PRACE ORYGINALNE
ginekologia

Clinical application of HE4 and CA125 in ovarian cancer Type I and Type II detection and differential diagnosis

Wartość diagnostyczna HE4 i CA125 w wykrywaniu i różnicowaniu typu I i II raka jajnika

Emilia Gąsiorowska[1], Marcin Michalak[1], Wojciech Warchoł[2], Agnieszka Lemańska[1], Piotr Jasiński[3], Marek Spaczyński[1], Ewa Nowak-Markwitz[1]

[1] Klinika Onkologii Ginekologicznej Uniwersytetu Medycznego w Poznaniu, Polska
[2] Katedra i Zakład Biofizyki Uniwersytetu Medycznego w Poznaniu, Polska
[3] Pracownia Patomorfologiczna Ginekologiczno-Położniczy Szpital Kliniczny w Poznaniu, Polska

Abstract

Objectives: *The aim of this study was to assess the sensitivity and specificity of HE4 in detecting and differentiating between types I and II epithelial ovarian cancer (EOC) in comparison with CA125.*

Material and methods: *We measured HE4 and CA125 serum concentrations in 206 samples taken from patients operated in Gynecologic Oncology Department due to ovarian tumors. Ovarian cancer was confirmed in 89 cases divided into type I and type II. 52 healthy patients without any gynecological disease formed the control group. The sensitivity and specificity for type I and type II EOC detection and differentiating between both types was evaluated for HE4 and CA125.*

Results: *The HE4 and CA125 serum concentrations were significantly higher in type II than in type I EOC (p=0.008696, p=0.000243 respectively).The HE4 and CA125 sensitivity for type I and benign tumors differentiation was 63.16% for both of them and specificity was 87.29% vs 67.89% respectively. For CA125 these differences did not reach statistical significance. The HE4 sensitivity and specificity for type II and benign tumors differentiation were 87.14% and 96.61%, respectively, and for CA125 these values were 82.86% and 94.07%, respectively.*

Conclusions: *Pretreatment analysis of HE4 serum concentration is superior to CA125 in differential diagnosis of ovarian cancer subtypes (I and II). HE4 is superior to CA125 in detecting ovarian cancer type II. Neither HE4 nor CA125 is an effective diagnostic tool for type I ovarian cancer detection. A new highly specific and highly sensitive tumor marker for type I EOC is needed.*

Key words: **ovarian cancer** / **type I and type II** / **tumor marker** / **HE4** / **CA125** /

Address for correspondence:
Emilia Gąsiorowska
Klinika Onkologii Ginekologicznej, Uniwersytetu Medycznego w Poznaniu
Polska, 60-535 Poznań, ul. Polna 33
Tel. +48 61 8419271
E-malL: emilia.gasiorowska@gmail.com

Otrzymano: **20.03.2014**
Zaakceptowano do druku: **14.05.2014**

Streszczenie

Cel pracy: *Celem pracy było określenie czułości i swoistości białka HE4 w wykrywaniu i różnicowaniu typu I i II raka jajnika (EOC) w porównaniu z CA125.*

Materiał i metody: *Stężenia HE4 oraz CA125 zostały zmierzone w próbkach surowicy krwi pobranej od 206 pacjentek operowanych w Klinice Onkologii Ginekologicznej z powodu guzów jajnika. Rak jajnika został potwierdzony w 89 przypadkach podzielonych na typ I i II EOC. Grupę kontrolną utworzyły 52 zdrowe pacjentki bez schorzeń ginekologicznych. Została określona czułość i swoistość HE4 oraz CA125 w wykrywaniu oraz różnicowaniu typu I i II EOC.*

