, and the unit used for results is U/mL.

Calculation of the ROMA score

The ROMA utilizes the HE4 and CA125 concentrations obtained by ECLIA to generate a predictive index (PI) for EOC calculated by the following formulas (Moore et al., 2010):

For premenopausal women: $PI = -12.0 + 2.38 \times LN[HE4] + 0.0626 \times LN[CA125]$;

For postmenopausal women: $PI = -8.09 + 1.04 \times LN[HE4] + 0.732 \times LN[CA125]$.

Then, the ROMA score is calculated using the following equation: $\text{ROMA value } (\%) = \exp(PI)/[1 + \exp(PI)] \times 100$.

Assessment of the predictive capacity of HE4 in treatment response and progression compared with CA125

A total of 372 serum samples (patients: 46; mean samples per patient: 8, range: 4–14) were tested for each of the biomarkers, HE4 and CA125. To date, the definitions of treatment response to clinical therapy and disease progression have been based on the Gynecological Cancer Intergroup (GCIG) recommendations for disease monitoring using CA125 (Rustin et al., 2011; Schummer et al., 2012). As a new similar marker of EOC, these rules were also applied to HE4 analysis.

The response to clinical therapy, including surgical therapy, for the two markers measured was defined as a 50% reduction in marker levels in two continuous determinations. Conversely, HE4 and CA125 marker elevation was defined by two methods that are commonly used for disease recurrence (Rustin et al., 2011; Hynninen et al., 2011): (1) Marker increases two-fold above the lowest value or a 20% increment measured during the remission period. (2) Marker rises above a standard population threshold. Because of the relative infrequency of blood draws, conditions were relaxed to require a single measurement above threshold to count as a positive marker increase.

Statistical analysis

In terms of diagnostic accuracy, the performance was assessed by the evaluation of the receiver operating characteristic (ROC) curve for ovarian cancer cases (study group) versus non-ovarian cancer subjects (reference group). The area under the ROC curve was calculated by SPSS Software Version 13.0. In all analyses, *P*-values of less than 0.05 were considered to be statistically significant. All statistical calculations were performed with SPSS, version 13.0.

Results

ROMA is useful for malignant prediction in EOC patients

To ensure that the findings of the current study provided an accurate tool for clinical application at our hospital, we set the ROMA score cut-off value at a specificity of 75%. The concentrations of HE4 and CA125 in serum samples of 192 patients with ovarian disease were detected by ECLIA. The data were further divided into two groups: premenopause (n = 108) and menopause (n = 84). ROMA scores were 12.2% and 25.8% in the premenopausal and postmenopausal groups, respectively. Correspondingly, the sensitivity was 88.9% in the former group and 91.3% in the latter (Table 1). The total coincidence rate with

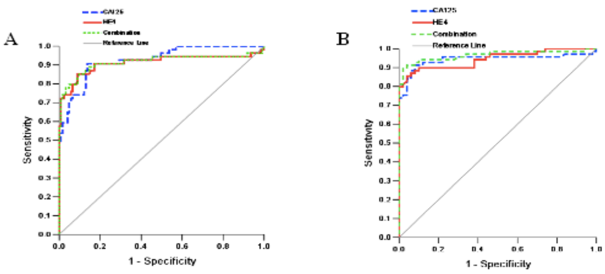

Figure 1. ROC Curve Analysis of HE4, CA125 and ROMA in Serums of the Patients. The comparison of ROMA score for the malignancy in either (A) the premenopausal group (n = 54), or (B) the postmenopausal group (n = 69)

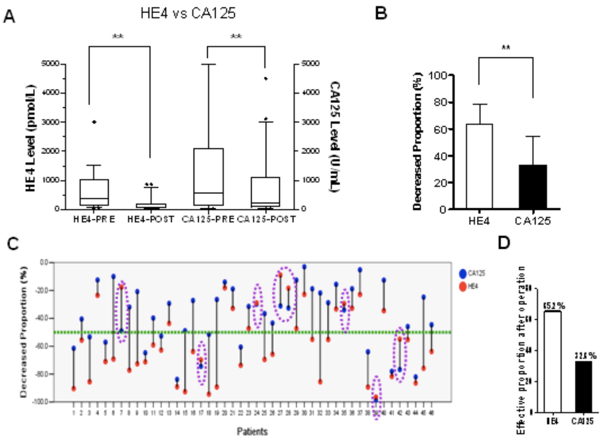

Figure 2. Analysis HE4 and CA125 Levels Before after Surgery. (A) Comparison of HE4 and CA125 levels pre- and post-surgery. Bar represents median level. (B) Comparison of the proportional changes in HE4 and CA125 levels after surgery. (C) Changes in HE4 and CA125 levels of each patient analyzed post-surgery (n = 46). (D) The effective rate for therapy response indicated by HE4 and CA125

