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RĪGAS STRADIŅA UNIVERSITĀTE

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DIAGNOSTIC SENSITIVITY AND SPECIFICITY OF BIOMARKERS IN COMBINATION WITH ULTRASONOGRAPHIC FINDINGS AND CLINICAL SYMPTOMS IN OVARIAN CANCER

Summary of promotion thesis for obtaining
a degree of a Doctor of Medicine
Speciality – Gynaecology and Obstetrics

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CONTENT

INTRODUCTION

Relevance of the subject matter of the study

According to The National Health Service data, ovarian cancer incidence from year 2007 to 2010 was 23.0-29.0 cases on 100 000 women each year. Ovarian cancer is diagnosed in advanced stages in 70% of patients. In such cases patients can be cured in only 20-30%, however, when disease is diagnosed in early stages, 5-year survival can be observed in 70-90% of patients (*Jemal et al.,* 2006; *Jemal et al.,* 2007; *Heintz et al.,* 2006). Earlier detection of the disease can be improved by establishing more sensitive and specific ovarian cancer diagnostic tests and algorithms.

In the 80-ties and 90-ties of the 20th century platinum and paclitaxel chemotherapy was introduced and signifficantly improved survival of ovarian cancer patients, but, despite intensive progress in the research and science, there are no noteworthy development in the next decades in chemotherapy for patients suffering from ovarian cancer (*Bookman et al.,* 2009).

In the last years attention has been paid to more agressive surgery that has improved overall survival of ovarian cancer patients. These procedures include comprehensive peritonectomies, splenectomy, bowel resections and pelvic exenteration, that leads to optimal cytoreduction with no macroscopic disease. To facilitate optimal cytoreduction, patients with ovarian cancer should be centralised and treated in specialized oncology centers, that can be achieved by developing algorithm how patients should be stratified using sensitive and specific ovarian cancer detection tests.

Sensitivity and specificity of ovarian cancer associated antigen CA125 is not sufficient, especially in the diagnostics of early stage disease. Ovarian cancer diagnostic can be improved by using standardized ultrasonographic investigation and biomarker detection algorithms. Distinct clinical symptoms, that returns frequently and have appeared in the last year, probably, allow to

diagnose disease earlier, although use of ovarian cancer symptom index can be recomended only in combination with ultrasonographic investigations and biomarker tests.

Diagnostic tests and algorithm with high sensitivity and specificity will allow stratify patients with ovarian tumors and avoid unnecessary surgical manipulations. This aspect is very important in patients with severe comorbidities, when surgical interventions can be replaced with surveillance.

Topicality of the thesis confirms ongoing international trials in early ovarian cancer detection.

Biomarkers and diagnostic tests investigated in the study are analyzed according to previous studies that warrants necessity for further investigations.

Aim of study

To develop a new ovarian cancer diagnostic algorithm using most sensitive and specific biomarkers in combination with clinical symptoms and ultrasonographic findings.

Work assignments

1. To analyze sensitivity and specificity of ovarian cancer symptom index alone and in combination with biomarkers in ovarian cancer diagnostics.
2. To analyze sensitivity and specificity of different risk of malignancy indexes in ovarian cancer diagnostics.
3. To analyze sensitivity and specificity of apolipoprotein A1, transthyretin, transferrin, beta-2-microglobulin, human epidydimis secretory protein-4 and CA125 in ovarian cancer diagnostics.
4. To analyze diagnostic tests with single biomarkers or their combinations in conjunction with ultrasonographic findings and menopausal status.

5. To develop recommendations for biomarker, ultrasonographic findings and ovarian cancer symptom index application in the clinical practice to identify ovarian cancer patients.

Novelty of the study

1. Analysis of sensitivity and specificity of ovarian cancer symptom index in combination with CA125 concentration in the serum.
2. Analysis of different biomarker combinations in ovarian cancer diagnostics.
3. Comparison of different risk of malignancy indexes to develop a new ovarian cancer diagnostic test.
4. Development of a new ovarian cancer risk of malignancy indexes including single biomarkers or combination of biomarkers.
5. Analysis of ability to detect human epidydimis secretory protein 4 (HE4) concentration in urine and sensitivity and specificity in ovarian cancer detection.

Working hypothesis

1. Measurement of several biomarker concentrations improves sensitivity and specificity of ovarian cancer diagnostic tests.
2. Assessment of ovarian cancer symptom index allows to identify women who needs further investigations.
3. Assessment of ultrasonographic apearences in combination with biomarker combinations significantly improves sensitivity and specificity of ovarian cancer detection algorithm.

Practical diagnostic novelty

Assessment of ovarian cancer symptom index by physician will improve earlier patient referral to gynecologist to perform additional inestigations.

Assessment of several biomarker concentrations will improve sensitivity and specificity of diagnostic tests to identify patients with ovarian cancer, furthermore consequent ultrasonographic investigation will allow gynecologist to decide whether patient should be sent to gynecological oncologist or simple gynecologist.

Implementation of uniform and standardized ovarian cancer diagnostic algorithm in clinical practice will improve care of patients suffering from ovarian cancer.

More accurate differentialdiagnosis of ovarian tumors will reduce unnecessary surgical interventions, especially in patients with severe comorbidities.

Personal input

Analysis of literature, development of study design and coordination in the Ethics committee of Riga Stradins university, sample collection, patient survey, patient treatment, sample processing, analysation, analyzis of the results, writing of articles, thesis and promotional work.

Ethical concerns

Study has been approved by the Ethical committee of Riga Stradins University in 12th of February 2009.

1. MATERIALS AND METHODS

1.1. Study design, study groups, inclusion and exclusion criteria

Starting from 15th of February of 2009 till 15th of May of 2011 a prospective case-control study has been conducted. Initially also a pilot project has been performed in which 109 women were included with an aim to select biomarkers who can be analyzed in a wider research groups. After pilot study patient groups were expanded involving 83 patients with malignant ovarian tumor, 77 patients with benign ovarian tumor and 83 women in control group.

All consecutive patients with ovarian tumor who were sent to Riga Eastern University hospital Latvian Oncology center for operative therapy were included in the study.

Patients in the study groups were stratified after surgical intervention into group of patients with benign and malignant ovarian tumors. In the group of benign ovarian tumors also patients with stromal ovarian tumors and mature teratomas were included. Separate analysis for patients with benign endometrioid ovarian tumors and endometriosis was performed. In the control group healthy women who attended gynecologist for prophylactic visits in Riga Eastern University hospital Outpatient unit and voluntary agreed to participate in study either responded to an announcement that was published in the network website www.tvnet.lv were includednas.

Patients were stratified in the study groups after analysis of pathology specimens.

Inclusion criteria for patients with malignant ovarian tumors:

1) a written consent to participate in study;

2) patients regardless of age and menopausal status who were sent to the Latvian Oncology center of Riga Eastern University hospital for surgical treatment and with histologically proven ovarian cancer regardless of stage and tumor differentiation (*Grade*);

3) with no contraindications for surgical treatment;

4) with no oncological diseases in medical history.

Inclusion criteria for patients with benign ovarian tumors:

1) a written consent to participate in the study;

2) patients regardless of age and menopausal status who were sent to Latvian Oncology center of Riga Eastern University hospital for surgical treatment and histologically proven benign ovarian tumor;

3) with no contraindications for surgical treatment;

4) with no oncological diseases in medical history.

Inclusion criteria for women in the control group:

1) patients who attended gynecologist for prophylactic visits in Riga East University hospital Outpatient department;

2) patients who responded to an announcement that was published in a network website www.tvnet.lv to participate in study;

3) a written consent to participate in study;

4) women after age of 40 years regardless to their menopausal status;

5) with no oncological diseases in medical history;

6) without pathological findings during gynecological check-up and ultrasonographic investigation that could influence symptoms and concentration of biomarkers in sera.

Exclusion criteria of women from the control group:

1) women with diagnosed Echo-negative plane cyst more than 2 cm in diameter during gynecological ultrasonography;

2) women with diagnosed uterine fibroids more than 3 cm in diameter during gynecological ultrasonography.

1.2. Patient survey

Patients were asked to participate in the study in the day before the surgery. All patients completed written consent about voluntary involvement in the study, after that inquiry of "Clinical data form of the study" and "Ovarian cancer symptom index" inquiry were also filled.

In inquiry "Clinical data form of the study" information about patient's age, gynecological history, co-morbidities and ultrasonographic findings were included. In "Ovarian cancer symptom index" inquiry questions regarding ovarian cancer symptom index were included.

Transvaginal gynecological ultrasonographic examination was performed to all women in involved in the control group to exclude gynecological pathology. All women which participated in the control group were examined in a telephone interview in 5-18 month period after the study to reassure of their wellbeing.

1.3. Clinical methods of investigation

1.3.1. Assessment of Risk of malignancy index (RMI)

Four different risk of malignancy index models were calculated. These models differ with interpretation of ultrasonographic appearances, menopausal status. Risk of malignancy index is calculated similarly in all models by multiplying ultrasound score „U" with menopausal score „M" and biomarker CA125 concentration in the sera (Table 1.1.).

During ultrasonographic investigation attention was paid to certain ultrasonographic findings: multilocular cyst, solid areas; ascites; bilateral lesions and metastasis.

