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The clinical value of the combined detection of sEGFR, CA125 and HE4 for epithelial ovarian cancer diagnosis

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Abstract. – OBJECTIVE: This study aims to investigate the clinical value of combined detection of serum soluble epidermal growth factor receptor (sEGFR), cancer antigen 125 (CA125), and human epididymis protein 4 (HE4) in the diagnosis of epithelial ovarian cancer (EOC).

PATIENTS AND METHODS: From December 2017 to October 2018, the serum samples were obtained from the Affiliated Hospital of Xuzhou Medical University, with 30 patients as EOC group, 30 patients with benign ovarian neoplasms as benign group, and 17 healthy subjects as healthy group. Besides, among 30 EOC patients, 9 serum samples were obtained from pre-operative and post-operative EOC patients. The levels of serum sEGFR were detected by enzyme-linked immunosorbent assay (ELISA), while CA125 and HE4 were detected by enhanced chemiluminescence immunoassay (ECLIA). The diagnostic value was evaluated by receiver operating characteristic (ROC) curve analysis.

RESULTS: The levels of serum sEGFR, CA125, and HE4 in EOC group were significantly higher than those in benign group ($p<0.05$) and healthy group ($p<0.05$). When using a single tumor marker, the CA125 shows the highest sensitivity (93.30%) and HE4 shows the highest specificity (97.87%). The specificity of combined detection of serum sEGFR, CA125, and HE4 was 100%, which was significantly higher than that using a single tumor marker. The area under the ROC curve (AUC) of combined detection of serum sEGFR, CA125, and HE4 (0.965) was much higher than that of the single detection and higher than that of combined detection of CA125 and HE4 (0.940). Moreover, the level of serum sEGFR in post-operative EOC group was significantly lower than that in the corresponding pre-operative EOC group ($p<0.05$).

CONCLUSIONS: Our study shows that combined detection of serum sEGFR, CA125, and HE4 increases the specificity and efficiency in EOC diagnosis, indicating that sEGFR could be a potential biomarker for the diagnosis and prognosis of EOC.

Key Words:
sEGFR, CA125, HE4, Epithelial ovarian cancer, Diagnosis.

Introduction

Ovarian cancer is the most lethal gynecologic cancer, in which epithelial ovarian cancer (EOC) accounts for 90%[1]. About one hundred and twenty-five thousand people worldwide die from ovarian cancer each year, with a five-year survival rate of approximately 46%[2,3]. This is primarily due to the lack of specific signs and symptoms in the early stage[4]. Currently, CA125 and HE4 are well-established biomarkers in ovarian cancer diagnosis[5]. CA125 is a large transmembrane glycoprotein, which is encoded by Mucin 16 (MUC16) gene[6]. CA125 is overexpressed in EOC and could be detected in serum[7]. Some studies[5,8] have shown that the sensitivity of CA125 is only 50%-62% in early-stage ovarian cancer diagnosis. Additionally, CA125 is reported to be higher in other physiological or pathological conditions, such as menstruation, pregnancy, endometriosis, and inflammatory diseases of the peritoneum, making its specificity low (73%-77%)[5,8]. Another

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serum biomarker named human epididymis protein 4 (HE4), known as WAP four disulfide core 2 (WFDC2), has been shown to be overexpressed in some ovarian tumors[9]. Yanaranop et al[10] have shown that HE4 has a higher specificity than CA125. Nevertheless, the level of serum HE4 is reported to be increased in multiple abdominal tumors[11,12], endometrial cancer[13], and pancreatic cancer[14]. Hence, detection of HE4 alone in the diagnosis of EOC has some shortcomings[15]. Moreover, it is reported that the five-year survival rate of patients with stage I EOC is in the range of 90% to 95%[16]. Therefore, it is quite necessary to exploit new biomarkers for improving the early diagnosis of EOC.

Epidermal growth factor receptor (EGFR), a membrane-bound tyrosine kinase glycoprotein, is widely expressed on the surface of multiple cell types and belongs to the human epidermal receptor family[17]. Gui and Shen[18] have suggested that the overexpression of EGFR is detected in 30%-98% of EOC. Besides, the sEGFR and the extracellular ligand-domains of EGFR could be detected in the blood stream[19]. Therefore, EGFR might be a potential biomarker for the EOC diagnosis. However, there are controversial data on the expression level of serum sEGFR in EOC patients[20]. Baron et al[21] found that the level of serum sEGFR was lower in patients with ovarian cancer than in healthy subjects, but Tas et al[22] reported no significant difference in the baseline levels of serum sEGFR between EOC patients and controls. The purpose of this study is to evaluate the level of serum sEGFR, CA125, and HE4 in EOC patients and their role in EOC diagnosis.

