Diagnostic value of CA125, HE4, ROMA and logistic regression model in pelvic mass diagnostics – our experience

Wartość diagnostyczna CA125, HE4, ROMA oraz modelu regresji logistycznej w diagnistyce guzów miednicy mniejszej – doświadczenia własne

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Abstract

Objectives: *The aim of this study was to compare and evaluate the quality of CA125, HE4, logistic regression model based on CA125 and HE4, and ROMA algorithm in preoperational differential diagnostics of the ovarian tumors.*

Material and methods: *To the study 110 patients enrolled. Based on histopatological examination of removed tumors, they were divided into study group (56 cancer patients) and control one (nonmalignant 54 patients). Serum CA125 and HE4 concentrations were measured following a standard procedure.*

Results: *A commonly accepted referential value for CA125 is 35 IU/ml. In our study, this cut-off value yielded very low sensitivity and specificity results (85.2% and 63.6%, respectively).*

When we adopted HE4 normal value to be 140 pM,the sensitivity and specificity obtained in the investigated population was 68.5% and 94.6%, respectively.

When the cut-off value for HE4 was adopted as 74 pM, the sensitivity improved considerably (88.9%), but specificity decreased to 85.7%. In case of CA125 when we adopted Ca125 normal value to be 77 IU/ml, the sensitivity and specificity obtained in the investigated population was 81.5% and 83.6%, respectively. In analysis based on combination of biomarkers, the highest sensitivity was obtained for the logistic regression model based on CA125 and HE4 (89.5%). A little bit lower sensitivity was achieved for HE4 used as a single diagnostic test (88.9%). The highest specificity was observed for ROMA algorithm (94.5%). This means that ROMA algorithm is the best diagnostic tool to differentiate between the malignant and non-malignant ovarian tumors.

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Conclusions:

1. *ROMA algorithm yielded the highest specificity and slightly lower sensitivity in the case of differential diagnosis between malignant and non-malignant ovarian tumors. Therefore, it should become a basic tool in the ovarian tumors diagnosis prior to a surgery.*
2. *HE4 as a single diagnostic test (based on one marker) was found to be better suited to the ovarian tumor differential diagnosis than CA125 test.*
3. *Combined test, based on double marker analysis, should be applied and then the risk of the ovarian cancer should be calculated. This approach is more effective than single marker analysis.*

Key words: **ovarian cancer** / **ROMA** / **HE4** / **CA 125** /

Streszczenie

Cel pracy: *Celem niniejszego badania było porównanie i ocena wartości CA125, HE4, modelu regresji logistycznej oraz algorytmu ROMA w przedoperacyjnej diagnostyce różnicowej guzów przydatków.*

Materiał i metody: *Do badania włączono 110 pacjentki, które na podstawie wyniku badania histopatologicznego usuniętych guzów podzielono na grupę badaną (56 pacjentek z nowotworami złośliwymi) i grupę kontrolną (54 pacjentek ze zmianami niezłośliwymi). Oznaczenie osoczowych stężeń CA125 oraz HE4 wykonano zgodnie z standardową procedurą.*

Wyniki: *Powszechnie uznaną wartością graniczną dla CA125 jest 35 IU/ml. W naszym badaniu, przyjęcie tej wartości punktu odcięcia zaowocowało niskimi wartościami czułości i swoistości – odpowiednio 85,2% oraz 63,6%.*

Wyjściowo uznaliśmy wartość 140 pM jako punkt odcięcia (wartość sugerowana przez producenta). Dla takiego punktu odcięcia, czułość i swoistość osiągnęły odpowiednio wartość 68,5% i 94,6%. Gdy wartość punktu odcięcia dla HE4 została zmieniona na 74 pM, czułość testu wzrosła do 88,9% a swoistość zmniejszyła się do 85.7%. W przypadku CA125 zmiana wartości punktu odcięcia na 77 IU/ml spowodowała spadek czułości do 81,5% przy jednoczesnym wzroście swoistości do 83,6%. W analizach obejmujących jednocześnie obydwa markery (CA 125 i HE4), model oparty na regresji logistycznej osiągnął najwyższą czułość (89,5%). Niewiele mniejszą wartość czułości osiągnął test oparty na oznaczeniu HE4 (88,9%). Natomiast najwyższą wartość swoistości osiągnął algorytm ROMA (94,5%). Oznacza to że algorytm ROMA jest najlepszym narzędziem diagnostycznym w różnicowaniu złośliwych i niezłośliwych guzów jajnika.