Table 1.1.

Calculation of four risk of malignancy index models

Risk of malignancy index models	Figure „U" number of ultrasonographic features			Figure „M"	
	none	one	Two and more	premeno-pausal	meno-pausal
RMI1 (*Jacobs et al.*,1990)	0	1	3	1	3
RMI2 (*Tingulstad et al.*, 1996)	1	1	4	1	4
RMI3 (*Tingulstad et al.*,1999)	1	1	3	1	3
RMI4 (*Yamamoto et al.*, 2009)	1	1	4	1	4

Fourth model of Risk of malignancy index (RMI4) had additional variable - the resulting number was multiplied by tumor size (1 if tumor diameter ≤7cm; 2 if tumor diameter >7 cm).

A CA125 concentration in the calculation of Risk of malignancy index was added as absolute concentration in serum (U/ml).

Sensitivity and specificity of Risk of malignancy indexes were calculated at cutoff levels of 200, 300 and 450.

1.3.2. Assessment of Ovarian cancer symptom index

Ovarian cancer symptom index was considered positive if patient had at least one positive symptom recurring at least 12 days per month and which appeared during last 12 months – not earlier. Ovarian cancer symptom index combines fallowing symptoms: abdominal or pelvic pain, increase in abdominal size, abdominal bloating, eating disorders and feeling full quickly *(Goff et al.,* 2007). Number of symptoms included in ovarian cancer symptom index was updated with two symptoms which are very frequently observed in ovarian cancer patients – frequent urination and urgency (*Ovarian cancer symptom consensus statement*, 2007). Urinary symptoms were considered positive if they met criteria proposed by *Goff et al.* – have appeared in period of last 12 month and repeated at least 12 days per month (*Goff et al.,* 2007).

1.4. Laboratory investigations

Concentration of CA125 in sera was determined using automatized analyzer *Abbott Architect* 2000 with matching reagent *Abbott ARCHITECT* CA125 II. For dilution of sera *ARCHITECT* buffer was used to determine CA125 concentration in range of 0-10000 U/ml.

Concentration of apolipoprotein A1 (ApoA1) quantitative determination in sera was performed using immunoturbidometry and automatic analyzer *Roche Cobas* and reagent *Tina-quant Apolipoprotein A-I ver.2*.

Transferrin (TF) concentration has been determined using immunoturbidometry method, using automatized *Roche Cobas* analyzer and reagent *Tina-quant Transferrin ver.2*.

Transthyretin (TT) concentration quantitative determination has been made using immunoturbidometry method, using automatic analyzer *Abbott Architect* and matching reagent set (*Abbott Laboratories*).

Beta-2-microglobulin (β-2-MG) concentration determination in sera has been performed using standardized hemiluminiscence method, using automatic *Siemens* analyzer *Immulite 2000* with matching reagent set (*Siemens Diagnostics*).

Human *epididymis* secreted protein-4 (HE4) has been determined in sera using automatic analyzer *Abbott Architect* with matching set of reagents (*Abbott Diagnostics*).

1.5. Statistical processing

Descriptive statistic was used to characterize study groups. To analyze differences in appearance of symptoms included in the ovarian cancer symptom index *Kruskal-Wallis* test was applied. Separately diagnostic sensitivity and specificity of ovarian cancer symptom index were determined, including and excluding symptoms that were associated with urination.

Sensitivity and specificity of the risk of malignancy indexes were analyzed at different cut-off levels.

Correspondence of mean biomarker concentrations to normal (Gaussian) distribution *Shapiro-Wilk* test was applied. In cases when concentration of biomarkers did not correspond to normal distribution, data normalization was performed using natural logarithm function. Central tendency (mean), standard deviation and differences in the biomarker concentrations among the study groups were analyzed using t-test and 95% confidence interval as well as nonparametric *Mann-Whitney* test.

Biomarkers were analyzed together with ultrasonographic features and menopausal status using multivariate logistic regression analysis and novel diagnostic tests were compared by calculating and comparing area under curve (AUC).

In the multivariate logistic regression analysis ultrasonographic appearances were classified in correspondence to the third model of risk of malignancy index (*Tingulstad et al.*, 1999). To measure differences of diagnostic test accuracies areas under curves were compared using *McNemar* test.

Statistical analysis and graphical data processing was done using professional software SPSS 20.00 (*SPSS Inc.*, ASV).

Sensitivity and specificity were calculated using professional software *Vassarstat* (*Vassar College*, ASV).

The null hypothesis was denied if statistical probability was similar or lower than 0.05.

2. RESULTS

Mean age in the study group was 60.8 years (range: 36–87 years) for ovarian cancer patients, 54.5 years (range: 18–79 years) for patients with benign ovarian tumors and 53.0 years (range: 37–81 years) for women in the control group (p=0.09). High grade, serous adenocarcinoma was most common morphological type in patients with malignant ovarian tumors. In patients with benign ovarin tumors serous cystadenoma was the most common morphological finding (Table 2.1.).

Table 2.1.

Characteristics of tumor morphological types, differentiation and stage distribution in patients included in the study

Patient distribution	Distribution according to morphological tumor type, differentiation and stage	Number of cases	
		abs.	%
Morphological types of malignant tumors (n=83)	Serous adenocarcinoma	71	85.5
	Endometrioid adenocarcinoma	1	1.2
	Mucinous adenocarcinoma	3	3.6
	Clear cell adenocarcinoma	1	1.2
	Borderline epithelial ovarian tumors	7	8.5
Morphological types of benign tumors (n=77)	Serous cystadenoma	36	46.8
	Endometrioid cystadenoma	12	15.6
	Mucinous cystadenoma	10	13.0
	Mature teratoma	7	9.0
	Stromal tumors (tekoma, fibroma)	12	15.6
Tumor differentiation (Grade) (n=76)	Low grade (Grade I)	14	18.4
	Intermediate grade (Grade II)	30	39.5
	High grade (Grade III)	32	42.1
Stage distribution (n=76)	Stage I	16	19.3
	Stage II	5	6.0
	Stage III	49	59.0
	Stage IV	13	15.7

Patients with borderline ovarian tumors were combined in one group with malignant ovarian tumors, but patients with non-epithelial origin (stromal tumors and mature teratomas) were combined in one group with benign epithelial ovarian tumors.

Menopausal patients were more frequently observed in the study group of patients with malignant ovarian tumors, but less frequently among women included in the control group (Table 2.2.).

Table 2.2.

Patient distribution in the study groups according to menopausal status

Menopausal status	Patients with malignant ovarian tumors (n=83; p<0.001*)	Patients with benign ovarian tumors (n=77; p=0.009*)	Control group (n=82; p=0.659*)
Menopause	59 (71.1%)	50 (64.9%)	40 (48.8%)
Premenopause	24 (28.9%)	27 (35.1%)	42 (51.2%)

**Chi-square tests*

2.1. Ovarian cancer symptom index

More frequently symptoms included in the ovarian cancer symptom index were observed in patients with malignant ovarian tumors. In patient group with benign ovarian tumors urinary symptoms were observed most often (Figure 2.1.).

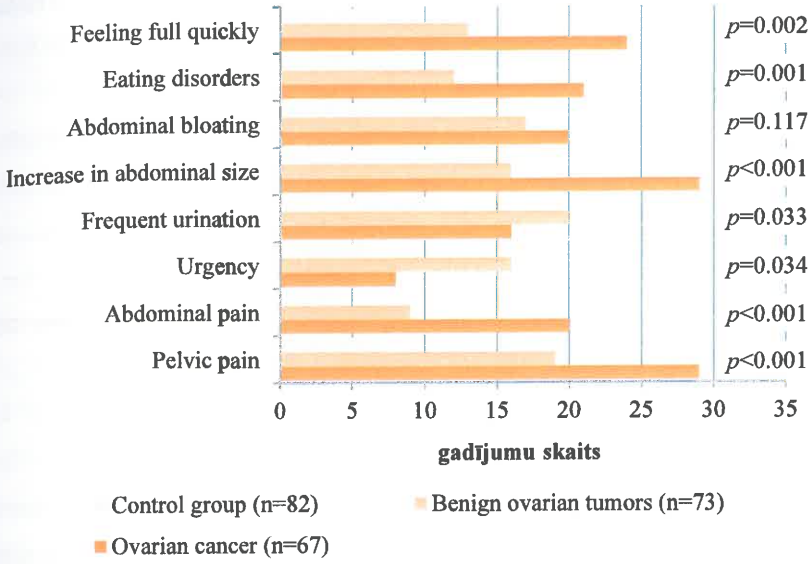


Fig. 2.1. Frequency of symptoms in the study groups included in the ovarian cancer symptom index*

* *Kruskal-Wallis* tests

First symptoms appear 5.4 months before the diagnosis of ovarian cancer is established. Symptoms recur 21.3 days in a month. Positive ovarian cancer symptom index (without 2 urinary symptoms) was observed in 76.12% (51/67) ovarian cancer patients, 56.16% (41/73) patients with benign ovarian tumors and 28.92% (24/83) in women included in the control group ($p<0.001$). Sensitivity and specificity of ovarian cancer symptom index (without 2 urinary simptoms) was 76.12% and 58.33%, respectively.