Patients and Methods

Patients

30 EOC patients who were diagnosed and surgically treated in the Affiliated Hospital of Xuzhou Medical University from December 2017 to October 2018 were recruited. Among these, there were paired 9 pre-operative and post-operative EOC patients. In addition, 30 patients with benign ovarian neoplasms who were admitted to the Affiliated Hospital of Xuzhou Medical University were included as the benign group, and 17 healthy women were included as the healthy group. All patients were pathologically confirmed. This investigation was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University.

Detection Methods

The samples were collected during the non-menstrual period. 5 mL elbow vein of the above study objects were collected and placed in a yellow test tube (Guangzhou Yangpu Biotechnology Co., Ltd., Guangzhou, China). After standing for 10 min, the samples were centrifuged at 3500 r/min for 10 min at 4°C. Then, the supernatant was added to the sterile Eppendorf tube (EP; Jiangsu Kangjian Biotechnology Co., Ltd., Jiangsu, China) and stored in the refrigerator at –80°C. The level of serum sEGFR was detected by sEGFR ELISA Kit (Beijing Yiqiao Shenzhou Technology Co., Ltd, Beijing, China). The levels of serum HE4 and CA125 were detected by ECLIA from Roche COBASE 601 automatic chemiluminescence instrument (Roche Pharmaceutical Ltd, Shanghai, China) and original matching reagent. Moreover, all procedures were performed according to the kit instructions, and all controls were within the scope stated in the instructions.

Positive Judgment

The level of sEGFR>277.84 pg/ml was defined as positive according to the ROC curve. The levels of CA125>35.00 U/mL, and HE4>92.10 pmol/l (premenopausal) or HE4>121.00 pmol/l (postmenopausal) were defined as positive based on the reference range provided by the kit.

Statistical Analysis

All the data were analyzed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7.0 software (Version VII; La Jolla, CA, USA). Categorical data were evaluated using the *t*-test. Count data were evaluated between groups using χ^2-test. The measurement data were expressed as the means of the data ± standard deviation (SD). The ROC curves and AUC were used to assess the diagnostic value of sEGFR, CA125, and HE4. When the Youden index is maximum, the cutoff value of sEGFR is determined. When the *p*-value is less than 0.05, the difference is considered statistically significant.

Results

Comparisons of the Levels of Serum sEGFR, CA125, and HE4 in the EOC Group, the Benign Group and the Healthy Group

As shown in Table I, the mean levels of serum sEGFR in EOC group, benign group, and healthy

Table I. Comparisons of the levels of sEGFR, HE4, and CA125 in the EOC group, benign group, and healthy group.

Group	sEGFR (pg/ml)	CA125 (U/ml)	HE4 (pmol/ml)
EOC Group (n = 30)	282.26 ± 35.42	1664.67 ± 3717.74	430.66 ± 503.80
Benign Group (n = 30)	265.89 ± 16.60	33.35 ± 51.31	48.76 ± 11.47
Healthy Group (n = 17)	262.86 ± 12.03	18.20 ± 17.87	47.02 ± 19.63

group were (282.26±35.42) pg/ml, (265.89±16.60) pg/ml, and (262.86±12.03) pg/ml, respectively. The mean levels of serum CA125 in EOC group, benign group, and healthy group were (1664.67±3717.74) U/ml, (33.35±51.31) U/ml, and (18.20±17.87) U/ml, respectively. The mean levels of serum HE4 in EOC group, benign group, and healthy group were (430.66±503.80) pmol/ml, (48.76±11.47) pmol/ml, and (47.02±19.63) pmol/ml, respectively. The level of sEGFR in EOC group was significantly higher than that in benign group and healthy group (p<0.05, Figure 1A). Meanwhile, the levels of CA125 and HE4 were significantly higher than those in benign group and healthy group (p<0.001, Figure 1B, 1C). The levels of the three markers in benign group and healthy group were not significantly different (p>0.05, Figure 1).

