Cancer Biomarkers -1 (2017) 1–7
DOI 10.3233/CBM-170720
IOS Press

The clinical significance of the combined detection of serum Smac, HE4 and CA125 in endometriosis-associated ovarian cancer

Xin-Ran Xu[a,1], Xin Wang[b,1], Hong Zhang[a,*], Ming-Yan Liu[a] and Qi Chen[a]
[a]*Department of Gynaecology, Tianjin Central Hospital of Gynecology Obstetrics, Tianjin 300100, China*
[b]*Department of Assisted Reproductive Pregnancy, Yantai Yuhuangding Hospital, Yantai, Shandong 264000, China*

Abstract.
OBJECTIVE: This study aims to investigate the clinical significance of serum Smac, HE4 and CA125 alone or combined for detecting endometriosis-associated ovarian cancer (EAOC).
METHODS: The level of serum Smac, HE4 and CA125 in 40 healthy controls, 40 cases of benign endometriosis ovarian tumor, and 60 cases of EAOC were detected by ELISA and electrochemical immune method.
RESULTS: Serum Smac expression level was significantly lower in the EAOC group than in the control group and benign ovarian tumor group ($P < 0.05$), while HE4 and CA125 expression levels were significantly higher in the EAOC group than the other two groups. The sensitivity of Smac single detection was up to 91.67%, and the specificity of HE4 was up to 98.75%. Furthermore, the sensitivity of Smac + HE4 + CA125 combined was the highest, which reached up to 98.33%; but the specificity was low, which reached up to 75%. The serum expression level differences before and after surgery were statistically significant. As the number of chemotherapies increases, the Smac level increased, and HE4 and CA125 levels gradually decreased. Furthermore, Smac increased to normal at the end of the 2nd period of chemotherapy, while HE4 and CA125 decreased to normal in 2nd and 3rd period of chemotherapy, respectively.
CONCLUSION: Serum Smac, HE4 and CA125 may play an important role in predicting EAOC and in monitoring the prognosis of postoperative EAOC.

Keywords: Smac, HE4, CA125, endometriosis-associated ovarian cancer, chemotherapy

1. Introduction

Endometriosis is one of the most common gynecological diseases, which can cause infertility, dysmenorrhea, dyspnea, chronic abdominal pain, as well as pelvic pain [1]. It is estimated that the incidence of endometriosis in women of childbearing age is 5–10% [2]. The disease belongs to benign lesions, but its biological behavior has the characteristics of a malignant tumor, manifesting something in common with ovarian malignancies in terms of cell proliferation, apoptosis, angiogenesis and invasion [3]. A large number of studies have revealed that endometriosis increases the risk of epithelial ovarian cancer (EOC) [4, 5]. EOC is insidious and has a high mortality. Since patients with endometriosis-associated ovarian cancer (EAOC) have the symptoms of endometriosis, they are more likely to have early diagnosis. If serological detection can early detect the canceration of endometriosis, it will be the key means to improve the survival rate of these patients [6,7].

Cancer antigen 125 (CA125) is a serological marker for diagnosis of EOC, which has high sensitivity, but low specificity [8,9]. HE4 is overexpressed in ovarian cancer, and thereby has high diagnostic specificity. In addition, it significantly increases in EAOC, and is

[1]Contributed equally.
*Corresponding author: Hong Zhang, Department of Gynaecology, Tianjin Central Hospital of Gynecology Obstetrics, No. Three, No. 156, Nankai Road, Nankai District, Tianjin 300100, China. Tel./Fax: +86 22 58287068; E-mail: zhanghong159@21cn.com.

not affected by the menstrual cycle [10]. In 2015, Dobrzycka first identified Smac in the serum of EOC patients [11]. It is an apoptosis-promoting factor, which can promote the apoptosis of tumor cells by specifically binding with apoptosis inhibitory proteins [12]. The formation of ovarian cancer is the result of cancer cells successfully escaping from apoptosis. Therefore, Smac may be more valuable in the diagnosis of EAOC.

The aim of this study was to determine the serum levels of CA125, HE4 and Smac, and investigate its roles as EAOC tumor markers. This would provide important clinical value for the early diagnosis and condition monitoring of EAOC, and improving the survival rate of patients.