Positive ovarian cancer symptom index (including all 8 symptoms) was observed in 77.61% (52/67) ovarian cancer patients, 64.38% (47/73) patients with benign ovarian tumors and 34.94% (29/83) women includen in the control group (p<0,001). Sensitivity and specificity of ovarian cancer symptom index was 77.61% and 51.28%, respectively. When ovarian cancer symptom index was analyzed only in women with benign and mailgnant ovarian tumors

sensitivity and specificity was 76.12% and 43.84%, respectively (for ovarian cancer symptom index without 2 urinary symptoms) and 77.61% and 35.61%, respectively (for ovarian cancer symptom index with all 8 symptoms).

No differences in distribution according to menopausal status of positive and negative ovarian cancer symptom index among ovarian cancer patients (p=0.473 for 6 symptom index and p=0.744 for 8 symptom index), patients with benign ovarian tumors (p=0.081 for 6 symptom index and p=0.166 for 8 symptom index) and control women (p=0.847 for 6 symptom index and p=0.643 for 8 symptom index) were observed.

2.2. Ovarian cancer symptom index in combination with CA125

The highest diagnostic sensitivity was observed when ovarian cancer symptom index was combined together with assesment of CA125 concentration in the sera with condition that at least one of the tests should be positive, although specificity decreased dramaticaly with such condition. Specificity of the diagnostic test was improved in analysis where both of diagnostic tests need to be positive – positive ovarian cancer symptom index and elevated CA125 in sera. Also specificity increased when women from control group were added to the analysis – 68.85% and 88.28%, respectively.

Ovarian cancer symptom index identified patients with early stage ovarian cancer with sensitivity ans specificity of 66.6% and 36.6%, respectively, but whan combined with CA125 concentration in sera, sensitivity and specificity was 53.3% and 74.24%, respectively.

According to positive predictive values of ovarian cancer symptom index in general population published by *Rossing et al.*, at sensitivity of 75.41% ovarian cancer would be found in one women from 133 with positive ovarian cancer symptom index (*Rossing et al.,*2010). When ovarian cancer symptom index would be tested alone, additional investigations would be necessary to all 133 women. But when ovarian cancer symptom index would be tested

following mesurement of CA125 concentration in sera, despite decrease of sensitivity (68.85%; one true positive case from 145 women with positive ovarian cancer symptom index), specificity would be increased to 83.07%, and there would be 121 true negative cases and only 24 women who need additional investigations instead of 133.

2.3. Assesment of four Risk of malignancy index models

Sensitivity and specificity of the first risk of malignancy index (RMI 1) was lower when compared to CA125 – area under the curves were 0.852 vs. 0.878, respectively. The second model of risk of malignancy index revealed higher accuracy in patient discrimination with ovarian cancer when compared to CA125 – area under the curves were 0.899 and 0.878, respectively. The third model of risk of malignancy index similarly had higher accuracy than CA125 – area under the curves were – 0.901 and 0.878, respetively.

The fourth model of risk of malignancy index had the highest accuracy from all risk of malignancy indexes – area under the curve 0.902.

When risk of malignancy indexes were analyzed at particular cut-off levels, highest sensitivity was observed for the fourth risk of malignancy index at cutoff level of 200, but highest specificity was observed at cut-off level of 450 for first three malignancy indexes (Table 2.3.).

Table 2.3.

Sensitivity and specificity of four risk of malignancy indexes at different cut-off levels

Risk of malignancy indexes	Cut-off level 200		Cut-off level 300		Cut-off level 450	
	sensitivity	specificity	sensitivity	specificity	sensitivity	specificity
RMI 1	78.2%	83.1%	73.1%	88.3%	71.8%	89.6%
RMI 2	85.9%	77.9%	83.3%	85.7%	79.5%	89.6%
RMI 3	84.6%	83.1%	78.2%	88.3%	75.6%	89.6%
RMI 4	89.7%	72.7%	88.5%	80.5%	85.9%	88.3%

2.4. Mean biomarker concentrations in study groups

Mean serum concentrations of CA125, HE4 and beta-2-microglobulin concentrations were elevated, but decreased for apolipoprotein A1, transferrin and transthyretin (Table 2.4.).

Table 2.4.

Mean biomarker concentrations in sera in patients with ovarian cancers and benign epithelial ovarian tumors

Biomarkers	Mean concentrations in sera ± SD		
	ovarian cancers (n=76)	benign epithelial ovarian tumors (n=58)	*p***
CA125, U/ml*	5.85±1.60	3.26±1.16	<0.001
b-2-MG, mg/l*	0.79±0.35	0.52±0.36	<0.001
HE4, pmol/l*	5.80±1.38	3.87±0.51	<0.001
Apo A1, mg/dl	122.59±38.19	140.64±27.70	<0.001
TF, g/l	2.21±0.52	2.65±0.46	<0.001
TT, g/l	0.18±0.08	0.24±0.05	<0.001

* transformed absolute serum levels; ** t-test

In patients with benign epithelial ovarian tumors mean concentrations of CA125, HE4 and beta-2-microglobulin were elevated, but concentrations of apolipoprotein A1 and transthyretin decreased when compared to mean serum concentrations in control group (Table 2.5.).

Table 2.5.

Mean biomarmer concentrations in serum in patients with benign epithelial ovarian tumors and control group women

Biomarkers	Mean concentration in sera ± SD		
	benign epithelial ovarian tumors (n=77)	control group (n=82)	*p***
CA125, U/ml*	3.26±1.16	2.49±0.53	<0.001
b-2-MG, mg/l*	0.52±0.36	0.28±0.22	<0.001
HE4, pmol/l*	3.87±0.51	3.59±0.29	<0.001
Apo A1, mg/dl	140.64±27.70	168.47±26.17	<0.001
TF, g/l	2.65±0.46	2.68±0.39	0.689
TT, g/l	0.24±0.05	0.26±0.05	0.035

* transformed absolute serum levels; ** t-test

In patients with benign endometrioid ovarian tumors higher CA125 and beta-2-microglobulin concentrations in sera were observed when compared to patients with benign serous epithelial ovarian tumors (Table 2.6.).

Table 2.6.

Mean biomarmer concentrations in serum in patients with benign serous epithelial ovarian tumors and patients with benign endometrioid epithelial ovarian tumors

Biomarkers	Mean concentration in sera ± SD		
	benign serous ovarian tumors (n=36)	benign endometrioid ovarian tumors (n=11)	*p***
CA125, U/ml*	3.27±1.31	3.92±1.02	0.042
b-2-MG, mg/l*	0.68±0.41	0.32±0.28	0.024
HE4, pmol/l*	4.02±0.57	3.64±0.35	0.060
Apo A1, mg/dl	132.64±29.79	149.76±31.52	0.138
TF, g/l	2.58±0.37	2.70±0.27	0.156
TT, g/l	0.23±0.06	0.25±0.03	0.545

* * transformed absolute serum levels; ** *Mann-Whitney* tests

In patients with early stage ovarian cancers higher CA125 and HE4, but lower transthyretin concentrations were obseved when compared to patients with benign ovarian tumors (Table 2.7.).

Table 2.7.

Mean biomarmer concentrations in serum in patients with early stage ovarian cancers stadijās and patients with benign ovarian tumors

Biomarkers	Vidējās koncentrācijas serumā ± SD		
	ovarian cancer, Stage I/II (n=18)	benign ovarian tumors (n=77)	*p***
CA125, U/ml*	4.11±1.78	3.26±1.16	0.014
b-2-MG, mg/l*	0.66±0.32	0.52±0.36	0.180
HE4, pmol/l*	4.38±1.06	3.87±0.51	0.003
Apo A1, mg/dl	134.37±54.96	140.64±27.70	0.487
TF, g/l	2.47±0.54	2.65±0.46	0.146
TT, g/l	0.21±0.08	0.24±0.05	0.050

transformed absolute serum levels; ** t-tests

When biomarkers were analyzed in multivariate logistic regression analysis, HE4 ($p<0.001$) and CA125 ($p=0.004$) where the only independent

biomarkers (apolipoprotein A1 p=0.093; transferrin p=0.417; transthyretin p=0.323; beta-2-microglobulin p=0.218).

2.5. Biomarkers in the conjunction with ultrasonographic findings and menopausal status

In order to develop diagnostic test for clinical purposes, biomarkers where combined together with ultrasonographic appearances and menopausal status. Highest diagnostic accuracy for diagnostic tests with human epididymis secretory protein 4 and CA125 was observed (Table 2.8.).

Table 2.8.

Accuracy of individual biomarkers in combination with ultrasonographic findings and menopausal status irrespective of tumor

Biomarkers	AUC (SE)	95% CI	Equations of multivariate logistic regression analysis
HE4+U+M	0.930 (0.020)	0.891-0.969	Ln(HE4)*2.359+U*0.685-M*0.387-10.839
CA125+U+M	0.902 (0.024)	0.855-0.949	Ln(Ca125)*1.084+U*0.636+M*0.551-7.011

When two biomarkers were included in the logistic regression analysis together with ultrasonographic appearances and menopausal status, highest diagnostic accuracy for combination of human epididymis secretory protein 4 and ovarian cancer associated antigen CA125 was observed. Second most accurate test included serum concentrations of human epididymis secretory protein 4 and beta-2-microglobulin (Table 2.9.).