Wnioski:

1. *Algorytm ROMA osiągnął najwyższą wartość swoistości i niewiele niższą wartość czułości jako narzędzie diagnostyczne w różnicowaniu złośliwych od niezłośliwych guzów jajnika. Dlatego powinien zostać podstawowym narzędziem diagnostycznym przed planowanym leczeniem chirurgicznym.*
2. *HE4 jako pojedynczy test diagnostyczny osiągnął wyższe wartości czułości i swoistości w porównaniu z CA 125.*
3. *Jednoczesne oznaczanie dwóch markerów (CA 125 i HE4) oraz obliczanie ryzyka wystąpienia nowotworu złośliwego jest zalecanym postępowaniem u pacjentek z guzami przydatków. Analizy oparte na dwóch markerach są bardziej efektywne niż analizy oparte na pojedynczych markerach.*

Słowa kluczowe: **rak jajnika** / **algorytm ROMA, HE4** / **CA 125** /

Background

Ovarian cancer is the fourth cause of death from malignant neoplasms in women and a leading cause of death from a gynaecologic cancer. The highest incidence of ovarian cancer is observed in highly developed countries in North America, Europe, Australia and New Zealand [1].

Despite intensive research, ovarian cancer is still the greatest challenge in the gynaecologic oncology. Lack of effective screening system and asymptomatic onset of the disease mean that the ovarian cancer is usually diagnosed only at advanced clinical stage. Treatment results are still unsatisfactory. About 70-75% of the ovarian cancer cases are diagnosed at FIGO stage III and IV.

The overall survival (OS) in the advanced ovarian cancer patients reaches about 46%. In contrary, 5 year OS in the patients diagnosed at early stages is 94%. [2-4]

The most important prognostic factor for the ovarian cancer is optimal cytoreduction and correct surgical staging. Considering the technical requirements of an abdominal surgery, surgical cytoreduction should be performed in designated centers by well-trained and experienced staff [3, 5].

Given the technical difficulties of advanced ovarian cancer surgery, the methods for detecting and diagnosing the ovarian cancer at early clinical stages seem to be greatly desired. They should enable optimal cytoreduction prior to a surgical treatment and improve the overall survival. To this end, a number of tests based on biochemical markers have been introduced to the clinical practice. These tests should enable the detection of the ovarian cancer at its early stages [6,7].

The most commonly analyzed ovarian cancer biomarker is CA125, despite its low sensitivity at early stages of the disease.

Due to its low sensitivity, CA125 is not recommended for the ovarian cancer screening. It is a recommended biomarker only in the follow-up and diagnosis of the ovarian cancer relapse. Only high-risk ovarian cancer population is the target population for bimodal screening based on CA125 and annual transvaginal ultrasound [8, 9].

CA125 is a particularly ineffective biomarker of the ovarian cancer in premenopausal women. This is due to high incidence of uterine myomas, endometrial cysts and serous cysts in this group that are also manifested by increased CA125 serum concentration, thus producing false positive results in the screening tests [8].

One of the most promising new biomarkers seems to be HE4. The sensitivity of HE4 is much higher than CA125, and its increase by more than 50% was observed in the ovarian cancer patients with normal range of CA125 [10-12].

However, the literature data indicate that HE4 is not a perfect biomarker. Its concentration increased in the case of lung cancer, endometrial cancer and renal failure, thus lowering HE4 specificity and producing false positive results [13, 14].