2. Materials and methods

2.1. Material

2.1.1. Clinic pathological data

The clinical data of ovarian tumor patients who received surgical treatment at Tianjin Central Obstetrics and Gynecology Hospital from December 2015 to January 2017 were collected. Among these patients, 40 patients had benign ovarian endometriosis cysts (group II). The age of these patients ranged within 19–63 years old, with an average age of 38 years old. In addition, 60 patients had EAOC (group III). The age of these patients ranged within 27–69 years old, with an average age of 54 years old. These 60 patients comprised of 46 patients with clear cell carcinoma, eight patients with endometrial carcinoma, five patients with serous carcinoma, and one patient with mucinous carcinoma. Diagnostic criteria: (1) the cancer tissue and ectopic endometrial tissue coexist in the same ovary; (2) there is histological correlation between the cancer tissue and ectopic endometrial tissue, and resembling endometrial stromal cells surround the characteristic endometrial glands or preexisting bleeding was present; (3) tumors that originate from endometriotic tumors, and primary or other metastatic malignancies are excluded; (4) microscopically, the progressive morphological changes of benign ectopic endometrium to malignant metastasis can be observed. The clinical staging was based on the 2009 International Federation of Obstetricians and Gynecologists (FIGO) staging criteria. Among these patients, 11 patients were at stage I, five patients were at stage II, 39 patients were at stage III, and five patients were at stage IV. Furthermore, ascites were found in 37 patients, and not present in 23 patients. In addition, lymph node metastasis was found in 25 patients, and was not present in 35 patients. Moreover, 30 patients had a neutrophil/lymphocyte ratio (NLR) of $\geqslant 2.62$, and 32 patients had a platelet/lymphocyte ratio (PLR) of $\geqslant 173$. Exclusion criteria: (1) Patients who have had infectious diseases in the past two weeks; (2) patients who underwent radiotherapy or chemotherapy before surgery; (3) patients who were accompanied by serious liver and kidney diseases; (4) patients who were accompanied by diseases of blood, immune system and thrombosis, or hemorrhagic diseases; (5) patients who were accompanied by other tumors. In addition, 40 subjects in good health were selected as controls (group I). The age of these subjects ranged within 26–52 years old, with an average age of 36 years old. All controls had no history of liver, kidney and blood diseases, as well as other tumors, and had no recent history of medication.

Fasting serum was collected from subjects in group I early in the morning, and fasting serum was collected from all patients with ovarian masses early in the morning before and after the operation. All patients with epithelial ovarian cancer underwent satisfactory primary cytoreductive surgery, and the diameters of the residual tumors were all < 1 cm. Patients received 4–8 courses of combined chemotherapy after the operation every three weeks for one course of treatment. Fasting serum was collected early in the morning before each chemotherapy session.

2.1.2. Main reagents and instruments

Smac Kit (Tianjin WoSunBio Technology Co., Ltd.), HE4 electrochemiluminescence Immunoassay kit (Tianjin WoSunBio Technology Co., Ltd.), CA125 electrochemiluminescence immunoassay kit (Tianjin WoSunBio Technology Co., Ltd.), enzyme micro-plate reader (Hangzhou MultiSciences [Lianke] Biotech Co., Ltd.), microplate washer (Beijing Qianming Gene Technology Co., Ltd.), high-speed constant-temperature centrifuger (Eppendorf, Germany), Roche Cobase601 fully automatic electrochemiluminescence immunoassay system (Roche, Switzerland), and ELISA data analysis software ReaderFit (Hangzhou Emerald Biotech Co., Ltd.).

2.2. Methods

2.2.1. Sampling

Fasting serum was collected from all subjects early in the morning: 2 ml blood was collected using a blood collection tube without anticoagulant, coagulated at

Table 1
The expression levels of Smac, HE4 and CA125 in serum in the three groups ($\overline{X} \pm s$)

Group	Cases	Smac (pg/ml)	HE4 (pmol/l)	CA125 (U/mL)
I group	40	300.78 ± 140.58	45.23 ± 17.94	24.73 ± 11.97
II group	40	283.1 ± 107.13	51.48 ± 20.01	35.85 ± 19.1
III group	60	97.52 ± 43.53	243.76 ± 142.01	316.95 ± 178.83
F		36.208	41.248	68.208
P		0.000	0.000	0.000

room temperature at 25°C for 30 minutes, and centrifuged at 1,500 rpm for 15 minutes. The serum was collected using a 0.5 ml EP tube, packed, placed in a refrigerator, and preserved at −80°C for testing. In the experiment, serum Smac was detected using enzyme-linked immunosorbent assay (ELISA), and serum HE4 and CA125 were detected using electrochemiluminescence immunoassay (ECLIA). All procedures were strictly carried out according to manufacturer's instructions.