Table 2.9.

Most accurate ovarian cancer diagnostic tests with combination of two biomarkers, ultrasonographic findings and menopausal status irrespective of tumor stage

Biomarkers	AUC (SE)	95% CI	Equations of multivariate logistic regression analysis
HE4+CA125+U+M	0.939 (0.019)	0.902-0.977	Ln(CA125)*0.607+Ln(HE4)*1.660 +U*0.622-M*0.013-11.106
HE4+B2MG+U+M	0.935 (0.022)	0.891-0.979	Ln(HE4)*2.612-Ln(B2MG)*1.428 +U*1.097-M*0.013-12.140

2.6. Comparison of most sensitive and specific ovarian cancer diagnostic tests

When all diagnostic tests were compared, tests with HE4+CA125 and HE4+B2MG had highest area under the curves which reached statistical difference when compared to accuracy of fourth risk of malignancy index (Table 2.10.).

Table 2.10.

Comparison of most sensitive and specific ovarian cancer diagnostic tests consisting of biomarkers, ultrasonographic findings and menopausal status irrespective of tumor stage

Ovarian cancer diagnostic tests	AUC (SE)	Sensitivity at 75% specificity	Tests positive, when ≥	*p** (vs. RMI 4)
HE4+CA125+U+M	0.939 (0.020)	95.0%	-1.505	0.031
HE4+B2MG+U+M	0.935 (0.023)	94.8%	-0.918	0.029
HE4+U+M	0.930 (0.022)	90.0%	-1.318	0.577
Ca125+B2MG+U+M	0.925 (0.023)	89.6%	-0.774	0.031
RMI4	0.911 (0.023)	90.0%	233.900	1.000

* *McNemar* test

When diagnostic tests where applied for early stage ovarian cancer detection, highest accuracy was observed for the same tests - HE4+B2MG and HE4+CA125 (Table 2.11.)

Table 2.11.

Comparison of most sensitive and specific ovarian cancer diagnostic tests consisting of biomarkers, ultrasonographic appearances and menopausal status in detection of early stage ovarian cancer

Ovarian cancer diagnostic tests	AUC (SE)
B2MG+CA125+U+M	0.714 (0.078)
HE4+CA125+U+M	0.709 (0.074)

2.7. Evaluation of the new ovarian cancer diagnostic tests in two independent study populations

Diagnostic tests developed in the study were evaluated in validation setting of Asian Pacific ovarian cancer biomarker research group study

population with 271 patient with benign ovarian tumor, 49 patients with malignant ovarian tumors and 9 patients with metastatic and nonepithelial malignant tumors.

Highest accuracy was observed for diagnostic test with human epidydimis secretory protein 4, ultrasonographic findings and menopausal status (Figure 2.2.).

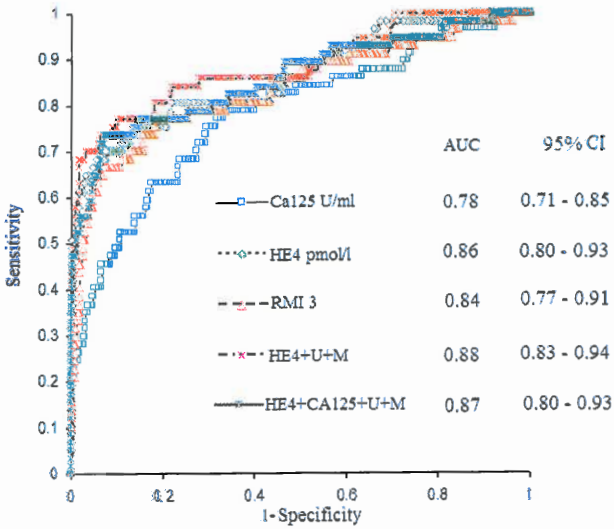


Fig. 2.2. Accuracy of ovarian cancer diagnostic tests in Asian Pacific ovarian cancer biomarker research group study population irrespective of tumor stage

Ovarian cancer diagnostic tests HE4+U+M and HE4+CA125+U+M had higher accuracy in tumor discrimination when compared to conventional third model of risk of malignancy index in Asian pacific ovarian cancer biomarker research group study population (Table 2.12.).

Table 2.12.

Comparison of ovarian cancer diagnostic test accuracy in Asian Pacific ovarian cancer biomarker research group study population

Biomarkers and diagnostic tests	Area under the curve (AUC), *p**			
	CA125	HE4	RMI3	HE4+U+M
CA125	1.000	0.008	< 0.001	< 0.001
HE4	0.008	1.000	0.344	0.077
RMI3	< 0.001	0.344	1.000	0.031
HE4+U+M	< 0.001	0.077	0.031	1.000
HE4+CA125+U+M	< 0.001	0.098	0.010	0.156

* *McNemar* test

Ovarian cancer diagnostic HE4+CA125+U+M was validated also in „Danish Pelvic Mass Project" population with age matched 74 patients with ovarian cancers and 77 patients with benign ovarian tumors. Accuracy of diagnostic test in „Danish Pelvic Mass Project" population was even higher when compared to training setting – 95.9% vs. 93.9% (Figure 2.3.).

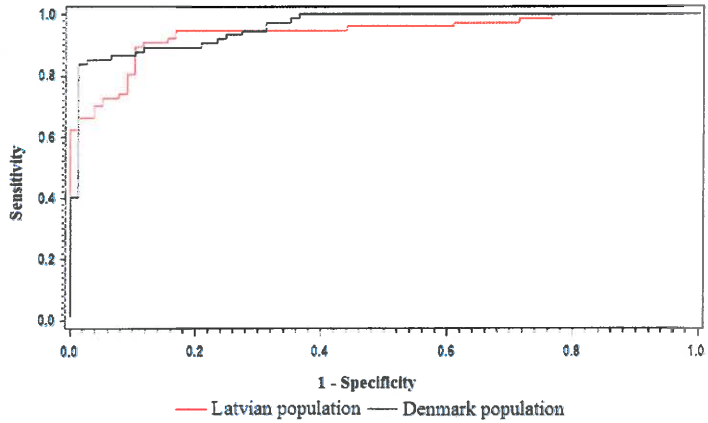


Fig. 2.3. Comparison of accuracy for discovered ovarian cancer diagnostic test (HE4+CA125+U+M) in validation setting of „Danish Pelvic Mass Project" population and training setting

3. DISCUSSION

To reduce the mortality from ovarian cancer, accurate discrimination of patients with genign and malignant ovarian tumors is very important. Correct differentiation of patients contributes precise tumor staging according to FIGO guidelines and optimal cytoreductive surgery (*Shih et al.,* 2010). Patients with suspected malignant ovarian tumors should be sent to specialized treatment centers, but patients with benign ovarian tumors are treatable within general hospitals (*Morgan et al.,* 1996). Such approach of patient stratification improves the quality of surgery in specialized centers favoring more extensive surgery, which allows to extend survival of patients suffering from ovarian cancer (*Vernooij et al.,* 2008).

In some studies patients with borderline ovarian tumors are delineated separately, because these tumors are less aggressive, more commonly found in early stages and biomarker concentrations in serum are less altered than in cases with invasive ovarian tumors (*Goff et al.,* 2000; *Lataifeh et al.,* 2005; *Ryerson et al.,* 2007). In contrast, other authors consider that patients with borderline ovarian tumors should be allocated in one group with invasive ovarian tumors (*Friedman et al.,* 2005).

In our study patients with borderline ovarian tumors were analysed together with invasive ovarian cancers because, according to FIGO recommendations, tumor stage in patients with borderline ovarian tumors should be assessed similarly as in case of invasive ovarian cancer (*Benedet et al.,* 2000). Therefore it is essential that patients with borderline ovarian tumors for correct staging and tumor debulking procedures are sent to specialized centers.

3.1. Ovarian cancer symptom index

Sensitivity and specificity of ovarian cancer symptom index differs in the various studies. One reason is the different inclusion criteria for the study and control groups – *Friedman et al.* and *Reyerson et al.* to include patients in the study groups used medical records, *Olson et al.* and *Attanucci et al.* included information only from patients which were hospitalized, but *Goff et al.* included women which participated in screening program (*Friedman et al.*, 2005; *Reyerson et al.*,2007; *Olson et al.*,2001, *Attanucci et al.*,2004, *Goff et al.*, 2007). Even more important is women selection for the control group. *Smith et al.* and *Wynn et al.* in their studies for the control group choosed women included in medical documentation records, but *lson et al.* and *Goff et al.* used patients which were hospitalized as well as women invited for screening during the study period (*Smith et al.*,2005; *Wynn et al.*, 2007; *Olson et al.*, 2001; *Goff et al*, 2007).

It is possible that patients who responded and were included in the control group, had more symptoms if compared with the general population. This explains the fact that symptoms included in the ovarian cancer symptom index were rather common in the control group.

Goff et al. believes that patients included in the control group from general population is less informative than patients who has come to visit doctor (*Goff et al.*,2004). However, *Rossing et al.* believes that the assesment of proper ovarian cancer symptom index sensitivity, specificity, and positive predictive value should be carried out in women from the general population (*Rossing et al.*, 2010).