Wyniki: *Stężenia HE4 i CA125 były istotnie wyższe w typie II niż w typie I EOC (p=0,008696, p=0,000243). Czułość w różnicowaniu typu I EOC i guzów niezłośliwych wynosiła 63,16% dla obydwu markerów, HE4 wykazało swoistość 87,29% a CA125 67,89%. Dla CA125 nie stwierdzono jednak istotności statystycznej. Czułość i swoistość HE4 w różnicowaniu typu II EOC i zmian niezłośliwych wynosiła 87,14% i 96,61%, natomiast dla CA125 wynosiła 82,86% i 94,07%.*

Wnioski: *Przedoperacyjne określenie stężenia HE4 ma większą wartość w różnicowaniu typu I i II raka jajnika niż CA125. HE4 jest lepszym markerem w diagnostyce typu II raka jajnika. Żaden z badanych markerów nie ma zadowalającej czułości i swoistości w wykrywaniu typu I EOC. Do diagnozowania tego typu nowotworu jest potrzebny nowy wysoce czuły i swoisty marker.*

Słowa kluczowe: **rak jajnika** / **typ I i II** / **markery nowotworowe** / **HE4** / **CA125** /

Introduction

In 2004 Kurman introduced a dualistic model of ovarian cancer pathogenesis based on clinical and molecular analysis [1, 2, 3]. In this model EOC is divided into type I and type II. Type I ovarian cancer includes highly differentiated (GRADE 1) serous and endometrial EOC and all grade mucinous and clear cell cancer. In this type mutations in *KRAS, BRAF, PTEN, TFGβR 11* and *β-catenin* genes were detected [1, 2]. Type I ovarian cancer is usually diagnosed in earlier stages, tumor growth is slower and prognosis is better compared to type II ovarian cancer.

Type II EOC consists of poorly differentiated (GRADE 2 and 3) serous and endometrial cancer, ovarian carcinosarcoma and undifferentiated cancer. It is characterized by mutations in *TP53, BRCA1, BRCA2, MLH1* and *MSH2* genes [1, 2]. It constitutes 75% of all ovarian cancer cases and is usually advanced at the time of diagnosis (FIGO stage III and IV). Disease progression is fast, distant metastases appear earlier and five year survival rate is substantially lower compared to type I ovarian cancer.

CA125 is an acknowledged marker for advanced ovarian cancer. Unfortunately, its sensitivity and specificity for early clinical stages is highly unsatisfactory. Moreover, it is often elevated in physiological conditions (e.g. pregnancy) and non-malignant lesions.

HE4 is a protein of Mullerian origin and belongs to the family of serine protease inhibitors. This group of proteins is responsible for increasing the rate of cell-cycle and cell differentiation and thus may play an important role in carcinogenesis and disease progression [4, 5]. HE4 is known to be a more specific early stage (FIGO I i II) ovarian cancer marker than CA125 and therefore HE4 is more useful in differential diagnosis between malignant and non-malignant ovarian tumors [4, 5].

The aim of this study was to evaluate the usefulness and accuracy of HE4 and CA125 in prediction of ovarian cancer type I and type II occurrence, as well as in differential diagnosis between those two types.

Materials and methods

Pretreatment serum samples from 206 patients with ovarian tumors qualified for surgical treatment were collected in Gynecologic Oncology Department of Poznan University of Medical Sciences. In 89 cases EOC was diagnosed. Ovarian cancer staging (in accordance with FIGO classification system) and grading were performed based on standard microscopic examination performed by an experienced pathologist using standard hematoxylin+eosin staining (Table I). Patients diagnosed with ovarian carcinosarcoma with predominant epithelial components confirmed on microscopic examination (type II EOC) were also included into this study (Table I). The study group was divided into two EOC types based on histopathological diagnosis and grading. 19 patients were included into type I EOC subgroup and 70 to type II EOC subgroup (Table III). The rest of 117 operated patients without EOC formed the group of benign tumors (Table II). We also examined serum concentrations of HE4 and CA125 in samples taken from 52 healthy patients without any gynecological disease who formed the control group (Table IV).