The Correlation of sEGFR, CA125, and HE4 with Clinicopathological Factors in the EOC Group

In EOC group, the levels of sEGFR in high-grade and advanced patients were higher than those in low-grade and early patients (p>0.05, Table II). However, the levels of CA125 and HE4 were significantly related to age, Malpica grade, and FIGO stage (p<0.05, Table II). Additionally, the level of CA125 was significantly related to Menopause state (p<0.05, Table II).

Sensitivity, Specificity, and Diagnostic Performance of Single or Combination of sEGFR, CA125, and HE4 in the Diagnosis of EOC

ROC curves of single or combination of sEGFR, CA125, and HE4 were shown in Figure 2. The ROC curve analysis revealed that when the level of sEGFR was 277.84 pg/ml, the maximum of the Youden index was 0.342, and the AUC of sEGFR was 0.666. The sensitivity, specificity, Youden Index, and AUC of single or combination of sEGFR, CA125, and HE4 in the diagnosis of EOC were listed in Table III. The sensitivity of sEGFR, CA125, and HE4 were 53.33%, 93.33%, and 60.00%, respectively (Table III). The specificity of sEGFR, CA125, and HE4 were 80.85%, 85.11%, and 97.87%, respectively (Table III). Furthermore, the sensitivity and specificity of combined detection of CA125 and HE4 were 86.67% and 95.75% (Table III). The sensitivity and specificity of combined detection of three

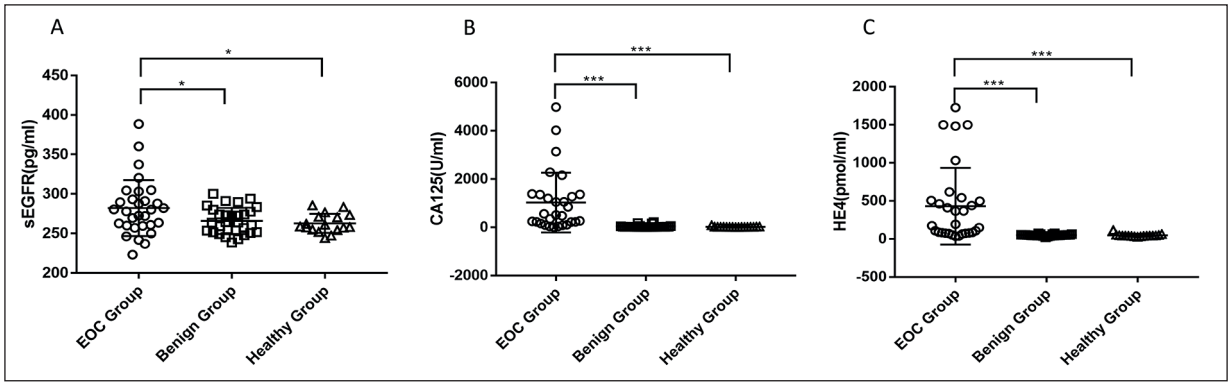

Figure 1. The levels of serum sEGFR, CA125, and HE4 in the EOC group, benign group, and healthy group. **A,** Level of serum sEGFR was significantly higher in the EOC group than that in the benign group and healthy group (*p<0.05, compared with the EOC group). **B,** Level of serum CA125 was significantly higher in the EOC group than that in the benign group and healthy group (***p <0.001, compared with the EOC group). **C,** Level of serum HE4 was significantly higher in the EOC group than that in the benign group and healthy group (***p <0.001, compared with the EOC group).

Table II. The correlation of sEGFR, HE4, and CA125 with clinicopathological factors in the EOC group.

Clinicopathological factors	No.	sEGFR (pg/ml)	*p*	CA125 (U/ml)	*p*	HE4 (pmol/ml)	*p*
Age							
<=50	11	273.24 ± 29.57	0.162	687.56 ± 1038.58	0.027	185.57 ± 175.77	0.041
> 50	19	287.49 ± 38.17		2230.37 ± 4554.95		572.56 ± 577.75	
Menopause state							
Premenopausal	9	265.54 ± 26.92	0.081	441.30 ± 479.17	0.044	173.01 ± 176.60	0.067
Postmenopausal	21	289.43 ± 36.74		2188.97 ± 4357.44		541.09 ± 559.35	
Malpica grade							
Low grade	8	274.77 ± 26.86	0.606	125.34 ± 115.42	< 0.001	73.62 ± 36.09	0.001
High grade	22	284.98 ± 38.25		2224.43 ± 4225.11		560.50 ± 532.77	
FIGO stage							
I-II	8	275.74 ± 26.89	0.743	340.28 ± 451.53	0.022	72.34 ± 24.87	0.002
III-IV	22	284.64 ± 38.33		2146.27 ± 4255.33		560.96 ± 532.54	

markers were 83.30% and 100% (Table III). Additionally, the AUC of combined detection of CA125 and HE4 was 0.940, while the AUC of combined detection of sEGFR, CA125, and HE4 was 0.965 (Figure 3).