Although in our study, as well as *Goff et al. Reyerson, et al., Smith et al.* and *Wynn et al.* have different inclusion criteria, patients with ovarian cancers have one or more symptoms included in the ovarian cancer symptom index lasting several months before the diagnosis (*Goff et al.*, 2007; *Ryerson et al.*,

2007; *Smith et al.*, 2005; *Wynn et al.*, 2007). In addition, in patients with ovarian cancer symptoms are more pronounced, more frequent and appeared recently when compared to women in the control group (*Goff et al.*, 2007).

In June 2007, Foundation of Gynecological cancers, Society of Gynecological oncologists and American Cancer Society declared a statement on ovarian cancer symptoms, which were identified in patients with ovarian cancer – intestinal bloating (flatulence), abdominal or pelvic pain, eating disorders, quick sense of fullness and frequent or urgent urination, which lasts for several weeks and repeats every day.

By contrast, *Goff et al.* the same year published a study describing six most common symptoms characteristic in patients with ovarian cancer - pain in the abdomen or pelvis, increasing abdominal size, or an increase in flatulence, eating disorders and quick sense of fullness (*Goff et al.*, 2007). In the study of *Goff et al.*, no differences in study groups were observed in the frequent urination or urgency between the study groups (*Goff et al.*, 2007).

In our study patients with benign ovarian tumors urinary symptoms were observed more frequently than in women with malignant ovarian tumors - 27.4% vs. 23.9% (frequent urination; $p = 0.033$) and 21.9% vs. 11.9% (urinary urgency; $p=0.034$). In this case, urinary symptoms disclose the importance of the methodology of women inclusion in the control group. Frequent urination and urgency in ovarian cancer patients were seen in 23.9% and 11.9% of cases, what is essential proportion of patients and, therefore, the urinary symptoms should be used to identify patients with ovarian cancer.

In the *Friedman et al.* study symptoms associated with ovarian cancer were analyzed from patient medical records, in the control group there were no differences in frequent urination, but symptom was observed in 7.25% patients with ovarian cancer (*Friedman et al.*, 2005).

Low prevalence of urinary symptoms has been also reported by *Rossing et al.,* where only 4.3% of the 1313 women included in the control group had frequent or urgent urination (*Rossing et al.,* 2010).

Appearance of symptoms before the disease is detected, theoretically, could improve the prognosis for ovarian cancer patient survival. In the study of Smith et al., the median time from onset of symptoms to diagnosis was 6 months - this correlates with the data from our study (*Smith et al.,* 2005).

In the study of Rossing et al. 70% of 812 ovarian cancer patients time from onset of symptoms untill the diagnosis was less than 6 months *(Rossing et al.,* 2010).

In our study symptoms included in the ovarian cancer symptom index appeared 5.4 months before clinical diagnosis. So far, there is no evidence of ovarian cancer symptom index effect on survival rates, however, it has been prooven that 5-year survival rates are twice as good for patients who have undergone optimal tumor debulking surgery - 30-40% vs. 15-20% (*Ozols et al.,* 2005).

Symptoms included in the ovarian cancer symptom index arise from tumor spread in the abdominal cavity. Most of these patients with positive ovarian cancer symptoms will have FIGO stage IIIC tumor, but even then patients can have dissease of completely different volume, therefore ovarian cancer symptom index could contribute in earlier women referral to oncogynecological centers to achieve optimal tumor debulking.

Ovarian cancer tumor size at the time of hospitalization is the most important predictive factor to achieve optimal cytoreduction (*Hoskins,* 1994).

Doubling time of ovarian cancer load has been proposed as less than 3 months,therefore it is likely that the interval of 3-6 month before a diagnosis can be very important (*Miller*, 2007).

Although ovarian cancer symptom index more often is found positive in

late stage disease, in the study of *Goff et al.* positive ovarian cancer symptom index was found in 56.7% of patients with early stage disease. These data correspond to the data reported by *Rossing et al.* where sensitivity of ovarian cancer symptom index in patients with early stage disease was 62.3% (*Goff set al.*,2007; *Rossing et al.*, 2010).

Previously mentioned studies completely reflects results found in our study population - sensitivity of ovarian cancer symptom index among patients with early stage ovarian cancer 66.6% with specificity of 36.6%, but when combined together with CA125 serum concentrations (>35U/ml), sensitivity decreased to 53.3%, but specificity increased to 74.24%.

In the study of *Andersen et al.* sensitivity of ovarian cancer symptom index in combination with CA125 reached 80.6% in patient detection with early stage disease, and 95.1% in patients with late stage ovarian cancer (*Andersen et al.,* 2008). In addition, in that study 50% of ovarian cancers were identified using ovarian cancer symptom index, when CA125 values in the serum were not increased (*Andersen et al.*, 2008).

3.2. Risk of malignancy index (RMI)

Risk of malignancy index is a simple examination that allows to identify patients at high risk of ovarian cancer. Society of Canadian gynecologists and obstetricians and Society of Canadian Gynecologic Oncologists recommends application of risk of malignancy index before patient referral to the oncological gynecologist (*Le et al.,* 2009).

Risk of malignancy index is standardized investigation with well defined ultrasound appearences, menopausal status, and concentration of CA125 should be mesured to calculate the individual risk of ovarian cancer.

Risk of malignancy index originally developed by *Jacobs et al.* in our study did not identify the patients who did not have ultrasonographic

appearances suspicious for malignancies even when the concentrations of serum CA125 were markedly increased (*Jacobs et al.,* 1990).

Risk of malignancy index modified by *Tingulstad et al.* and *Yamamoto et al.* in cases with no ultrasonographic appearances suspicious for malignancy ultrasound score should be noted as 1 instead of 0. This diffrence contributes the identification of ovarian cancer patients with elevated CA125 serum concentrations and no ultrasonographic features suspicious for malignancy (*Tingulstad et al.,* 1996; *Yamamoto et al.,* 2009).

The first reported sensitivity and specificity of risk of malignancy index by Jacobs et al. was 85.0% and 97.0% at cutoff level of 200 (*Jacobs et al.,* 1990). In the meta-analysis of eleven studies the first RMI model had sensitivity of 78.0% (95% CI, 72.0 to 84.0%) specificity of 90.0% (95% CI, 81.0 to 95.0%) (*Myers et al.,* 2006).

In our study the first risk of malignancy index model had lower diagnostic accuracy than reported in original study, but it was close to the results of meta-analysis (78.2% sensitivity at 83.1% specificity).

In the *meta-analysis* conducted by *Myers et al.* the second model of risk of malignancy index at the cutoff level of 200 had sensitivity of 77.0% (95% CI, 71.0 to 82.0%) at 89.0% (95% CI, 85, 0 to 91.0%), specificity (*Myers et al.,* 2006). In our study the second model of risk of malignancy index at cutoff level of 200 showed a higher sensitivity, but lower specificity compared with previously reported results by *Tingulstad et al.* and *Myers et al.* - 85.9% sensitivity at 77.9% specificity (data from meta analysis).

When the third model of risk of malignancy index was developed by *Tingulstad et al.* lower rates of sensitivity were reported in comparison to previously developed risk of malignancy index - 71.0% sensitivity at 92.0% specificity (*Tingulstad et al.,* 1999). Myers et al. in their meta-analysis found similar results of sensitivity and specificity reported in the study of *Tingulstad*

et al. - 74.0% sensitivity (95% CI, 65.0 to 83.0%) and 91.0% (95% CI, 83.0 to 99.0%), specificity (*Tingulstad et al .*, 1999, *Myers et al.*, 2006).

In our study the third model of RMI had higher sensitivity at cutoff level of 200 when compared to the first RMI model and higher specificity when compared to both previously developed RMI models - sensitivity 84.6% at 83.1% specificity.

The fourth model of RMI discovered by *Yamamoto et al.* showed a sensitivity of 86.8% at specificity of 91.0% (*Yamamoto et al.*, 2009). Since there is additionally one more variable reflecting tumor size in the fourth model of RMI, the resulting number is greater, therefore the threshold to discriminate benign and malignant ovarian tumors of 450 has been proposed (*Yamamoto et al.*, 2009).

The fourth model of RMI in our study reached sensitivity of 85.9% and specificity of 88.3% at treshold of 450 - higher than the third RMI model at cutoff level of 200.

3.3. Assessment of biomarker serum levels in the study groups

In the study serum concentrations of biomarkers included in the comercially available ovarian cancer diagnostic tests "ROMA" and "OVA1" were measured - HE4, CA125 , apolipoprotein A1, transferrin, transthyretin and beta-2-microglobulin.

"OVA1" with "ROMA" algorithm are currently the only officially approved ovarian cancer diagnostic tests that are approved by the U.S. Food and drug administration.

In the literature there are quite few data available about the biomarkers included in the "OVA1" biomarker panel in context of ovarian cancer diagnostics, but in studies where they have been validated in patients with malignant ovarian tumors, reduced apolipoprotein A1, transferrin and

transthyretin concentrations, and elevated CA125 and beta-2-microglobulin concentrations have been reported (*Nosov et al.*, 2009; *Kozak et al.,* 2005).