HE4 protein is a member of WFDC protein family (Whey acidic protein Four Disulfide Core) and its most likely biological activity is an inhibition of serine proteases. This protiein was discovered in the epithelium of distal part of epididymis. HE4 is also expressed in the epithelium that lines the respiratory and genital tract. High expression of HE4 was reported in the ovarain cancer. This discovery was a milestone in recognizing HE4 as an ovarian cancer biomarker, especially in a differential diagnosis of pelvic masses [10, 15].

HE4 reveals 78.6% sensitivity and 95% specificity in the differential diagnosis between the ovarian cancer and ovarian endometriosis (endometrioma or chocolate cysts) [16].

The concept of significant increase of CA125 and HE4 in the malignant ovarian cancer, has been strongly supported by literature reports [10, 11, 16-23].

An introduction of ROMA *(Risk of Ovarian Malignancy Algorithm)* into the clinical practice is a huge step forward in the ovarian cancer detection. This algorithm enables an assessment of individual ovarian cancer risk. Calculation of the risk is based on CA125 and HE4 serum concentration and hormonal status (premenopausal or menopausal) of the patient. Application of the ROMA algorithm improved the sensitivity and specificity of combined diagnostic test, compared to separate analyses of CA125 and HE4 [14, 16, 19, 24].

Aim of the study

The aim of this study was to compare and evaluate the quality of CA125, HE4, logistic regression model based on CA125 and HE4, and ROMA algorithm in preoperational differential diagnostics of the ovarian tumors.

Material and methods

To the study enrolled 110 patients operated on in the Gynecologic Oncology Department of Poznan University of Medical Sciences due to ovarian tumors. Blood samples were collected from all the patients before surgery, after obtaining their written consents. Based on histopathological examination of the removed tumors, performed by an experienced pathologist, the patients were divided into two groups: study group diagnosed with ovarian carcinoma (n=56, median age 54 years, range 30-78 years) and control group diagnosed with benign tumors (n=54, median age 43 years, range 15-73 years). Serum CA125 and HE4 concentrations were measured in the Central Hospital Laboratory Unit by ECLIA COBAS System, following a standard procedure (Roche Diagnostics GmbH)

An individual risk of ovarian cancer occurrence was measured for each patient based on CA125 and HE4 levels, and was compared with the histopathological examination results after the surgery. The risk was calculated according to ROMA algorithm with menopausal status (on-line version [7]) and according to a logistic regression model without menopausal status.

ROMA algorithm was calculated as per the following formulae [44]:

$$\text{Risk (\%)} = \exp(PI)/[1+\exp(PI)]*100, :$$
$$\text{Exp(PI)} = e^{PI} \text{ and}$$

1. For the patients before the menopause:
   PI=-12.0+2.38*ln[HE4]+0.0626*ln[CA125],
2. For the patients after the menopause:
   PI=-8.09+1.04*ln[HE4]+0.732*ln[CA125],
   ln – natural logarithm e = 2.7182818

ROMA algorithm results above the cut-off suggest high risk of ovarian cancer occurrence:

1. ROMA ≥ 11.4% for women after the menopause,
2. ROMA ≥ 29.9% for women before the menopause

Table I. Characteristics of the study group – age of the patients enrolled in the study.

	Study group (n=56)	Control group (n=54)	*p*
Median age	54 years	43 years	0,01
Range	30-78 years	15-73 years	-

Table II. Characteristics of the study group – histopathology.

Study group (n=56)		Control group (n=54)	
Diagnosis	**Number of patients (%)**	**Diagnosis**	**Number of patients (%)**
Serous carcinoma	31 (55.3%)	Serous cyst	23 (42.6%)
Undifferentiated carcinoma	9 (16.1%)	Endometrial cyst	17 (31.5%)
Endometrial carcinoma	5 (9%)	Mature teratoma	7 (13.0%)
Clear cell carcinoma	5 (9%)	Adenoma	6 (11.1%)
Mucinous carcinoma	3 (5.3%)	Fibroadenoma	1 (1.8%)
Carcinosarcoma	3 (5.3%)		

The most common ovarian cancer types in the study group were serous carcinoma (55.3%) and undifferentiated carcinoma (16.1%).