2.2.2. Detection methods

The serum samples were centrifuged, serum HE4 and CA125 levels were detected by ECLIA, and the analysis was performed using the Roche Cobase601 full automatic ECLI system (Roche). Serum Smac was detected by sandwich ELISA and the data were analyzed using data analysis software ReaderFit (Hangzhou Emerald Biotech Co., Ltd.). All procedures were strictly conducted according to manufacturer's instructions. All controls were within the scope stated in the instructions. The positive values of serum Smac were determined according to the ROC curve. In this experiment, Smac $<$ 135.62 pg/ml was defined as positive, while the positive values of HE4 and CA125 were determined based on the reference range provided by the kit, wherein HE4 $>$ 140 pmol/L and CA125 $>$ 35 U/mL was defined as positive. Tumor marker levels higher than the cut-off value were defined as positive. In the combined detection, when either marker was above the cut-off value, the result was determined as positive.

2.3. Statistical methods

Data was analyzed using statistical software SPSS22.0. Categorical data were evaluated using t-test. Count data were evaluated between groups using X^2-test. With postoperative pathological results as the gold standard of diagnosis had a contrast from the control group and benign ovarian ectopic cysts group, the ROC curves for serum Smac, HE4 and CA125 were drawnto determine the best critical value of serum Smac in EAOC. The sensitivity and specificity of the single and combined detection of these three indicators were calculated. $P < 0.05$ was considered statistically significant.

Table 2
Clinical value of single or combination of Smac, HE4 and CA125 in the diagnosis of EAOC

Index	Sensitivity (%)	Specificity (%)
Smac	90.00	92.68
HE4	81.67	98.75
CA125	86.67	76.25
Smac + HE4	93.33	95.00
Smac + CA125	96.67	76.25
HE4 + CA125	88.33	75.00
Smac + HE4 + CA125	98.33	75.00

3. Results

3.1. The expression levels of Smac, HE4 and CA125 in serum in the three groups

The serum levels of Smac, HE4 and CA125 were detected in groups I–III (Table 1).

3.2. ROC curve determines the cut-off value of Smac in the diagnosis of epithelial ovarian cancer

With groups I + II as the control group, the ROC curve of Smac was drawn. The area under the curve of Smac (Az) = 0.972 ($P < 0.05$; 95% CI: 0.950, 0.997). When Smac was 135.62 pmol/L, the maximum of the Youden index was 0.825. The Az of HE4 = 0.967, SAz $<$ 0.05, 95% CI was (0.933, 0.962), and the maximum of the Youden index was 0.825. The Az of CA125 = 0.873, SAz $<$ 0.05, 95% CI was (0.786, 0.961), and the maximum of the Youden index was 0.700. It can be observed that the Az of CA125 was greater than the Az of HE4 and Smac (Fig. 1).

3.3. Comparison of the sensitivity and specificity of single or combination of Smac, HE4 and CA125 in the diagnosis of EAOC

Smac had the highest (90%) sensitivity for single detection, which was higher than that of the single and

Table 3
Comparison of the positive rates of serum Smac, HE4 and CA125 in different FIGO stages of EAOC

Classification	Smac (%)	HE4 (%)	CA125 (%)
I + II group (16 cases)	87.50 (14 cases)	81.25 (13 cases)	75.00 (12 cases)
III + IVgroup (44 cases)	93.18 (41 cases)	81.82 (36 cases)	90.90 (40 cases)

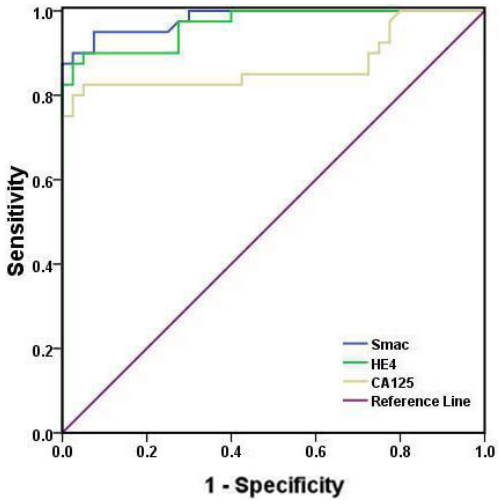

Fig. 1. ROC curve of Smac, HE4, CA125.