Comparison of the Levels of sEGFR, CA125, and HE4 in Pre-Operative and Post-Operative EOC Patients

The levels of sEGFR and CA125 were significantly higher in pre-operative patients than in post-operative EOC ones ($p<0.05$). However, the level of HE4 was slightly decreased in post-operative EOC patients ($p>0.05$; Figure 3, Table IV).

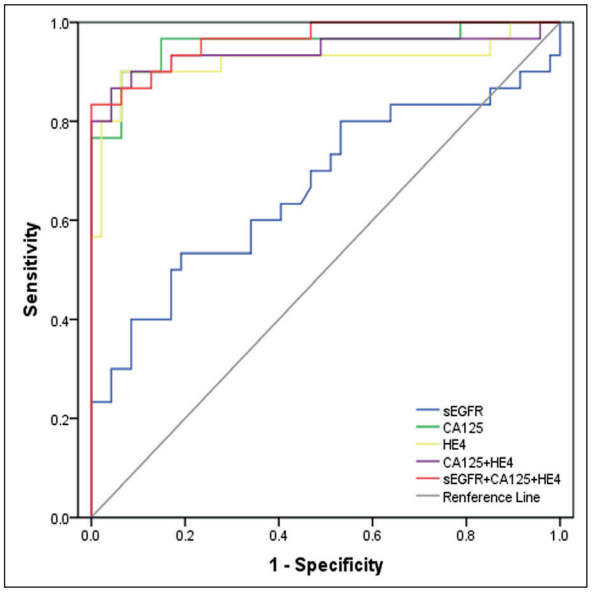

Figure 2. The ROC curves of serum sEGFR, CA125, HE4, CA125+HE4 and CA125+HE4+sEGFR.

Discussion

The aim of this study is to explore the clinical value of sEGFR, CA125, and HE4 in the detection of EOC and to explore which single or combined tumor markers has the highest diagnosis sensitivity and specificity in EOC patients.

So far, CA125 and HE4 are widely regarded as serum biomarkers for the diagnosis of ovarian cancer, which have practical clinical significance for differential diagnosis, curative effect assessment, as well as prognosis monitoring of ovarian cancer[23,24]. However, CA125 is mainly elevated in advanced ovarian cancer, and its specificity is low[25]. HE4 improved the specificity of the diagnosis of EOC, but the sensitivity is poor[26]. Therefore, it is believed that the combined detection of various serum tumor markers in the early diagnosis of ovarian cancer could make up the deficiencies of the single detection.

Authors[20,27] have shown that EGFR is low expressed in normal ovarian epithelial tissues, but higher in EOC tissues. In addition to the existence of EGFR in the form of transmembrane glycoprotein, its extracellular segment can be released into the blood in the form of sEGFR[28]. We found that the level of sEGFR in EOC group was significantly higher than benign group and healthy group, but other studies showed different results. Baron et al[29] determined sEGFR of 144 healthy women and 225 patients with EOC by acridinium-linked immunosorbent assay (ALISA). Compared with healthy controls, they found that the level of sEGFR was higher in healthy subjects than that in patients with EOC[20,21,29]. Moreover, the level of sEGFR was

Table III. Sensitivity, specificity and AUC of sEGFR, CA125 and HE4 in the diagnosis of EOC.