In the control group, the most common were serous cysts (42.6%) and endometrial cysts (31.5%).

Table III. Ovarian cancer FIGO stage in the study cohort.

FIGO	N
I – II	17 (31.5%)
III - IV	37 (68.5%)

The results above show that the study group was composed of 68.5% advanced FIGO stage patients and 31.5% low-stage patients. Statistical analysis, logistic regression model and ROC curves were calculated using MedCalc (MedCalc Software bvba, Belgium, version 14.8.1).

Results

Sensitivity and specificity were calculated based on CA125 and HE4 serum concentration in the patients enrolled into the study. Then ROC curves were drawn and presented in Figure 1. The figure shows ROC curves for CA125, HE4, logistic regression model based on CA125 and HE4, and ROMA algorithm.

The most accurate tool to compare and validate the resulting ROC curves is AUC (Area Under Curve). This parameter is calculated automatically during an analysis performed by statistical software used in data analysis.

Table IV. Tabular presentation of ROC curve parameters that enable comparison of the applied diagnostic tests.

Diagnostic test	AUC	SE	95% CI	*p*
ROMA	0.940	0.0227	0.877 to 0.976	<0.0001
CA125 + HE4	0.939	0.0220	0.876 to 0.976	<0.0001
HE4	0.939	0.0221	0.876 to 0.976	<0.0001
CA125	0.889	0.0316	0.814 to 0.941	<0.0001

AUC – Area Under Curve
SE – Standard Error
95% CI – 95% Confidence Interval

This tabular presentation of ROC curve parameters revealed the highest AUC value for ROMA algorithm. This means that ROMA algorithm showed the highest diagnostic value in this study. Slightly lower AUC value was obtained for the logistic regression model (based on CA125 and HE4) and HE4. The lowest AUC was determined for CA125, making it the lowest quality diagnostic tool used in the differential diagnosis of the ovarian tumors (malignant or non-malignant).

Based on the ROC curve analysis, sensitivity and specificity of the investigated tests were calculated. Results were assembled and presented in Table V below.

Sensitivity and specificity for CA125 presented in Table V was calculated for a cut-off value of 77 IU/ml. The cut-off value for HE4 was 74 pM.

A commonly accepted referential value for CA125 is 35 IU/ml. In our study, this cut-off value yielded very low sensitivity and specificity results (85.2% and 63.6%, respectively). This may explain high rate of false positive results observed in the clinical practice.

Table V. Tabular presentation of sensitivity, specificity, and likelihood for positive and negative results.

Diagnostic test	Sensitivity	Specificity	LR +	LR -
ROMA algorithm	83.3 %	94.5 %	15.14	0.18
Logistic regression model CA125+HE4	89.5 %	85.2 %	6.04	0.12
CA 125	81.5 %	83.6 %	4.98	0.22
HE4	88.9 %	85.7 %	6.22	0.13

LR+ Likelihood Ratio for positive results
LR- Likelihood Ratio for negative results

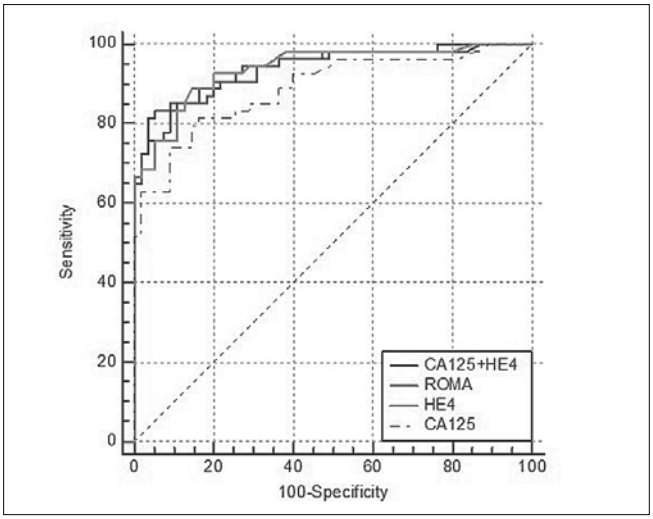

Figure 1. ROC curves for CA125, HE4, ROMA algorithm and logistic regression model based on CA125 and HE4.