combined detection of HE4 and CA125, but lower than that of the other combined detection. HE4 has the highest specificity, which was up to 98.75%; and this was significantly higher than that of the single and combined detection of the other two. After the combination of each two of serum Smac, HE4 and CA125, the sensitivity was lower than that of the combination of these three, and the specificity was lower than that of the highest among the three single indicators. However, the sensitivity and specificity of the combination of Smac and HE4 were relatively high, and the sensitivity of Smac + HE4 + CA125 was the highest (98.33%), which was higher than that of all others, but the specificity was only 75% (Table 2).

3.4. *Comparison of the positive rates of serum Smac, HE4 and CA125 in different FIGO stages of EAOC*

The 60 patients with EOC were divided into two groups according to FIGO staging: stage I + II group and stage III + IV group. The positive rates of Smac, HE4 and CA125 were calculated, respectively. Smac had the highest positive rate among the three indicators in both the stage I + II and stage III + IV groups, followed by HE4; while the positive rate of CA125 was significantly higher in the stage III + IV group than in the stage I + II group (Table 3).

3.5. *The correlation of Smac, HE4 and CA125 with clinicopathological factors in EAOC*

In EAOC, serum Smac was not correlated with age, pathological type, the presence of ascites, NLR and PLR; but was closely related to FIGO staging and lymph node metastasis. In addition, the lower the Smac level was, the later the staging was, and the higher the rate of lymph node metastasis became. Serum HE4 level was not correlated with the presence of ascites, lymph node metastasis, NLR and PLR; but was correlated to age, FIGO staging and pathological type. In addition, serum HE4 level was significantly higher in patients who were > 55 years old, had clear-cell carcinoma and were at stages III–IV, compared with other patients. CA125 was not correlated with age, the presence of ascites, lymph node metastasis and PLR; but was correlated to FIGO staging, pathological type and NLR. In addition, the later the staging was, the higher the level of inflammation was, and the higher the CA125 level became. CA125 level in patients with clear-cell carcinoma was significantly higher than patients with other types of carcinoma (Table 4).

3.6. *Comparison of serum levels of Smac, HE4 and CA125 before and after the operation and during chemotherapy in EAOC*

Serum Smac, HE4 and CA125 before and after the operation were showed in Table 5.

The serum levels of Smac (pg/ml), HE4 and CA125 before and after the operation and after 1–6 courses of treatment are shown in Table 6. Smac level gradually increased with the increase in the number of chemotherapy courses, while HE4 and CA125 levels gradually decreased. Smac level increased to the normal range at the end of the second course of treatment, while HE4 and CA125 levels decreased to its normal ranges at the end of the second and third course of treatment, respectively (Table 6).

4. Discussion

Ectopic endometrial cells implanting and surviving outside the uterine cavity is related to changes in the

Table 4
The correlation of Smac, HE4 and CA125 with clinicopathological factors in EAOC

Clinicopathological factors	n	Smac (pg/ml)	P	HE4 (pmol/l)	P	CA125 (U/mL)	P
Age							
$\leqslant 55$	34	102.38 ± 56.31	0.372	178.50 ± 85.07	0.015	255.16 ± 206.53	0.427
> 55	26	92.37 ± 27.96		262.19 ± 168.35		300.18 ± 200.10	
FIGO staging							
I–II	16	162.00 ± 79.42	0.000	156.79 ± 58.13	0.000	174.59 ± 133.07	0.016
III–IV	44	87.70 ± 26.72		303.82 ± 165.67		311.97 ± 207.87	
Pathological type							
Clear	46	102.10 ± 50.80	0.254	247.85 ± 164.76	0.037	304.19 ± 213.47	0.042
Other	14	87.91 ± 21.30		188.34 ± 97.44		192.2 ± 160.38	
Ascites							
Yes	37	101.90 ± 52.13	0.336	233.84 ± 175.99	0.342	298.50 ± 209.94	0.107
No	23	90.83 ± 30.26		200.34 ± 91.98		205.28 ± 179.02	
Neutrophil-to-lymphocyte ratio (NLR)							
< 2.62	30	173.91 ± 72.16	0.631	199.81 ± 96.10	0.373	182.49 ± 165.74	0.028
$\geqslant 2.62$	30	130.38 ± 47.33		231.11 ± 170.62		308.48 ± 209.24	
Platelet-to-lymphocyte ratio (PLR)							
< 173	28	90.64 ± 28.36	0.247	193.16 ± 94.52	0.172	224.73 ± 191.26	0.262
$\geqslant 173$	32	105.24 ± 56.46		240.82 ± 169.58		289.96 ± 208.66	
Lymph node metastasis							
Yes	25	76.43 ± 22.85	0.015	229.55 ± 157.54	0.282	290.60 ± 196.901	0.191
No	35	107.88 ± 50.72		192.39 ± 89.45		220.28 ± 205.46	