Ethics Statement

The current study was designed in 2012 and was started after receiving an approval of Local Bioethics Commission. The study protocol and information for the patients were accepted by the Local Bioethics Commission of the Poznan University of Medical Sciences (No. 695/12). Written informed consent was obtained from all patients enrolled for this study.

The analysis of CA125 and HE4 serum concentrations was performed in the Central Hospital Laboratory Unit following a standard procedure. Blood samples were centrifuged (2000 rpm, 10 minutes, at 4°C) to separate the serum from the blood cells. Serum HE4 and CA125 levels were quantitatively measured by ECLIA on Roche Cobas System. Electrochemiluminescence (ECL) is a specific chemiluminescence reaction on the electrode surface induced by the electrochemical reaction. The serum HE4

Table I. Characteristics of the study group.

Study group		n=89
Age (years): Median (range)		54 (30-78)
FIGO Stage	I-II	17
	III-IV	37
Histology	Serous	49
	Mucinous	6
	Endometrial	15
	Clear cell cancer	4
	Undifferentiated	12
	Carcinosarcoma	3
Grading	G1	13
	G2	27
	G3	49

Table II. Characteristics of the benign group.

Benign group	n=117
Age (years) Median (range)	43(15-80)
Endometrial cysts	40
Serous cysts	29
Mature teratomas	20
Fibroadenomas	6
Adenomas	10
Borderline tumors	12

Table III. Histology of EOC type I and II.(According to the proposed by Kurman division).

Epithelial Ovarian Cancer Type I	Number of patients n= 19	Epithelial Ovarian Cancer Type II	Number of patients n = 70
Serous G1	7	Serous G2 or G3	42
Endometrial G1	2	Endometrial G2 or G3	13
Clearcell	4	Undifferentiated	12
Mucinous	6	Carcinosarcoma	3

Table IV. Characteristic of the control group-healthy patients without gynecologic diseases.

Age (median) [years]	Age (range) [years]	Number of patients
47	20-75	n=52

and CA125 levels were measured according to the instructions of the manufacturer. HE4 detection kits, a CA125 quantitative determination kit, calibrators (HE4 CalSEt and CA125IICalSet) and controls (Elecsys PreciControl HE4 level 1, level 2 and Elecsys PreciControl Tumor Marker level 1, level 2) were provided by Roche Diagnostics GmbH.

Statistical analysis

For all statistical calculations and analyses MedCalc and Statistica software were used.

First step in statistical analysis was evaluation of data distribution in all data sets. This procedure was done by the use of Lillefors test. As distribution of all data sets appeared not to be normal, logarithm transformation procedure was applied to overcome limitations caused by non-normal distribution. After normalization of data sets, Kruskal-Wallis non-parametric ANOVA was performed. ANOVA could not be used because assessment of variances done by the use of Levene's test did not confirmed equality of variances. As a post-hoc test for Kruskal-Wallis non-parametric ANOVA, was used multiple range Duncan's test.

Correlation analysis was performed after normalization procedure. Sperman's test was used in this analysis.

Results

HE4 and CA125 distributions in analysed populations are shown on Figures 1 and 2. (Figure 1, Figure 2). For type I EOC the median of HE4 serum concentration was 85 pmol/l (range 40-121 pmol/l) whereas for type II EOC it equaled 621 pmol/l (range 58-6930 pmol/l) and the difference between both types was statistically significant (p=0.08696). Sensitivity and specificity of HE4 differentiation between the types was 85.71% and 94.74%. The medians of CA125 serum concentrations for EOC type I and type II were 45 IU/ml (range 9-309 IU/ml) and 936 IU/ml (range 27-5000 IU/ml), respectively. This difference was also statistically significant (p=0.000243) with 74.29% sensitivity and 94.74% specificity. In the benign group, the median of HE4 serum concentration equaled 52 pmol/l (range 32-212pmol/l) and for CA125 it was 25 IU/ml (range 7-745 IU/ml) In the control group, the median of HE4 was 46 pmol/l (range 29-81 pmol/l) and for CA125 17 IU/ml (range 5-232 IU/ml). (Table V, VI, VII).