According to human epidydimis secretory protein 4 in the literature there are available a number of studies that have described increased HE4 serum levels in patients with malignant ovarian tumors (*Hellstrom et al.,* 2003; *Moore et al.,* 2007; *Van Gorp et al.,* 2011).

Similarly, in our study patients with benign and malignant epithelial ovarian tumors showed statistically significant differences for all the biomarker concentrations in serum – patients with malignant ovarian tumors in contrast to patients with benign ovarian tumors had elevated ovarian cancer associated antigen CA125, human epidydimis secretory protein 4 and beta-2-microglobulin serum levels and reduced apolipoprotein A1, transferrin and transthyretin concentrations. Also in patients with benign ovarian tumors concentrations of CA125, human epidydimis secretory protein 4 and beta-2-microglobulin were higher, but lower concentrations of apolipoprotein A1and transthyretin when compared to the control group.

When there were compared mean biomarker concentrations in serum in patients with benign serous and endometrioid ovarian tumors, statistically significant differences were observed in mean values of CA125 and beta-2-microglobulin - in patients with benign endometrioid tumors concentrations of CA125 were higher, but concentrations of beta-2-microglobulin were lower than those in patients with benign serous ovarian tumors.

Also *Moore et al.* have reported about elevated serum CA125 concentrations, but unchanged concentrations of HE4 in patients with endometrioid ovarian tumors and endometriosis (*Moore et al.,* 2012). Recent studies confirm that human epidydimis secretory protein 4 can be used in differential diagnosis of endometriosis and malignant epithelial ovarian tumors (*Kadija et al.,* 2012).

3.4. Sensitivity and specificity of biomarkers in diagnostics of early stage ovarian cancer

When the average concentrations of biomarkers where analysed in the early and late stages ovarian cancer patients statistically significant differences were observed only for biomarkers CA125, HE4 and TF. When comparison of mean biomarker concentratiosn was performed between patient groups with early stage ovarian cancer patients and patients with benign ovarian tumors, statistically significant difference only for mean concentrations of HE4 and CA125 was observed, indicating that the two biomarkers have higher sensitivity and specificity in detection of patients with early stage ovarian cancer.

Also lower concentrations of transthyretin had patients with early stages ovarian cancer, but this difference was less pronounced ($p = 0.050$) when compared to HE4 ($p=0.003$) and CA125 ($p=0.014$).

In the study conducted by *Lenhard et al.* HE4 had higher diagnostic sensitivity and specificity than CA125 in detection of early stage ovarian cancer that correcponds to our results (*Lenhard et al.,* 2011). In contrast, *Partheen et al.* discovered higher accuracy for CA125 in comparison to HE4 in discrimination of patients with early stage ovarian cancer - 86.8% for CA125 and 84.4% for HE4 (*Partheen et al.,* 2011). In the separate analysis where only patients with first stage ovarian cancer were analysed together with benign ovarian tumors, area under the curve for HE4 was 72%, but 76% for CA125 (*Partheen et al.,* 2011).

In our study area under the curve for human epidydimis secretory protein 4 was 64.4%, and 64.0% for ovarian cancer associated antigen CA125 in discrimination of patients with early stage ovarian cancer. Lower ovarian cancer diagnostic accuracy can be explained by addition of patients with borderline ovarian tumors together in one group with early stage ovarian cancer patients.

In the study of *Zhen et al.* sensitivity of CA125 in the diagnosis of early stage ovarian cancer for menopausal women at the cutoff level of 35 U/ml was 44.4%, which correlates with the findings of our study – 40.0% (*Zhen et al.*, 2010).

3.5. Combined biomarker tests in conjunction with ultrasonographic findings and menopausal status in ovarian cancer diagnostics

Since ultrasonography is one of the most important investigation in differential diagnosis of ovarian tumors, in the logistic regression analysis together with biomarker concentrations ultrasonographic findings and menopausal status were included. The highest ovarian cancer diagnostic accuracy involving only a biomarker was observed for human epidydimis secretory protein 4.

Logistic regression analysis with the ultrasonographic characteristics, menopausal status and concentration of CA125 revealed diagnostic test with comparable diagnostic accuracy to the accepted risk of malignancy indexes - area under the curve for model obtained in logistic regression analysis in our study population was 90.2%, 89.9% for the second RMI model and 90.1 % for the third RMI model.

A similar diagnostic accuracy was also observed in other studies - *Tingulstad et al.* reported area under the curve of 86.0% for the second RMI model and van den *Akker et al.* reported about area under the curve of 89.3% for the third RMI model (*Tingulstad et al.*, 1996, *van den Akker et al.*, 2010).

These findings suggest similarity of patients included in our study with patient populations included in studies by other authors and the relatively high diagnostic sensitivity and specificity of risk of malignancy index, which includes HE4 concentration.

The highest diagnostic accuracy from tests including ultrasonographic

score, menopausal status and two biomarkers, was observed for model with HE4 and CA125 serum concentrations (AUC 93.9%). Addition of second biomarker in the diagnostic assay slightly increased the diagnostic accuracy in comparison to model with one biomarker (HE4).

3.6. Evaluation of the new ovarian cancer detection algorithm in two independent study populations

Differentialdiagnostic tests developed in our study showed similar results in Asian Pacific ovarian cancer biomarker research group study population - area under the curve of human epidydimis secretory protein 4 was 86.0%, but only 78.0% for CA125. In our study, regardless of tumor stage and menopausal status area under the curve for HE4 was 88.0% and 86.5% for CA125.

When analysis was performed for diagnostic tests, which include ultrasonographic findings, menopausal status and a single biomarker, diagnostic test with HE4 in the Asian Pacific ovarian cancer biomarker research group study population had higher area under the curve (88.0%) than third RMI model (84.0%), where CA125 is included ($p = 0.031$). Superior diagnostic accuracy of diagnostic test with HE4 corresponds to findings of our study.

Area under the curve of diagnostic test HE4 + U + M in our study was 93.0%, but for the third and fourth RMI model as well as for RMI developed in our study (CA125+U+M) area under the curve ranged from 89.9% to 90,2%.

Analysis of diagnostic assay with concentrations of both biomarkers (HE4+CA125+U+M) in the Asian Pacific ovarian cancer biomarkers research group study population showed a lower diagnostic sensitivity and specificity (area under the curve 87.0%) when compared to a diagnostic test whith only HE4, ultrasound score and menopsausal status (area under the curve 88.0%). This difference can be explained by differences in patient proportion with

benign and malignant dideases in training and validation setting where in training setting proportion was 1:1, but in validation setting 1:5. And also possible biological differences in Latvian and Asian population, which can influence biomarker concentrations can not be ruled out. Addition of second biomarker to the diagnostic test improved its sensitivity and specificity – area under the curve for CA125 + HE4 + U + M was 93.9%, but 93.0% for HE4 + U + M test.

In the separate analysis with early stage ovarian cancers highest sensitivity and specificity was observed for diagnostic tests which included CA125 and beta-2-microglobulin serum concentrations - area under the curve 71.4%. Area under the curve for ovarian cancer diagnostic test with only concentration of HE4 (HE4+U+M) was little lower (70.6%) than accuracy of test with both biomarkers – HE4 and CA125 (area under the curve 70.9%).

As concentrations of the beta-2-microglobulin were not determined in the Asian Pacific ovarian cancer biomarkers research group study population, combination of B2MG+HE4+U+M can not be compared between patient settings.

Diagnostic test HE4+CA125+U+M was analysed in another independent "Danish Pelvic Mass Project" population, where even higher diagnostic accuracy was observed – area under the curve was 95.9% vs. 93.9%.Equally high diagnostic accuracy can be explained with accurate patient selectio n in the validation setting - patients with malignant ovarian tumors were selected according to tumor stage, age, menopausal status and ultrasonographic findings, but patients with benign ovarian tumors were selected according to age, menopause and ultrasound findings.

4. CONCLUSIONS

1. Ovarian cancer symptom index with eight symptoms has higher diagnostic sensitivity.

2. Assessment of CA125 concentration in serum increases specificity of ovarian cancer symptom index.

3. The fourth risk of malignancy index model had the highest sensitivity and specificity in identification of ovarian cancer patients at treshold of 450.

4. Biomarker concentrations of HE4 and CA125 were the only statistically significant independent biomarkers in ovarian cancer patient identification from multivariate logistic regression analysis of six biomarkers analysed in the study.

5. Diagnostic assay consisting of human epidydimis secretory protein 4 and CA125 serum concentrations together with ultrasonographic findings and menopausal status have the highest sensitivity and specificity in differential diagnosis of ovarian tumors.