In the case of HE4, the manufacturer recommends the evaluation and setting the normal values individually for each analytical laboratory that uses HE4 kits. When we adopted HE4 normal value to be 140 pM (recommended initial value of the cut-off point), the sensitivity and specificity obtained in the investigated population was 68.5% and 94.6%, respectively.

When the cut-off value for HE4 was adopted as 74 pM, the sensitivity improved considerably (88.9%), but specificity decreased to 85.7%.

In practice, if the cut-off value was 140 pM, high rate of false negative results would be produced. This means that many of the ovarian cancer patients would not be diagnosed and the risk of the ovarian cancer would be considered low.

According to the results presented in Table 5, the highest sensitivity was obtained for the logistic regression model based on CA125 and HE4 (89.5%). A little bit lower sensitivity was achieved for HE4 used as a single diagnostic test (88.9%). The highest specificity was observed for ROMA algorithm (94.5%). This means that ROMA algorithm is the best diagnostic tool to differentiate between the malignant and non-malignant ovarian tumors.

Discussion

This study showed an imperfection of both biomarkers (CA125 and HE4), manifested by the presence of false positive results. The rate of false positive results was 16% for CA125 and 14% for HE4. Endometriosis remains a serious problem in the differential diagnosis of the ovarian tumors, as positive results were obtained in 40% of the patients (CA125 cut-off value of 77 IU/ml). When the cut-off value was 35 IU/ml, the false positive results rate reached 66.7%.

In the case of HE4, false positive results were obtained in 20% of the patients. Our data regarding CA125 were comparable with the previously published reports (33%) [25]. On the other hand, 20% false positive result rate for HE4 did not match the results published for the Spanish , South Korean, and Italian population [26-28]. These data did not confirm the HE4 increase in endometriosis [27, 28].

The cut-off value for HE4 of 74 pM was approved not only following the sensitivity and specificity analysis of our own material, but also after an analysis of the previously published data. In the reviewed papers, the cut-off value varied from 70 to 75.75 pM [29-36].

The data published by other teams were ambiguous. Our data, obtained for a general population, indicated that ROMA algorithm based on CA125 and HE4 was superior to the analysis based on a single marker. Similar results were presented by Bandiera, Kim, Moore and Sandri [17, 19, 26, 36-38].

Other teams reported that the advantage of a double marker algorithm was limited to either premenopausal or postmenopausal population [30, 34, 36, 39]. The papers published by Montagana and Jackob questioned any advantage of two-marker protocols over HE4 [32, 40].

The sensitivity of ROMA algorithm in our study was 83.3%. Similar results were obtained by Kim (88%) and Montagnana (75%), but higher values were reported by Moore, Jackob and Bandiera (94%, 90% and 91%, respectively).

In our study ROMA algorithm specificity reached 94.5%. Similar value was observed by Kim (94%), but Moore, Montagnana, Jackob and Bandiera claimed lower specificity of the test (75%, 82%, 87% and 83%, respectively). The reason for this discrepancy could not be just the HE4 cut-off value. In all the reviewed works it was between 70 and 74 pM.

Contrary to HE4, the CA125 cut-off value was fixed at 35 IU/ml. When CA125 cut-off value was changed to 77 IU/ml, sensitivity was improved, but as ROMA algorithm does not account for the reference values, its sensitivity and specificity cannot be altered by simply changing the reference values.

Discrepancies in the results published by different teams could be caused by different sets of pathological types of the ovarian cancer available. An increase in HE4 serum concentration was described only in serous, endometrial and clear cell types of the ovarian cancer [41].

A likelihood ratio analysis enabled more individual interpretation of the obtained data. This parameter revealed how strong were the positive (LR+) or negative (LR-) results in the confirmation (LR+) or exclusion (LR-) of the ovarian cancer risk, calculated individually for four analyzed diagnostic tests.