We compared sensitivity and specificity of HE4 and CA125 serum concentrations in diagnosing type I and type II epithelial ovarian cancer. There was a statistically significant difference in HE4 serum concentration between the benign group and ovarian cancer type II (p=0.000000) with 87.14% sensitivity and 96.61% specificity. The difference was also significant between the control group and Type II EOC (p=0.000000) with 91.43% sensitivity and 100% specificity. In contrast, the difference between the control group and the benign group was not significant (p=0.497074) with 63.6% sensitivity and 58.82% specificity. HE4 was able to separate the benign group from type I EOC (p=0.011954), but its utility was poor because of low 63.16% sensitivity and 87.29% specificity. A significant difference in HE4 concentration was also observed between the control healthy group and type I EOC (p=0.000515) with better 89.47% sensitivity and 75.00% specificity for EOC detection. (Table VI, VII)

On the contrary, the differences in CA125 concentrations between EOC type I and benign ovarian tumors proved insignificant. However, we did observe a statistically significant

Table V. HE4 and CA125 serum concentrations in the benign group (patients with non- malignant ovarian tumors), control group, type I and type II EOC.

	CA125 [IU/ml]		HE4 [pmol/l]	
	Median	**Range**	**Median**	**Range**
Type I EOC	45	9-309	85	40-121
Type II EOC	936	27-5000	621	58-6930
Benign group	25	7-745	52	32-212
Control group	17	5-232	46	29-81

Table VI. Differences in HE4 and CA125 serum concentrations between type I EOC patients, type II EOC patients, the control and benign groups (Kruskall -Wallis test).

CA 125		**HE 4**	
Group	***p***	**Group**	***p***
Type I *vs* Type II	0.000243	**Type I *vs* Type II**	0.008696
Type I *vs* benign group	**0.425338**	**Type I *vs* benign group**	**0.011954**
Type I *vs* control group	0.009973	**Type I *vs* control group**	0.000515
Type II vs benign group	0.000000	**Type II vs benign group**	0.000000
Type II vs control group	0.000000	**Type II vs control group**	0.000000
Benign vs control group	0.103213	**Benign vs control group**	0.497074

Table VII. Sensitivity and specificity of HE4 and CA125 for ovarian cancer type I and type II detection in comparison to benign and control groups.

	CA125		HE4	
	Sensitivity (%)	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**
Type I Vs control	73.68	78.85	89.47	75.00
Type II Vs control	94.29	98.08	91.43	100
Type I Vs benign	63.16	67.80	63.16	87.29
Type II Vs benign	82.86	94.07	87.14	96.61
Type I Vs Type II	74.29	94.74	85.71	94.74
Benign Vs control	28.81	96.08	63.6	58.82

difference between the control group and type I EOC (p=0.009973) and the CA125 sensitivity and specificity reached 73.68% and 78.85%, respectively. We also calculated the 82.86% sensitivity and 84.07% specificity of CA125 in differentiating between type II EOC and the benign group (p=0.000000) and between the control group and type II EOC cohort sensitivity and specificity equaled 94.29% and 98.08%, respectively (p=0.000000). The differences between the control and benign cohort for CA125 were insignificant. (p=0.103213) (Table VI, VII).

Discussion

The analysis of numerous ovarian cancer biomarkers provided a conclusion that an ideal single specific marker for early ovarian cancer detection has not yet been found [6, 7].

CA125 is an already acknowledged ovarian cancer biomarker and, despite its low specificity, it is widely used in differential diagnosis of ovarian tumors (malignant vs nonmalignant).

Recently, a brand new marker, HE4, has been introduced into clinical practice as it was proven to significantly increase the accuracy of ovarian tumor differential diagnosis [8, 9].