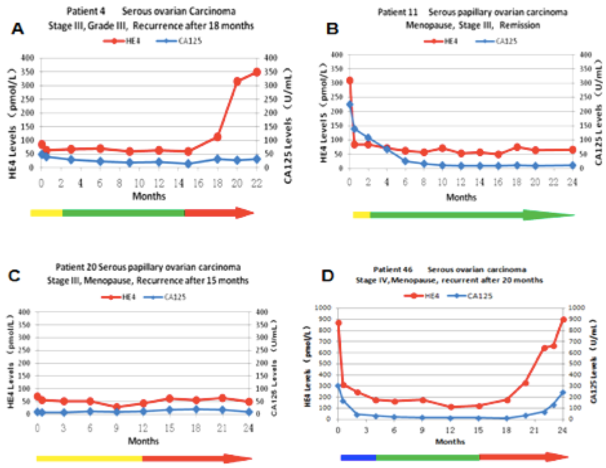

Figure 3. Representative Patient Profiles Showing the Varying Range of CA125 and HE4 Concentrations from Operation to Recurrence or Remission. HE4 (red) and CA125 (blue) serum concentration levels were measured at the indicated time points post-surgery. (A) The patient had no rising CA125 on the process of recurrence, while HE4 was hovered around threshold during remission and rose 2 months before recurrence; (B) This patient showed that both markers were above 4x threshold before surgery; CA125 and HE4 dropped more than 50% after surgery, remained below threshold and maintainable remission; (C) The patient had consistently normal HE4 and CA125 levels, but exhibited recurrence after 15 months from primary therapy; (D) HE4 and CA125 dropped after operation while HE4 was above threshold during remission, and further rose 3 months before recurrence

pathological outcome was 79.6% (86/108) and 89.3% (75/84) in these two groups. We also found the AUC of ROMA risk prediction was 0.884 and 0.938 in the premenopausal and postmenopausal groups, respectively (Figure 1).

Serum levels of HE4 are closely associated with EOC treatment response and progression

The GCIG recommends that to determine EOC therapy response and progression the definitions for CA125 should be used in addition to the updated Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 response criteria. A CA125 response is defined as at least a 50% reduction in CA125 levels compared with a pretreatment sample. To calculate the response, we analyzed all patients with an initial CA125 level who underwent first-line therapy (surgery) and compared this with levels in serum derived from blood samples collected before this primary surgery, 1/2 to 1 month after surgery, then another follow-up within 2- 3 months. PET-CT or contrast-enhanced CT was used for the evaluation of disease status. In 46 patients with EOC that received primary surgery, post-operative levels of both HE4 and CA125 were strongly decreased from an average of 395.6 pmol/L to 89.3 pmol/L, and 575.1 U/mL to 213.5 U/mL, respectively ($P < 0.01$). Furthermore, the reduction of HE4 (63.3%) was more significant than that for CA125 (33.3%, $P < 0.01$) (Figure 2A and 2B). Specifically, 38 patients with EOC exhibited a greater decline of HE4 compared with CA125 (Figure 2C). Therefore, the effective rate for therapy response indicated by HE4 (65.2%) was greater than that of CA125 (32.6%, Figure 2D).

In addition, the behavior of the serum tumor markers, HE4 and CA125, in 46 patients were also followed for several months post-surgery or chemotherapy (mean follow-up months/patient: 22, range: 8 -29 months). The patients were categorized according to the time of progression or recurrence. The date of progression after primary therapy was assigned on the basis of symptoms, RECIST and image analysis. Of these 46, 11 patients developed recurrence, and with it increased HE4 serum concentrations. In contrast, after effective surgical therapy, serum HE4 decreased in association with disease relief. Notably, the change in HE4 was more closely related to the therapy response and recurrence than that of CA125 as shown in the varying range of CA125 and HE4 concentrations from operation to remission or recurrence of the four representative patients (Figure 3). Interestingly, one patient's (Patient #20) serum HE4 and CA125 levels remained practically unchanged and within normal limits despite disease recurrence (Figure 3C).

The panel of representative graphs in Figure 4 focused on the clinically relevant expression range of 0 -1000 pmol/L of HE4. The majority of patients (30/46) showed a drop in HE4 concentration after surgery, which remained below threshold during remission but rose again 2 -3 months before recurrence. Notably, while some patients

A

HE4 alone	HE4 earlier	Both coincident	CA125 earlier	CA125 alone	None	Total
3	1	3	1	0	3	11