Table 5
Comparison of expression levels of Smac, HE4 and CA125 before and after the operation

Group	Cases	Smac (pg/ml)	HE4 (pmol/L)	CA125 (U/mL)
Before the operation	60	97.52 ± 45.53	243.76 ± 142.01	316.95 ± 178.83
After the operation	60	126.05 ± 74.01	117.72 ± 97.51	138.06 ± 103.83
t		-4.36	4.803	4.456
P		0.000	0.000	0.000

Table 6
Comparison of serum levels of Smac, HE4 and CA125 during chemotherapy in EAOC

Index	Before the operation	After the operation	1	2	3	4	5	6
Smac (pg/ml)	70.23	102.52	116.34	186.42	218.07	298.18	354.92	395.36
HE4 (pmol/L)	242.70	201.48	154.92	104.16	78.60	56.13	32.40	30.62
CA125 (U/mL)	369.7	187.64	102.39	64.56	25.74	18.63	11.26	6.18

immune environment of the peritoneal fluid, decrease the ability of immunocytes to induce apoptosis [13,14], and has the potential to become cancer cells. A number of studies have revealed that in both endometriosis cyst and ovarian cancer, immunocytes in peripheral blood and peritoneal fluid exhibit abnormal functions, and the expression of apoptosis-related genes is also abnormal [15,16]. A chromosome microsatellite analysis revealed that ovarian cancer and endometriosis have a common candidate gene [17]. A variety of gene alterations associated with tumor formation have been found in endometriotic tissues from patients with endometriosis accompanied by ovarian cancer, but few of these gene alterations have been found in simple endometrial tissues [18]. There is a decrease in immunocyte-induced apoptosis of ectopic endometrial cells in EAOC, and it may be one of the reasons for the development of endometriosis to EAOC. Therefore, serum tumor markers related to apoptosis may be more valuable in the early diagnosis and monitoring of the condition of EAOC. This study aims at investigate the role of these three EAOC tumor markers by detecting the serum levels of these indicators.

4.1. CA125

CA125 is the most common serological marker for the diagnosis of ovarian cancer, which has high sensitivity and low specificity; and CA125 only has a 50% positive rate in early EOC. In this experiment, the com-

parison of results of the three groups of serum detection revealed that the serum level of CA125 was significantly higher in the EAOC group than in the other two groups, and the difference was statistically significant ($P < 0.05$). The serum level of CA125 also slightly increased in the benign group, and the reason may be related to endometriosis; but the difference between the benign group and healthy group was not statistically significant ($P > 0.05$). Therefore, serum CA125 can be used for the identification of benign and malignant EAOC, but is easily interfered by other factors. The changes in NLR and PLR respectively reflected the changes in the ratios of neutrophils/platelets to lymphocytes. As biomarkers of inflammation in the body, these two can directly reflect the inflammatory state and immune level of the body [19]. Serum CA125 is related to FIGO staging, pathological type and NLR. The later the stage is, the higher the level of inflammation is, and the higher the CA125 becomes. Although CA125 can be used for the identification of benign and malignant ovarian tumors, its specificity is low, and it is also elevated in benign patients and healthy people. Therefore, it should be combined with other auxiliary examinations and individual conditions in the diagnosis.

