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

Wen-Ting Chen¹,²&, Xiang Gao¹,²&, Xiao-Dian Han¹, Hui Zheng¹, Lin Guo¹,², Ren-Quan Lu¹,²*

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
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 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×LN[HE4] + 0.0626×LN[CA125];
- For postmenopausal women: PI = -8.09 + 1.04×LN[HE4] + 0.732×LN[CA125].

Then, the ROMA score is calculated using the following equation: ROMA value (%) = exp (PI)/[1 + exp (PI)] × 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
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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
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 ovarian cancer (Moore 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 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.

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 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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