In 2012 Molina et al. proved that the sensitivity of CA125 and HE4 in ovarian cancer detection was similar and reached 79% and 83%, respectively, but HE4 was more specific (99%) compared to CA 125 (71%) [12]. Another study revealed sensitivity and specificity of HE4 of 73% and 88%, respectively, and concluded that it was the best single marker for early (stage I) EOC detection [13]. These results were consistent with the data provided by meta-analyses of Yang and Ferraro [14, 15]. Nevertheless, in all these studies the effectiveness of HE4 in EOC detection was still insufficient to establish it a single screening tool. Therefore, bearing in mind the variety of histological types of ovarian cancer, we decided to determine if its sensitivity and specificity differed between those types. We divided our patients for type I and type II EOC according to the model proposed by

Kurman in 2004 based on ovarian cancer pathogenetic origin. In our study CA125 and HE4 serum concentrations appeared to be significantly higher in patients who suffered from ovarian cancer type II than in those suffering from type I. Moreover, HE4 was more sensitive in ovarian cancer type II prediction compared to CA125 and HE4 serum concentration did not depend on the clinical stage of the disease. These results were confirmed by Kristjansdottir et al. in 2013 [17]. What is worth emphasizing, CA125 appeared not to be applicable for ovarian cancer type I detection due to its poor sensitivity and specificity. For HE4, the specificity in type I EOC detection was slightly higher but still poor.

Due to our knowledge and available papers the lack of CA125 usefulness in ovarian cancer type I detection has not yet been reported and confirmed. Unfortunately, the available scientific information did not explain why HE4 ability to detect type I EOC is limited. Results coherent with ours were obtained by Kristjansdottir et al. who concluded that the diagnostic performance of neither CA125 nor HE4 was satisfactory for type I EOC and benign cohort differentiation [17]. The mechanisms of pathogenesis of certain types of ovarian cancer still remain unclear [12, 14]. The type of cells releasing HE4 have not yet been identified and the signalling pathways that control HE4 gene expression and regulate antigen release in ovarian cancer cells are unknown. However, it has been discovered that HE4 is a protein of müllerian origin and, according to many studies, it is overexpressed in the tissues that derive from müllerian ducts as well as in type II EOC tissue which histologically resemble the müllerian epithelium rather than ovarian epithelium [1, 2]. Curiously, in type I EOC, which differs histologically from the müllerian epithelium, HE4 expression is minor and this provides a plausible explanation for its low concentration in type I EOC patients [1, 2].

Recently published data revealed a strong correlation between elevated HE4 serum level and fast disease progression and established HE4 serum concentration an independent prognostic factor in ovarian cancer [10]. This is consistent with our results as we proved that HE4 correlates better with type II EOC that is characterized by poorer survival rates [2, 4, 17] than in type I.

Moreover, a strong negative correlation between high serum concentration of HE4 and the feasibility of initial cytoreductive surgery has been reported [10]. Therefore, the possibility of ovarian cancer subtype evaluation before surgery can prove essential for optimal therapy planning. As type II EOC is often a spread disease at the moment of first diagnosis, optimal cytoreduction is less feasible than in the case of local or limited disease. Application of neoadjuvant chemotherapy in the cases of initially inoperable cancer may make optimal cytoreduction achievable which, in turn, is a crucial prognostic factor in EOC.

The results of our study clearly confirm the purposefulness of pre-operative HE4 serum level determination in patients with ovarian tumors. HE4 proved to be a useful biomarker in type II EOC detection and in pre-operative EOC type prediction. However, it has limited applicability in ovarian cancer type I detection, similarly to CA125 which fails to accurately diagnose type I EOC. Therefore, due to authors' opinion, our results indicate the need to search a new more efficient type I EOC single marker and it should be used in combination with HE4.