4.2. HE4

HE4 is overexpressed in ovarian cancer, is the only approved serum marker for ovarian cancer diagnosis in the past 25 years by the FDA, and its specificity for diagnosis is high [20]. At present, a number of studies have confirmed that HE4 plays an important role in the diagnosis and follow-up monitoring of ovarian cancer. However, in recent years, the clinical application of HE4 reveals that the 5-year survival rate of ovarian cancer patients remains at approximately 28%. The reason may be that the HE4 level in the body is also affected under some situations, in which age and smoking are the direct influencing factors [21], and postmenopausal status and renal function status can also affect the level of HE4 in the body [22]. The increase in HE4 may be related to the proliferation and apoptosis inhibition of ovarian cancer cells. Serum HE4 and HE4 mRNA levels in ovarian cancer tissues reflect the clinical progress and prognosis of ovarian cancer to a certain extent, and the overexpression of HE4 mRNA can be used as a marker of poor prognosis for ovarian cancer [23]. In a study, the expression of HE4 and CA125 was detected in peritoneal fluid in endometriosis patients; and the result revealed that the levels of these two in peritoneal fluid were significantly higher in the untreated group than in the control group. Different from CA125, HE4 is unaffected by menstrual cycles, is a tumor marker of endometriosis that is superior to CA125, and may become the diagnosis index of early endometriosis canceration [10]. However, in the present study, no significant difference in serum HE4 levels between the endometriosis group and control group was found. The relationship between serum HE4 and the clinic pathological factors of EAOC were further analyzed. The results revealed that serum HE4 was related with age, FIGO staging and pathological type; and HE4 significantly increased when patients were > 55 years old and were at stage III-IV. However, it was not correlated with the presence of ascites, lymph node metastasis, NLR and PLR. This indicates that serum HE4 level is unaffected by inflammation. Furthermore, this suggests that serum HE4 can be used to assess preoperative conditions and guide staging.

4.3. Smac

There are immune abnormalities and immune escape in ovarian cancer. The reason may be that the major genes that control apoptosis have become abnormal [24]. Smac is an apoptosis-promoting factor, which can promote the apoptosis of tumor cells by specifically binding with the inhibitor of apoptosis proteins. Dobrzycka first discovered Smac in EOC serum in 2015, and found that the serum level of Smac was significantly lower in EOC patients than in healthy controls, and that it was negatively correlated with tumor stage and pathological grade [11]. The present study revealed that the serum expression of Smac was low in EAOC, but high in the other two groups; and the difference was statistically significant. This can be used for identification between benign and malignant ovarian tumors. However, no difference was found between the benign and control groups. The positive rates of serum Smac, HE4 and CA125 among different FIGO stages of EAOC were further compared. The results revealed that Smac has a higher positive rate in the early and late stages than HE4 and CA125. Therefore, it has important clinical value for the early detection of EAOC.

4.4. The combined detection of the three indicators was used to monitor the curative effect and predict recurrence and metastasis

CA125 and HE4 are the most frequently used tumor markers in postoperative condition monitoring. Gener-

ally, after three courses of treatment, these falls back to the normal range. However, for the monitoring of recurrent ovarian cancer, HE4 increases at 5–8 months before the increase in CA125, which can better predict EAOC recurrence [25]. The present study revealed that the difference in the expression levels of these three indicators in serum before and after operation was statistically significant, the expression level of Smac was higher after the operation than before the operation, and HE4 and CA125 were contrary to this trend. Smac increased to normal levels at the end of the second course of treatment, while HE4 and CA125 decreased to normal levels at the end of the second and third course of treatment, respectively. These were consistent with the results of studies conducted by scholars.

In summary, the combination of CA125 and HE4 has been widely used in the diagnosis and monitoring of the prognosis for ovarian cancer, which remedies the limitations in the simple application of these two. There is no effective index for the early diagnosis of endometriosis-associated canceration. In the present study, by detecting the serum level of Smac, it was revealed that Smac has a certain screening value for early EAOC. The detection value of serum Smac combined with CA125 and HE4 for predicting the recurrence, monitoring of conditions and evaluation of curative effect for EAOC should be further studied, in order to discover the best indicator for the diagnosis of early EAOC, improve prognosis and improve the quality of life of patients.

Acknowledgments

Science and Technology Fund of Tianjin Health Bureau (2015KZ78), Science and Technology Research Projects of Tianjin Health and Family Planning Commission (16KG113).

References

[1] S. Park et al., Apigenin induces ROS dependent apoptosis and ER stress in human endometriosis cells, *J Cell Physiol* (2017).

[2] J.L. Liu and M. Zhao, A PubMed-wide study of endometriosis, *Genomics* **108**(3–4) (2016), 151–157.