Index	Sensitivity (%)	Specificity (%)	Youden Index	AUC (95% CI)
sEGFR	53.33	80.85	0.342	0.666 (0.533,0.800)
CA125	93.33	85.11	0.784	0.955 (0.901,1.000)
HE4	60.00	97.87	0.579	0.921 (0.841,1.000)
CA125+HE4	86.67	95.75	0.824	0.940 (0.871,1.000)
CA125+HE4+sEGFR	83.30	100.00	0.833	0.965 (0.926,1.000)

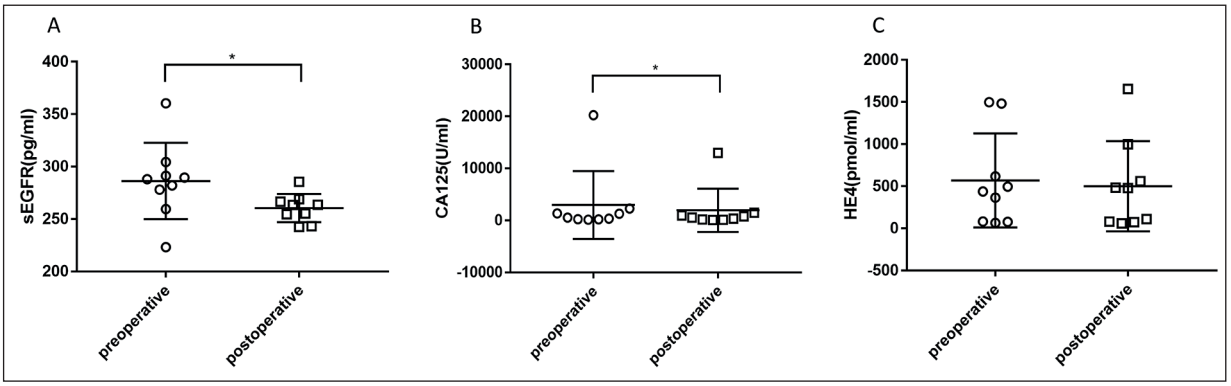

Figure 3. Levels of serum sEGFR, CA125, and HE4 in pre- and post-operative EOC patients. **A,** Level of serum sEGFR in pre-operative EOC patients was significantly higher than that in post-operative EOC patients (*p<0.05). **B,** Level of serum CA125 in pre-operative EOC patients was significantly higher than that in post-operative EOC patients (*p<0.05). **C,** There was no significant difference in the levels of serum HE4 between pre-operative and post-operative EOC patients (p>0.05).

negatively correlated with age, and the level of premenopausal women was higher than that of postmenopausal women. However, Tas et al[22] had shown that there was no significant difference in sEGFR between 55 patients with EOC and 20 healthy women by ELISA. The following reasons may account for the difference in our study and others. First of all, the subjects of our reserch were limited to Asian, while other studies focused mainly on Europe and the United States. Moreover, in terms of methodology, we used ELISA, while other studies used ALISA. Additionally, no other clinical variables, including age, menopause state, Malpica grade, and FIGO stage were found to be correlated with the sEGFR assay, which is consistent with that of Lafky et al[20]. Notably, we reveal that the level of serum sEGFR in high-grade and advanced patients was higher than that in low-grade and early patients. If the sample size is large enough, the results may have statistically clinical value in diagnosis. Therefore, it is suggested that the higher level of sEGFR may be a risk factor for EOC.

In this study, we compared the sensitivity and specificity of the single or combined tumor markers for the diagnosis of EOC. Results showed that the specificity of the combined detection of three tumor markers was the highest (100%), which was higher than that HE4 detection (97.87%). The sensitivity of CA125 was the highest (93.33%), but its specificity

Table IV. Comparison of the levels of sEGFR, HE4 and CA125 before and after operation.

Group	No.	sEGFR (pg/ml)	CA125 (U/ml)	HE4 (pmol/ml)
Preoperative	9	286.30 ± 36.46	2967.23 ± 6523.31	569.66 ± 558.92
Postoperative	9	260.43 ± 13.41	1944.02 ± 4168.14	500.72 ± 535.04
p		0.047	0.011	0.441

was low (85.11%), which is higher than in other studies[30,31]. This could be ascribed to the fact that most patients were in stages III/IV and the histologic type of EOC was serous ovarian cancer. Additionally, we compared the diagnostic performances of single or combined detection of sEGFR, CA125, HE4 using ROC analysis. We found that the sensitivity and specificity of combined detection of CA125 and HE4 could be improved to a certain extent. Nevertheless, their AUC (0.940) was lower than that of CA125 (0.955). Notably, the AUC of combined detection of sEGFR, CA125, and HE4 (0.965) was significantly higher than that of combined detection of CA125 and HE4 (0.940) or single marker, indicating that the combined detection of three tumor markers in the diagnosis of EOC was more effective. Moreover, the levels of sEGFR and CA125 were significantly higher in pre-operative than in post-operative EOC patients, and the level of sEGFR returned to the normal level after the operation. It is suggested that sEGFR could be a potential marker for prognosis evaluation. Further investigationss are needed to this confirm concern.