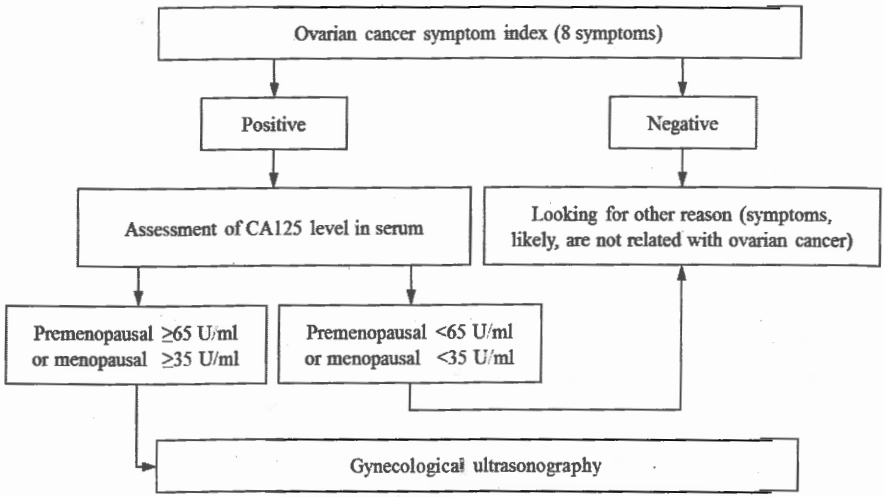
5. RECOMMENDATIONS

1. Ovarian Cancer Symptom Index with eight symptoms is recommended for use in clinical practice by general practitioners and gynecologists.
2. In patients with positive ovarian cancer symptom index measurement of CA125 concentration in serum should be followed.
3. Women with positive ovarian cancer symptom index and elevated serum CA125 concentrations (≥ 65U/ml in premenopause and ≥35 U/ml in menopause) should be referred for gynecological ultrasonographic examination (1.algorithm).
4. When ovarian tumor is found during gynecological ultrasonographic examination fourth risk of malignancy index should be assessed using a threshold of 450 (2.algorithm).
5. Calculation of fourth risk of malignancy index is recommended in patients with ovarian tumor found in gynecological ultrasonographic examination unrelated to ovarian cancer symptom index (2.algorithm).
6. Women with an increased risk of malignancy index value of 450 and more should be referred to the oncological gynecologist, but those with values below 450 should be referred to a gynecologist-obstetrician (2.algorithm).
7. In patients with a risk of malignancy index value of 450 and more, and a high surgical risk caused by co-morbidities or expectant technical difficulties to perform operation concentration of HE4 in serum should be determined.
8. Ovarian cancer risk is calculated by entering serum concentrations of HE4 and CA125 in absolute numbers, menopausal status (1 or 3) and ultrasonographic findings (1 or 3) in the ovarian cancer risk assessment calculator, which is available on the website www.onkoginekologija.lv (3.algorithm).
9. Patients with high risk of ovarian cancer, as determined by ovarian cancer

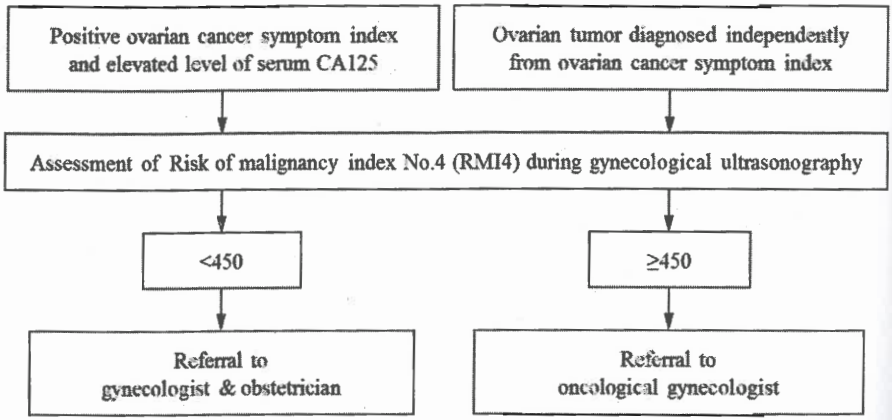
risk assessment calculator, should be operated, but patients with low risk of ovarian cancer should be observed (4.algorithm).

10. Ovarian cancer risk assessment calculator can be used in patients with symptoms or ultrasonographic findings typical for endometriosis with risk of malignancy index value of 450 and above (5.algorithm).

1.algorithm. Application of ovarian symptom index in the clinical practice



2.algorithm. Application of risk of malignancy index in clinical practice



3. algorithm. Ovarian cancer risk assesment calculator

(www.onkoginekologija.lv)

Ovarian cancer risk assessment calculator

If patient is premenopausal, enter 1; if menopausal, enter 3

Enter level of serum CA125, U/ml

Enter level of serum HE4, pmol/l

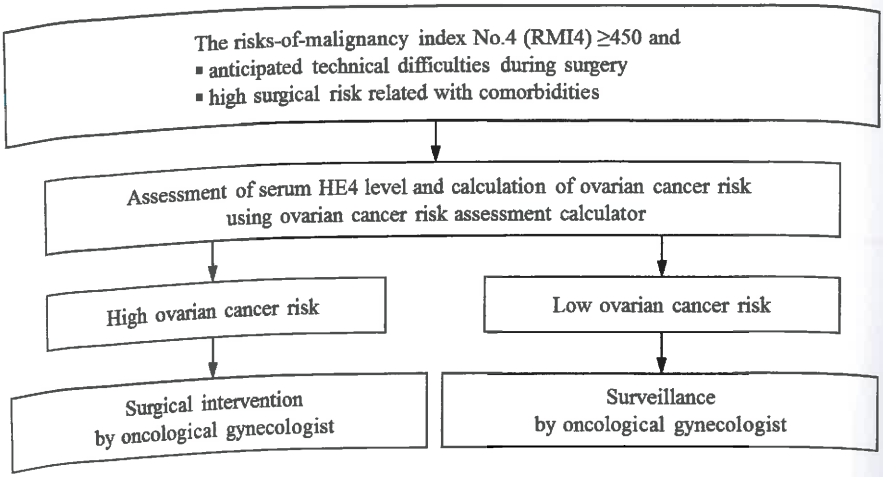
Enter ultrasonographic score:
1, if there are no findings suspicious for ovarian malignancy
1, if there is one finding suspicious for ovarian malignancy
3, if there are two or more findings suspicious for ovarian malignancy

Answer

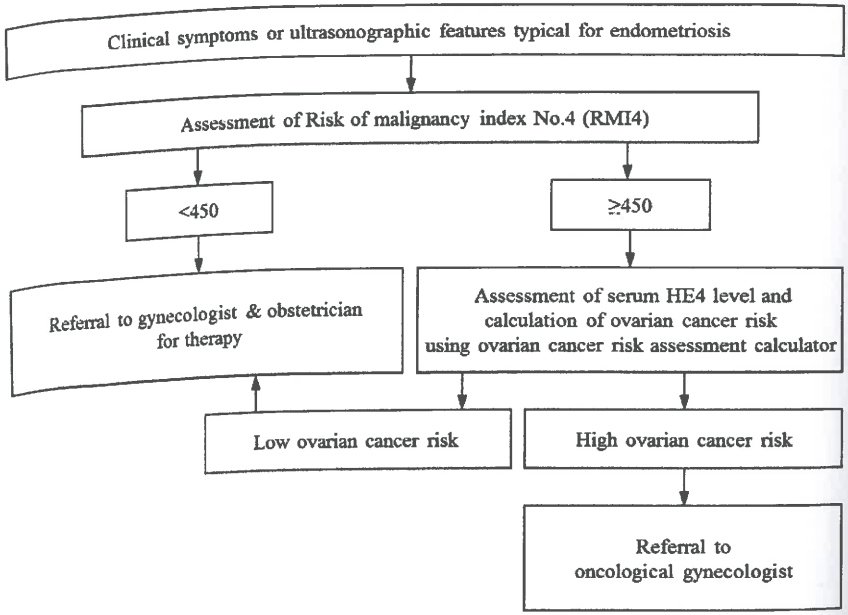
Calculate

Ultrasonographic findings indicating the possible ovarian malignancy:
– multilocular cyst
– solid area
– ascites
– bilateral lesions
– metastases

4.algorithm. Application of ovarian cancer risk assesment calculator in clinical practice



5.algorithm. Application of ovarian cancer risk assesment calculator in patients with clinical symptoms or ultrasonographic findings typical for endometriosis



6. PUBLICATIONS (SCIENTIFIC ARTICLES), ABSTRACTS AND PRESENTATIONS ON THE RESEARCH TOPIC

6.1. Publications (scientific articles) on the research topic

1. Macuks R, Baidekalna I, Donina S. Comparison of different ovarian cancer detection algorithms. Eur J Gynaecol Oncology, 2011; 32(4):408-10.
2. Macuks R, Baidekalna I, Donina S. Diagnostic test for ovarian cancer composed of ovarian cancer symptom index, menopausal status and ovarian cancer antigen CA125. Eur J Gynaecol Oncology, 2011; 32(3):286-8.
3. Macuks R, Baidekalna Ieva, Avdejeva Arina, Gritcina Julia, Donina Simona. Comparison of Two Novel Biomarker Panels in Ovarian Cancer Diagnosis for Patients with a Pelvic Mass. Eur J Clinical and Medical Oncology, 2011; 3:79-83.
4. Ronalds Macuks, Ieva Baidekalna, Ella Nesterenko, Renate Renemane, Simona Donina. Evaluation of ovarian cancer symptom index. RSU Research articles in Medicine & Pharmacy - Collection of Scientific papers, 2009:63-68.
5. Ronalds Macuks, Ieva Baidekalna, Julija Gritcina, Arina Avdejeva, Simona Donina. Apolipoprotein A1 and transferrin as biomarkers in ovarian cancer diagnostics. Acta Chirurgica Latiensis, 2010:16-20.
6. Ronalds Mačuks, Ludmila Eņgele, Inta Nuķe, Agnese Sudraba, Simona Doniņa. Comparative Analysis of Insulin-like Growth Factor I and Tumour-associated Antigens in Cancer Patients at the Time of Diagnosis. Proceedings of the Latvian Academy of Sciences. Section B, 2009; 4:186–190.
7. Ronalds Macuks, Ieva Baidekalna, Inta Nuke, and Simona Donina. Role of combined soluble interleukine-2 receptor alpha and CA125 serum marker assay for detection of ovarian cancer. Proceedings of Latvian Academy of Sciences. Section B, 2011; 1:20–30.
8. Ronalds Macuks, Ieva Baidekalna, Inta Nuke, Ludmila Engele, Simona Donina. Ovarian cancer specificity of combined biomarker assay consisting of soluble interleukine-2 receptor alpha and CA125. RSU Research articles in Medicine & Pharmacy - Collection of Scientific papers, 2010:66-72.