[3] J.S. Neto et al., Cellular, histologic, and molecular changes associated with endometriosis and ovarian cancer, *J Minim Invasive Gynecol* **21** (2014), 55–63.

[4] C.L. Pearce et al., Association between endometriosis and risk of histological subtypes of ovarian cancer: A pooled analysis of case-control studies, *Lancet Oncol* **13** (2012), 385–394.

[5] L.N. Heidemann et al., The relation between endometriosis and ovarian cancer – a review, *Acta Obstet Gynecol Scand* **93** (2014), 20–31.

[6] S. Wang et al., Prognostic analysis of endometrioid epithelial ovarian cancer with or without endome-triosis: A 12-year cohort study of Chinese patients, *Am J Obstet Gynecol* **209**(3) (2013), 241.e1–9.

[7] S. Wang et al., Clinica-l analysis of ovarian epithelial carcinoma with coexisting pelvic endometriosis, *Am J Obstet Gynecol* **208**(5) (2013), 413.e1–5.

[8] A. Babic et al., Predictors of pretreatment CA125 at ovarian cancer diagnosis: A pooled analysis in the Ovarian Cancer Association Consortium, *Cancer Causes Control* **28**(5) (2017), 459–468.

[9] F.M. Reis et al., Biomarkers of pelvic endometriosis, *Rev Bras Ginecol Obstet* **39**(3) (2017), 91–93.

[10] B. Mckinnon et al., Comparison of ovarian cancer markers in endometriosis favours HE4 over CA125, *Mol Med Rep* **12**(4) (2015), 5179–84.

[11] B. Dobrzycka et al., Prognostic significance of pretreatment VEGF, survivin, and Smac/DIABLO serum levels in patients with serous ovarian carcinoma, *Tumour Biol* **36**(6) (2015), 4157–4165.

[12] C. Du et al., Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition, *Cell* **102**(1) (2000), 33–42.

[13] V. Vetvicka et al., Regulation of apoptotic pathways during endometriosis: From the molecular basis to the future perspectives, *Arch Gynecol Obstet* **294**(5) (2016), 897–904.

[14] L. Jiang et al., Decreased cytotoxicity of peripheral and peritoneal natural killer cell in endometriosis, *Biomed Res Int* (2016), 2916070.

[15] X.Y. Liu et al., Expressions of Livin and PTEN in Cancerous Tissues of Ovary Endometriosis, *Sichuan Da Xue Xue Bao Yi Xue Ban* **47**(4) (2016), 512–515.

[16] E. Haidarali et al., Evaluation of the pathogenesis of tumor development from endometriosis by estrogen receptor, P53 and Bcl-2 immunohistochemical staining, *Asian Pac J Cancer Prev* **17**(12) (2016), 5247–5250.

[17] A.H. Prowse et al., Molecular genetic evidence that endometriosis is a precursor of ovarian cancer, *Int J Cancer* **119** (2006), 556–562.

[18] N. Krawczyk et al., Endometriosis-associated malignancy, *Geburtshilfe Frauenheilkd* **76**(2) (2016), 176–181.

[19] A. Badora-Rybicka et al., Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio before chemotherapy as potential prognostic factors in patients with newly diagnosed epithelial ovarian cancer, *ESMO Open* **1**(2) (2016), e000039.

[20] S.P. Gong et al., Diagnostic value of CA125, HE4 and Copenhagen Index in differentiating benign from malignant epithelial ovarian tumors, *Nan Fang Yi Ke Da Xue Xue Bao* **37**(5) (2017), 628–632.

[21] R.E. Gislefoss et al., HE4 as an early detection biomarker of epithelial ovarian cancer, *Int J Gynecol Cancer* **25**(9) (2015), 1608–1615.

[22] J. Kappelmayer et al., Human epididymis protein 4 (HE4) in laboratory medicine and an algorithm in renal disorders, *Clinica Chimica Acta* **438** (2015), 35–42.

[23] V. Nisenblat et al., Blood biomarkers for the non-invasive diagnosis of endometriosis, *Cochrane Database Syst Rev* **5** (2016), CD012179.

[24] M. Pal et al., Tumor-priming converts NK cells to memory-like NK cells, *Oncoimmunology* **6**(6) (2017), e1317411.

[25] T. Granato et al., HE4 in the differential diagnosis of ovarian masses, *Clinica Chimica Acta* **446** (2015), 147–155.