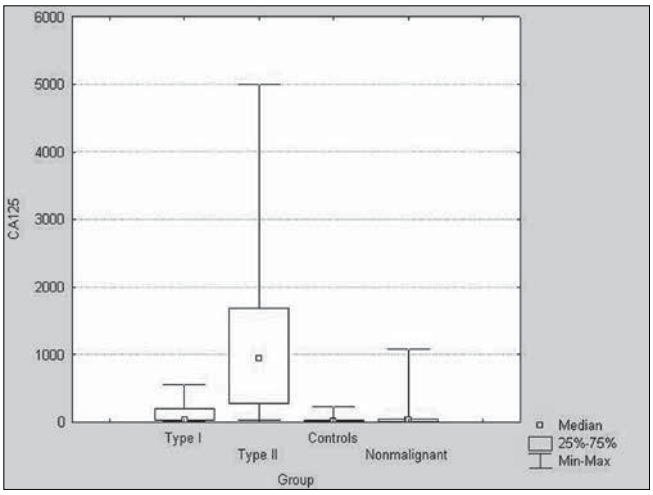

Figure 1. Graphic presentation of CA125 distribution in analysed populations, were: Type I – Type I ovarian cancer, Type II – Type II ovarian cancer, Controls – Healthy Controls, Nonmalignant – Nonmalignant ovarian tumor.

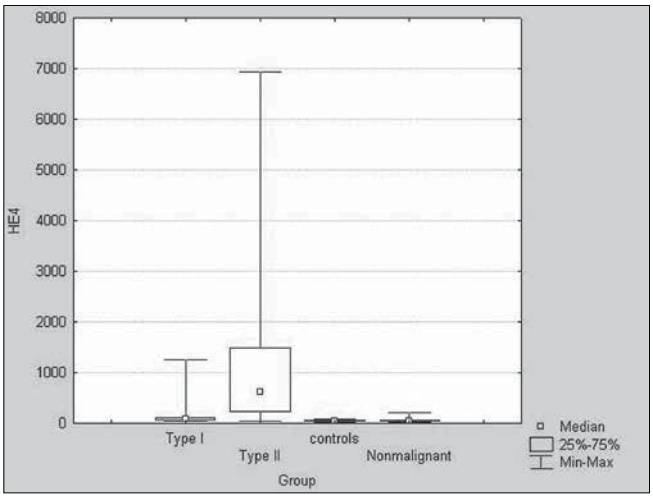

Figure 2. Graphic presentation of HE4 distribution in analysed populations, were: Type I – Type I ovarian cancer, Type II – Type II ovarian cancer, Controls – Healthy Controls, Nonmalignant – Nonmalignant ovarian tumor.

Conclusions

Pretreatment analysis of HE4 serum concentration is superior to CA125 in differential diagnosis of ovarian cancer subtypes (I and II) and thus may contribute to better therapy planning.

HE4 is superior to CA125 in detecting ovarian cancer type II. Neither HE4 nor CA125 is an effective diagnostic tool for type I ovarian cancer detection. A new highly specific and highly sensitive tumor marker for type I EOC is needed.

Oświadczenie autorów:

1. Emilia Gąsiorowska – autor koncepcji i założeń pracy, zebranie materiału, opracowanie i interpretacja wyników analiz statystycznych, przygotowanie manuskryptu i piśmiennictwa – autor zgłaszający i odpowiedzialny za manuskrypt.
2. Marcin Michalak – interpretacja wyników, korekta manuskryptu.
3. Wojciech Warchoł – wykonanie analizy statystycznej wyników.
4. Agnieszka Lemańska – tłumaczenie pracy na język angielski.
5. Piotr Jasiński – wykonanie badań histopatologicznych.
6. Marek Spaczyński – uzyskanie funduszy na realizację badań laboratoryjnych, przechowywanie dokumentacji, ostateczna akceptacja manuskryptu.
7. Ewa Nowak-Markwitz – współautor założeń pracy, ostateczna weryfikacja i akceptacja manuskryptu.