Conclusions

The combined detection of sEGFR, CA125, and HE4 could increase the specificity and efficiency in EOC diagnosis, which may provide a more accurate basis for clinical diagnosis. However, prospective investigations with a larger number of clinical samples should be performed to validate the importance of serum sEGFR for diagnosis and prognosis of EOC in the future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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References

1) Ren X, Wu X, Hillier SG, Fegan KS, Critchley HO, Mason JI, Sarvi S, Harlow CR. Local estrogen metabolism in epithelial ovarian cancer suggests novel targets for therapy. J Steroid Biochem Mol Biol 2015; 150: 54-63.
2) Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
3) Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. Lancet 2014; 384: 1376-1388.
4) Chien J, Fan JB, Bell DA, April C, Klotzle B, Ota T, Lingle WL, Gonzalez Bosquet J, Shridhar V, Hartmann LC. Analysis of gene expression in stage I serous tumors identifies critical pathways altered in ovarian cancer. Gynecol Oncol 2009; 114: 3-11.
5) Montagnana M, Benati M, Danese E. Circulating biomarkers in epithelial ovarian cancer diagnosis: from present to future perspective. Ann Transl Med 2017; 5: 276.
6) Williams KA, Terry KL, Tworoger SS, Vitonis AF, Titus LJ, Cramer DW. Polymorphisms of MUC16 (CA125) and MUC1 (CA15.3) in relation to ovarian cancer risk and survival. PLoS One 2014; 9: e88334.
7) Liu Q, Cheng Z, Luo L, Yang Y, Zhang Z, Ma H, Chen T, Huang X, Lin SY, Jin M, Li Q, Li X. C-terminus of MUC16 activates Wnt signaling pathway through its interaction with β-catenin to promote tumorigenesis and metastasis. Oncotarget 2016; 7: 36800-36813.
8) Sölétormos G, Duffy MJ, Othman Abu Hassan S, Verheijen RH, Tholander B, Bast RC, Gaarenstroom KN, Sturgeon CM, Bonfrer JM, Petersen PH, Troonen H, Carlotorre G, Kanty Kulpa J, Tuxen MK, Molina R. Clinical use of cancer biomarkers in epithelial ovarian cancer: updated guidelines from the European Group on Tumor Markers. Int J Gynecol Cancer 2016; 26: 43-51.
9) Drapkin R, Von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR, Hecht JL. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. Cancer Res 2005; 65: 2162-2169.
10) Yanaranop M, Anakrat V, Siricharoenthai S, Nakrangsee S, Thinkhamrop B. Is the risk of ovarian malignancy algorithm better than other tests for predicting ovarian malignancy in women with pelvic masses? Gynecol Obstet Invest 2017; 82: 47-53.
11) Gion M, Peloso L, Trevisiol C, Squarcina E, Zappa M, Fabricio AS. An epidemiology-based model as a tool to monitor the outbreak of inappropriateness in tumor marker requests: a national scale study. Clin Chem Lab Med 2016; 54: 473-482.
12) Mi D, Zhang YX, Wang CJ, Feng Q, Qi P, Chen SQ. Diagnostic and prognostic value of serum human

epididymis protein 4 in patients with primary fallopian tube carcinoma. J Obstet Gynaecol Res 2016; 42: 1326-1335.

13) Bignotti E, Ragnoli M, Zanotti L, Calza S, Falchetti M, Lonardi S, Bergamelli S, Bandiera E, Tassi RA, Romani C, Todeschini P, Odicino FE, Facchetti F, Pecorelli S, Ravaggi A. Diagnostic and prognostic impact of serum HE4 detection in endometrial carcinoma patients. Br J Cancer 2011; 104: 1418-1425.

14) Huang T, Jiang SW, Qin L, Senkowski C, Lyle C, Terry K, Brower S, Chen H, Glasgow W, Wei Y, Li J. Expression and diagnostic value of HE4 in pancreatic adenocarcinoma. Int J Mol Sci 2015; 16: 2956-2970.