Positive results of ROMA test mean a 15 times increase in the ovarian cancer risk (LR+ = 15.14) and positive results of HE4 test indicate 6.22 times increase (cut-off value 74 pM).

A likelihood ratio for negative results provides an answer to the question by how many times do the negative results decrease the risk of the ovarian cancer. Logistic regression based on CA125 and HE4 test showed the risk of the ovarian cancer was reduced by 8.3 times. Similar results were obtained for the single HE4 test. True negative results of HE4 test reduced the risk of the ovarian cancer by 7.7 times.

The worst results for all the analyzed diagnostic tests were obtained for CA125, even when its cut-off value was 77 IU/ml. CA125 yielded the worst results in the LR positive test – positive test results caused only 4.9 times increase in the ovarian cancer risk. Similarly, negative result of CA125 test reduced an individual ovarian cancer risk by only 4.5 times.

A correlation between CA125 and HE4 was weak (data not presented), but it was important from a practical point of view. Our analysis showed that sensitivity, specificity, LR+ and LR- were higher in the diagnostic tests based on two-marker analysis (ROMA and Logistic regression model). The sensitivity of ROMA algorithm is particularly important from the practical perspective. This parameter enabled and justified narrowing of the vast population of patients with positive results to a very limited group of patients at high risk of the ovarian cancer who really needed an invasive procedure (surgery) to confirm the ovarian cancer diagnosis. This means that the greatest benefit of ROMA algorithm was a reduction of false positive results and lower number of unnecessary surgical procedures.

The calculated values of AUC (0.94 for ROMA, 0.939 for HE4, and 0.889 for CA125) were very close to those previously published and available in original papers and meta-analyses [36, 40, 42].

The problem of early detection of the ovarian cancer remains unsolved. However, an application of diagnostic algorithms based on multi-marker models may improve the sensitivity and specificity of the diagnostic tests. Considering contrasting results and different conclusions presented by various research teams, the postulated superiority of ROMA algorithm over other diagnostic tests remains an open question. Ultimate benefits from using ROMA algorithm as a screening test are unclear and require further studies.

Conclusions

1. ROMA algorithm yielded the highest specificity and slightly lower sensitivity in the case of differential diagnosis between malignant and non-malignant ovarian tumors. Therefore, it should become a basic tool in the ovarian tumors diagnosis prior to a surgery.
2. HE4 as a single diagnostic test (based on one marker) was found to be better suited to the ovarian tumor differential diagnosis than CA125 test.
3. Combined test, based on double marker analysis, should be applied and then the risk of the ovarian cancer should be calculated. This approach is more effective than single marker analysis.

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References:

1. Jemal A, Siegel RXJ, Sala E. Cancer statistics CA. *Cancer J Clin.* 2010, 60 (5), 277-300.
2. Earle C.C, [et al.]. Effect of surgeon specialty on processes of care and outcomes for ovarian cancer patients. *J Natl Cancer Ins.* 2006, 93, 172-180.
3. Engelen MJ, Kos HE, Willemse PH, [et al.]. Surgery by consultant gynecologic oncologist improves survival in patients with ovarian carcinoma. *Cancer.* 2006, 106 (3), 589-598.
4. Paulsen T, Kjaerheim K, Kaern J, [et al.]. Improved short-term survival for advanced ovarian, tubal and peritoneal cancer patients operated at teaching hospitals. *Int J Gynecol Cancer.* 2006, 16, Suppl 1, 11-17.
5. Hoskins WJ, McGuire WP, Brady MF, [et al.]. The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with suboptimal residual epithelial ovarian carcinoma. *Am J Obstet Gynecol.* 1994, 170 (4), 974-979.
6. Eland FR, Desimone CP, Seamon LG, [et al.]. Effectiveness of a multivariate index assai In the preoperative assessment of ovarian tumors. *Obstet Gynecol.* 2011, 117 (6), 1289-1297.
7. Visintin I, Feng Z, Longton G, [et al.]. Diagnostic markers for early detection of ovarian cancer. *Clin Cancer Res.* 2008, 14 (4), 1065-1072.
8. Malkasian GD, [et al.]. Preoperative evaluation of serum CA 125 levels in premenopausal and postmenopausal patients with pelvic masses: discrimination of benign from malignant disease. Am J Obstet Gynecol. 1988, 159, 341-346.
9. Sturgeon CM, Duffy MJ, Stenman UH, [et al.]. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast and ovarian cancer. *Clin Chem.* 2008, 54 (12), 11-79.
10. Hellström I, Reycraft J, Hayden-Ledbetter M, [et al.]. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. *Cancer Res.* 2003, 63 (13), 3695-3700.
11. Moore RG, Brown AK, Miller MC, [et al.]. The use of multiple novel tumor biomarkers for detection of ovarian carcinomain patients with pelvic mass. *Gynecol Oncol.* 2008, 2, 402-408.
12. Shah CA, Lowe KA, Paley P, [et al.]. Influence of ovarian cancer risk status on the diagnostic performacne of the serum biomarkers mesothelin, HE4 and CA125. *Cancer Epidemiol Biomark Prev.* 2009, 18 (5), 1365-1372.
13. Escudero JM, Aule JM, Filella X, [et al.]. The utility of serum human epididymis protein 4 (HE4) in patients with malignant and non malignant diseases: comparison with CA125. *Clin Chem.* 2011, 57 (11), 1534-1544.
14. Hertlein L, Stieber P, Kirschenhofer A, [et al.]. Human epididymis protein (HE4) in benign and malignant diseases. *Clin Chem Lab Med.* 2012, 50 (12), 2181-2188.
15. Kirchhoff C. Molecular characterization of epididymal proteins. *Rev Reprod.* 1998, 3, 86-95.
16. Huhtinen K, Suvitie P, Hiissa J, [et al.]. Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. *Br J Cancer.* 2009, 100 (8), 1315-1319.
17. Bandiera E, Romani C, Specchia C, [et al.]. Serum human epididymis protein 4 and risk for ovarian malignancy algorithm as new diagnostic and prognostic tools for epithelial ovarian cancer management. *Cancer Epidemiol Biomark Prev.* 2011, 20, 2496-2506.
18. Molina R, Escudero JM, Auge JM, [et al.]. HE4 a novel tumor marker for ovarian cancer: comparison with CA 125 and ROMA algoritm in patients with gynaecological diseases. *Tumor Biol.* 2011, 32, 1087-1095.
19. Moore RG, MCMeekin DS, Brown AK, [et al.]. A novel multiple marker bioassay utilizing HE4 and CA 125 for the prediction of ovarian cancer in patients with a pelvic mass. *Gynecol Oncol.* 2009, 112, 40-46.
20. Moore RG, Jabre-Raughley M, Brown AK, [et al.]. Comparison of a novel multiple marker assay vs Risk of Malignancy Index for the prediction of epithelial ovarian cancer in patients with a pelvic mass. *Am J Obstet Gynecol.* 2010, 203, 228-236.
21. Nowak-Markwitz E, Spaczyński M. Ovarian cancer – modern approach to its origin and histogenesis. *Ginekol Pol.* 2012, 83 (06):454-457.
22. Park Y, Lee JH, Hong DJ, [et al.]. Diagnostic performances of HE4 and CA125 for the detection of ovarian cancer from patients with various gynecologic and non-gynecologic diseases. *Clin Biochem.* 2011, 44 (10-11), 884-888.