Źródło finansowania:

Część projektu finansowanego z badań statutowych Uniwersytetu Medycznego w Poznaniu – nr: 502011110140000257.

Konflikt interesów:

Autorzy nie zgłaszają konfliktu interesów oraz nie otrzymali żadnego wynagrodzenia związanego z powstawaniem pracy.

References

1. Shih IeM, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. *Am J Pathol.* 2004, 164 (5), 1511-1518.
2. Kurman RJ, Shih IeM. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. *Am J Surg Pathol.* 2010, 34 (3),433-443.
3. Nowak-Markwitz E, Spaczyński M. Ovarian cancer – modern approach to its origin and histogenesis. *Ginekol Pol.* 2012, 83 (06), 454-457.
4. Drapkin R, von Horsten HH, Lin Y, [et al.]. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Res.* 2005, 65 (6), 2162-2169.
5. Andersen MR, Goff BA, Lowe KA, [et al.]. Use of a Symptom Index, CA125, and HE4 to predict ovarian cancer. Gynecol Oncol. 2010, 116 (3), 378-383.
6. Nolen B, Marrangoni A, Velikokhatnaya L, [et al.]. A serum based analysis of ovarian epithelial tumorigenesis. *Gynecol Oncol.* 2009, 112 (1), 47-54.
7. Nolen B, Velikokhatnaya L, Marrangoni A, et al., Serum biomarker panels for the discrimination of benign from malignant cases in patients with an adnexal mass. *Gynecol Oncol.* 2010 Jun;117(3):440-5.
8. Havrilesky LJ, Whitehead CM, Rubatt JM, [et al.]. Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence. *Gynecol Oncol.* 2008, 110 (3), 374-382.
9. Partheen K, Kristjansdottir B, Sundfeldt K. Evaluation of ovarian cancer biomarkers HE4 and CA-125 in women presenting with a suspicious cystic ovarian mass. *J Gynecol Oncol.* 2011, 22, 4, 244-252.
10. Angioli R, Plotti F, Capriglione S, [et al.]. Can the preoperative HE4 level predict optimal cytoreduction in patients with advanced ovarian carcinoma. *Gynecol Oncol.* 2013, 128 (3), 579-583.
11. Kong SY, Han MH, Yoo HJ, [et al.]. Serum HE4 level is an independent prognostic factor in epithelial ovarian cancer. *Ann Surg Oncol.* 2012, 19 (5), 1707-1712.
12. Molina R, Escudero JM, Augé JM, [et al.]. HE4 a novel tumour marker for ovarian cancer: comparison with CA 125 and ROMA algorithm in patients with gynaecological diseases. *Tumour Biol.* 2011, 32 (6), 1087-1095.
13. Moore RG, Brown AK, Miller MC, [et al.]. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. *Gynecol Oncol.* 2008, 108 (2), 402-408.
14. Yang Z, Wei C, Luo Z, [et al.]. Clinical value of serum human epididymis protein 4 assay in the diagnosis of ovarian cancer: a meta-analysis. *Onco Targets Ther.* 2013, 23, 6, 957-966.
15. Ferraro S, Braga F, Lanzoni M, [et al.]. Serum human epididymis protein 4 vs carbohydrate antigen 125 for ovarian cancer diagnosis: a systematic review. *J Clin Pathol.* 2013, 66 (4), 273-281.
16. Yang J Sa M, Huang M, [et al.]. The reference intervals for HE4, CA125 and ROMA in healthy female with electrochemiluminescence immunoassay. *Clin Biochem.* 2013, 46 (16-17), 1705-1708.
17. Kristjansdottir B, Levan K, Partheen K, Sundfeldt K. Diagnostic performance of the biomarkers HE4 and CA125 in type I and type II epithelial ovarian cancer. *Gynecol Oncol.* 2013, 131 (52-58).