15) Van Gorp T, Cadron I, Despierre E, Daemen A, Leunen K, Amant F, Timmerman D, De Moor B, Vergote I. HE4 and CA125 as a diagnostic test in ovarian cancer: prospective validation of the risk of ovarian malignancy algorithm. Br J Cancer 2011; 104: 863-870.

16) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.

17) Hutchinson RA, Adams RA, Mcart DG, Salto-tellez M, Jasani B, Hamilton PW. Epidermal growth factor receptor immunohistochemistry: new opportunities in metastatic colorectal cancer. J Transl Med 2015; 13: 217.

18) Gui T, Shen K. The epidermal growth factor receptor as a therapeutic target in epithelial ovarian cancer. Cancer Epidemiol 2012; 36: 490-496.

19) Schippinger W, Dandachi N, Regitnig P, Hofmann G, Balic M, Neumann R, Samonigg H, Bauernhofer T. The predictive value of EGFR and HER-2/neu in tumor tissue and serum for response to anthracycline-based neoadjuvant chemotherapy of breast cancer. Am J Clin Pathol 2007; 128: 630-637.

20) Lafky JM, Wilken JA, Baron AT, Maihle NJ. Clinical implications of the ErbB/epidermal growth factor (EGF) receptor family and its ligands in ovarian cancer. Biochim Biophys Acta 2008; 1785: 232-265.

21) Baron AT, Cora EM, Lafky JM, Boardman CH, Buenafe MC, Rademaker A, Liu D, Fishman DA, Podratz KC, Maihle NJ. Soluble epidermal growth factor receptor (sEGFR/sErbB1) as a potential risk, screening, and diagnostic serum biomarker of epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 2003; 12: 103-113.

22) Tas F, Karabulut S, Serilmez M, Ciftci R, Duranyildiz D. Increased serum level of epidermal growth factor receptor (EGFR) is associated with poor progression-free survival in patients with epithelial ovarian cancer. Cancer Chemother Pharmacol 2014; 73: 631-637.

23) Lv XL, Zhu Y, Liu JW, Ai H. The application value of the detection of the level of tissue polypeptide antigen, ovarian cancer antigen X1, cathepsin L and CA125 on the diagnosis of epithelial ovarian cancer. Eur Rev Med Pharmacol Sci 2016; 20: 5113-5116.

24) Antonijevic A, Rancic N, Ilic M, Tiodorovic B, Stojanovic M, Stevanovic J. Incidence and mortality trends of ovarian cancer in central Serbia. J BUON 2017; 22: 508-512.

25) Xi QP, Pu DH, Lu WN. Research on application value of combined detection of serum CA125, HE4 and TK1 in the diagnosis of ovarian cancer. Eur Rev Med Pharmacol Sci 2017; 21: 4536-4541.

26) Dochez V, Caillon H, Vaucel E, Dimet J, Winer N, Ducarme G. Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. J Ovarian Res 2019; 12: 28.

27) Psyrri A, Kassar M, Yu Z, Bamias A, Weinberger PM, Markakis S, Kowalski D, Camp RL, Rimm DL, Dimopoulos MA. Effect of epidermal growth factor receptor expression level on survival in patients with epithelial ovarian cancer. Clin Cancer Res 2005; 11: 8637-8643.

28) Maramotti S, Paci M, Manzotti G, Rapicetta C, Gugnoni M, Galeone C, Cesario A, Lococo F. Soluble Epidermal Growth Factor Receptors (sEGFRs) in cancer: biological aspects and clinical relevance. Int J Mol Sci 2016; 17: 2-12.

29) Baron AT, Boardman CH, Lafky JM, Rademaker A, Liu D, Fishman DA, Podratz KC, Maihle NJ. Soluble epidermal growth factor receptor (sEGFR) [corrected] and cancer antigen 125 (CA125) as screening and diagnostic tests for epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 2005; 14: 306-318.

30) Munkarah A, Chatterjee M, Tainsky MA. Update on ovarian cancer screening. Curr Opin Obstet Gynecol 2007; 19: 22-26.

31) Park Y, Kim Y, Lee Ey, Lee JH, Kim HS. Reference ranges for HE4 and CA125 in a large Asian population by automated assays and diagnostic performances for ovarian cancer. Int J Cancer 2012; 130: 1136-1144.