23. Park Y, Kim Y, Lee EY, [et al.]. References 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.
24. Lenhard M, Stieber P, Hertlein L, [et al.]. The diagnostics accuracy of two human epididymis protein 4 (HE4) testing systems in combination wih CA125 in the differential diagnosis of ovarian masses. *Clin Chem Lab Med.* 2011, 49 (12), 2081-2088.
25. Ortiz-Munoz B, Aznar-Oroval E, Garcia Garcia A, [et al.]. HE4, Ca125 and ROMA algorithm for differential diagnosis between benign gynaecological diseases and ovarian cancer. *Tumor Biol.* 2014, 35 (7), 7249-7258.
26. 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, 117, 440-445.
27. Chung SH, Lee SY, Ju W, Kim S.C. Clinical efficacy of serum human epididymis protein 4 as a diagnostics biomarker of ovarian cancer: A pilot study. *Obstet Gynecol Sci.* 2013, 56 (4), 234-241.
28. Anastasi E, Granato T, Falzarano R, [et al.]. The use of HE4, CA125 and CA72-4 biomarkers for differential diagnosis between ovarian endometrioma and epithelial ovarian cancer. *J Ovarian Res.* 2013, 6 (1), 44.
29. Abdel-Azeez HA, Labib HA, Sharaf SM, Refai AN. HE4 and mesothelin: novel biomarkers of ovarian carcinoma in patients with pelvic masses. *Asian Pac J Cancer Prev.* 2010, 11 (1), 111-116.
30. Van Gorp T, Cadron I, Despierre E, [et al.]. 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.
31. Holcom K, Vucetic Z, Miller MC, Knapp RC. Human epididymis protein 4 offers superior specificity in the differentiation of benign and malignant adnexal masses in premenopausal women. *Am J Obstet Gynecol.* 2011, 205 (4), 358.ei-6.
32. Jackob F, Meier M, Caduff R, [et al.]. No benefit from combining HE4 and CA125 as ovarian tumormarkers in a clinical setting. *Gynecol Oncol.* 2011, 121 (3), 487-491.
33. Kondalsamy-Chennakesavan S, Hackethal A, Bowtell D, Obermair A. Diifferantiating stage 1 epithelial ovarian cancere from benign ovarian tumors using a combination of tumor markers HE4, CA125 and CEA and patient's age. *Gynecol Oncol.* 2013, 129 (3), 467-471.
34. Lawicki S, Bedkowska GE, Gacuta-Szumarska E, Szmitkowski M. The plasma concentration of VEGF, HE4 and CA125 as a New biomarkers panel In different stages and sub-types of epithelial ovarian tumors. *J Ovarian Res.* 2013, 6 (1), 45.
35. Montagnana M, Danese E, Ruzzenente O, [et al.]. The ROMA (Risk of Ovarian Malignancy Algorithm)for estimating the risk of ovarian cancer In women with pelvic mass. Is Ti really useful? *Clin Chem Lab Med.* 2011, 49, 521-525.
36. Sandri MT, Bottari F, Franchi D, [et al.]. Comparison of HE4, CA125, and ROMA algorithm in women with pelvic mass: correlation with pathological outcome. *Gynecol Oncol.* 2013, 128 (2), 233-238.
37. Kim YM, Whang DH, Park J, [et al.]. Evaluation of the accuracy of serum human epididymis protein 4 in combination with CA125 for detecting ovarian cancer as prospective case-control study in Korean population. *Clin Chem Lab Med.* 2011, 49, 527-534.
38. Li F, Tie R, Chang K, [et al.]. Does the risk of ovarian cancer malignancy algorithm excel human epididymis protein 4and CA125 in predicting ovarian cancer: a meta-analysis. *BMC Cancer.* 2012, 12, 258.
39. Ruggeri C, Bandiera E, Zanotti L, [et al.]. HE4 and epithelial ovarian cancer: comparison and clinical evaluation of two immunoassays and a combination algorithm. *Clin Chim Acta.* 2011, 412 (15-16), 1447-1453.
40. Montagnana M, Lippi G, Ruzzenente O, [et al.]. The utility of serum human epididymis protein 4 (HE4) in patients with pelvic mass. *J Clin Lab Annal.* 2009, 23, 331-335.
41. 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, 2162-2169.
42. Lin JY, Qin JinBao, Sangvatanakul V. Human epididymis protein 4 for differentia diagnosis between Benin gynecologic disease and ovarian cancer: a systematic review and meta-analysis. *Eur J Obstet Gyecol Reprod Biol.* 2013, 167, 81-85.