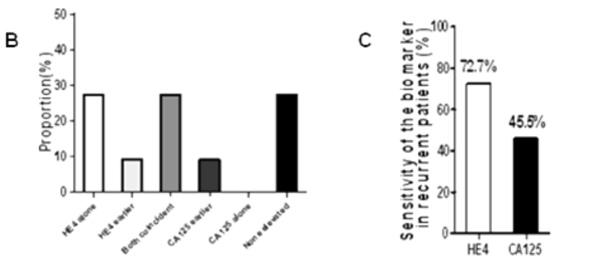

Figure 4. Patients with EOC Recurrence after Surgery Detected by HE4 and CA125. (A) The number of recurrent patients with EOC detected by elevation of HE4 and CA125. (B) Percentage of the recurrent patients detected by two biomarkers. (C) The sensitivity of HE4 and CA125 in recurrent EOC patients (n = 11)

had no rise in CA125 before recurrence, HE4 was above threshold or rose more than 20% before recurrence. Among the recurrent patients (n = 11), HE4 predicted 8 recurrences (72.7%) and CA125 predicted 5 (45.5%); while neither marker was elevated in 3 patients. Notably, Figure 5 shows that 3 recurrences were predicted by HE4 elevation alone. This also includes one patient, #4 (depicted in Figure 3A) who had HE4 levels at or below threshold during the entire remission period, but elevated at 2 months before recurrence. In another three patients, the recurrence was detected by both biomarkers at the same time. There was only one patient in which CA125 elevation occurred earlier than HE4 (Figure 4).

Discussion

CA125 is the most commonly used tumor marker for detecting and monitoring ovarian cancer in current clinical practice. However, CA125 effectiveness in the identification of the malignancy is limited by its low diagnostic specificity. In fact, this glycoprotein is widely distributed on the surface of cells of mesothelial origin in various benign and malignant conditions other than ovarian cancer (Miralles et al., 2003). Among the diverse biomarkers proposed to aid in the diagnosis of women with suspected ovarian cancer, HE4 is undoubtedly the most promising candidate (Havrilesky et al., 2008).

In the present study, we assessed the risk for EOC by the combinatory ROMA algorithm. To date, there are a growing number of patients who present with a pelvic mass; therefore, it is necessary to identify the potential malignant ovarian disease risk at this early stage for the delivery of primary clinical treatment. The calculation formula of the ROMA score is also different according to the menstrual status; therefore, the experiment was divided into premenopausal and postmenopausal groups. First, the cut-off ROMA scores for the combination of HE4 and CA125 were determined to be 12.2% and 25.8% for the premenopausal and postmenopausal groups, respectively. These critical values were used to evaluate the risk of ovarian cancer. The total accuracies with pathological outcome were 79.6% and 89.3% in these two groups. Therefore, the ROMA values were able to differentiate between benign and malignant ovarian status in both pre- and post-menopausal groups, suggesting that this quick approach could provide a strong basis for clinical diagnosis and treatment. According to the manufacturer of the HE4 and CA125 detection kits, in premenopausal women an index of 11.4% or higher (Elecsys HE4 + CA125) indicates a high risk for the presence of EOC, whereas in postmenopausal women a high risk index is given by values equal to or higher than 29.9%. However, the small deviations observed in the analysis presented here did not compromise the overall findings of the study. In the premenopausal group, the cut-off value of ROMA at 12.2% was established through the analysis of 54 patients with benign tumors and 54 malignances (the optimized Youden index, 0.742), while in the postmenopausal groups the cut-off was at 25.8%. Here, these differences with the HE4 kit may be related to the Chinese race and subjects enrolled in the study. Correspondingly, patients with low risk of malignancy may be treated in community hospitals by gynecologists or general surgeons, but patients with high risk of ovarian cancer should be managed in tertiary care centers with multidisciplinary teams specializing in ovarian cancer treatments (Moore et al., 2008).

In the 46 patients with EOC who received primary surgery followed by platinum-based chemotherapy in this study, post-operative levels of HE4 were significantly decreased after tumor removal from 395.6 pmol/L to 89.3 pmol/L ($P < 0.01$). Furthermore, the drop of HE4 (63.3%) was significantly deeper than that for CA125 (33.3%, $P < 0.01$). Indeed, 38 EOC patients exhibited significantly greater decreases of HE4 than in CA125. Therefore, the effective rate for therapy response indicated by HE4 that reached 65.2% was higher than that of CA125 (32.6%).

Optimal surgical outcome has proven to be one of the most powerful survival factors in the management of ovarian cancer patients. In fact, the degree of residual disease after surgical cytoreduction is the main factor that can be addressed by the surgeon (Allard et al., 2008). Among the recurrent patients in our study, HE4 predicted eight recurrences (72.7%) and CA125 predicted five (45.5%); while neither marker was elevated in the three remaining recurrent patients. Serum HE4 levels also reflected the course of the disease during and after chemotherapy in most cases. Interestingly, our initial results of 11 patients showed that three recurrences were detected by HE4 alone, this also included one patient with HE4 at or below threshold during the entire remission period, but elevated at two months before recurrence. An additional case of recurrence was detected earlier by HE4 than by CA125. In another three patients, the recurrence was detected by both markers at the same time, and in one patient, CA125 elevation occurred one month earlier than HE4. Thus, the serum HE4 profile was congruent with disease progression even when it differentiated from CA125 values. These data suggest that the velocities of HE4 early changes are useful as a predictor of EOC outcome. Therefore, our findings suggest that the determination of serum HE4 changes could help to evaluate the treatment response and early recurrence in EOC patients.

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