9. Ronalds Mačuks, Ludmila Eņģele, Inta Nuķe, Agnese Sudraba, Simona Doniņa. Comparative Analysis of Insulin-like Growth Factor I and Tumour-associated Antigens in Cancer Patients at the Time of Diagnosis. Proceedings of the Latvian Academy of Sciences. Section B, 2009; 4:186–190.

6.2. Abstracts on the research topic

1. Simona Doniņa, Ludmila Eņģele, Ronalds Mačuks, Inta Nuķe. Insulīnam līdzīgais augšanas faktors I – krūts un olnīcu vēža papildus biomarķieris. RSU Scientific conference thesis 2009, 185.
2. Macuks R, Baidekalna I, Doniņa S. Prolactin serum concentration among gynecological cancer and benign gynecological diseases. International Journal of Gynecological Cancer, Volume 19, October 2009, Supplement 2:128.
3. Macuks R, Baidekalna I, Doniņa S. Insuline – like growth factor-1 (IGF-1) and tumor associated antigens CEA and CA15-3 in breast and ovarian cancer. International Journal of Gynecological Cancer, Volume 19, October 2009, Supplement 2:157.
4. Macuks R, Baidekalna I, Doniņa S. Evaluation of an ovarian cancer symptom index consisting of 6 symptoms. International Journal of Gynecological Cancer, Volume 19, October 2009, Supplement 2:158.
5. Ronalds Mačuks, Ieva Baidekalna, Simona Doniņa. Šķīstošie interleikīna 2 receptori un CA125 olnīcu vēža diagnostikā. RSU Scientific conference thesis 2010, 287.
6. Ronalds Mačuks, Ieva Baidekalna, Simona Doniņa. Malignitātes riska indeksa un olnīcu vēža simptomu indeksa salīdzinoša analīze. RSU Scientific conference thesis 2010, 257.
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9. R.Mačuks, I.Baidekalna, S.Doniņa. Ar 4 nedēļu laika intervālu noteiktas Ca125 seruma koncentrācijas iekļaušana olnīcu vēža riska aprēķināšanas

algoritmā ROMA. Thesis of 69th Scientific conference of University of Latvia 2011, 28.
10. R.Mačuks, I.Baidekalna, S.Doniņa. ROMA un CA125 salīdzinoša analīze. RSU Scientific conference thesis 2011, 271.
11. Macuks R, Baidekalna I, Doniņa S. Ovarian cancer prediction test consisting of risk of ovarian malignancy algorithm followed by malignancy risk index. Thesis of 17th European Society of Gynecological Oncology congress 2011, 279.
12. Macuks R, Baidekalna I, Doniņa S. Combined biomarker test consisting of HE4, CA125 and Apolipoprotein A1 for ovarian cancer detection. Thesis of 17th European Society of Gynecological Oncology congress 2011, 278.
13. R. Macuks, Ieva Baidekalna, Simona Donina. Ovarian cancer prediction test composed of ovarian cancer symptom index and risk of ovarian malignancy algorithm. Thesis of International Ovarian cancer screening conference 2011, 29.
14. Baidekalna I., Macuks R., Nuke I., Engele L., Donina S. CA125 in comparison to risk of ovarian malignancy algorithm. Thesis of 6th Latvian Congress of Gynecologists and Obstetritians 2011, 38.
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16. Macuks R., Nuke I., Engele L., Donina S. Urinary concentrations of human epidydimis secretory protein 4 in ovarian cancer patients. Thesis of 6th Latvian Congress of Gynecologists and Obstetritians 2011, 37.
17. I.Baidekalna, R.Mačuks, S.Doniņa. ROMA un CA125 salīdzinoša analīze. RSU Scientific conference thesis 2012, 247.
18. Serumu HE4 un CA125 salīdzinoša analīze olnīcu vēža diagnostikā saistībā ar ultrasonogrāfiskajām pazīmēm un menopauzālo stāvokli. RSU Scientific conference thesis 2012, 246.

6.3. Oral presentations and posters at congresses and conferences

1. Simona Doniņa, Ludmila Eņģele, Ronalds Mačuks, Inta Nuķe. Insulīnam līdzīgais augšanas faktors I – krūts un olnīcu vēža papildus biomarķieris. Poster presentation at Scientific conference of Riga Stradins University. 2nd-3rd of April, 2009, Riga.

2. Macuks R, Baidekalna I, Doniņa S. Prolactin serum concentration among gynecological cancer and benign gynecological diseases. Poster presentation at 16th Congress of European society of gynecological oncologists. 11th-14th of October, 2009 in Belgrade, Serbia.
3. Macuks R, Baidekalna I, Doniņa S. Insuline – like growth factor-1 (IGF-1) and tumor associated antigens CEA and CA15-3 in breast and ovarian cancer. Poster presentation at 16th Congress of European society of gynecological oncologists. 11th-14th of October, 2009 in Belgrade, Serbia.
4. Macuks R, Baidekalna I, Doniņa S. Evaluation of an ovarian cancer symptom index consisting of 6 symptoms. Poster presentation at 16th Congress of European society of gynecological oncologists. 11th-14th of October, 2009 in Belgrade, Serbia.
5. Ronalds Mačuks, Ieva Baidekalna, Simona Doniņa. Šķīstošie interleikīna 2 receptori un CA125 olnīcu vēža diagnostikā. Oral presentation at Scientific conference of Riga Stradins University. 18th – 19th of march, 2010, Riga.
6. Macuks R, Baidekalna I, Nuke I, Engele L, Doniņa S. High diagnostic performance of ovarian cancer detection algorithm consisting of soluble interleukin – 2 receptor alpha and CA125 combined marker assay and malignancy risk index. Poster presentation at Congress of International society of oncology and biomarkers. 3rd-8th of September, 2010, Germany.
7. R.Mačuks, I.Baidekalna, S.Doniņa. Malignitātes riska indeksa un olnīcu vēža simptomu indeksa salīdzinoša analīze. Oral presentation at Scientific conference of Riga Stradins University. 18th-19th of March, 2010, Riga.
8. R.Mačuks, I.Baidekalna, S.Doniņa. Ar 4 nedēļu laika intervālu noteiktas Ca125 seruma koncentrācijas iekļaušana olnīcu vēža riska aprēķināšanas algoritmā ROMA. Oral presentation ar 69th Scientific cinference of University of Latvia. 3rd of February, 2011, Riga.
9. R.Mačuks, I.Baidekalna, S.Doniņa. ROMA un CA125 salīdzinoša analīze. Oral presentation at Scientific conference of Riga Stradins University. 14th-15th of April, 2011, Riga.
10. Macuks R, Baidekalna I, Doniņa S. Ovarian cancer prediction test consisting of risk of ovarian malignancy algorithm followed by malignancy risk index. Poster presentation at 17th Congress of European society of gynecological oncologists. 11th-14th of September, 2011, Milan, Italy.
11. Macuks R, Baidekalna I, Doniņa S. Combined biomarker test consisting of HE4, CA125 and Apolipoprotein A1 for ovarian cancer detection. Poster

presentation at 17th Congress of European society of gynecological oncologists. 11th-14th of September, 2011, Milan, Italy.

12. R.Mačuks, I.Baidekalna, S.Doniņa. Lizofosfatidilskābes un ar olnīcu vēzi asociētā biomarķiera Ca125 izmantošana olnīcu vēža diferenciāldiagnostikā. Oral presentation at Students scientific conference of University of Latvia. 3rd of December, 2010, Riga.
13. Simona Donina, Ronalds Macuks. Comparison of two novel biomarker combinations composed of human epidydimis secretory protein 4 and CA125 versus Apolipoprotein A1, transferrin, transthyretin and beta 2 microglobulin in ovarian cancer diagnosis. Poster presentation at Congress of International society of Oncology and biomarkers. 15th-19th of October, 2011, Italy.
14. Ronalds Macuks, Ieva Baidekalna, Simona Donina. Ovarian cancer prediction test composed of ovarian cancer symptom index and risk of ovarian malignancy algorithm. Poster presentation at International ovarian cancer screening conference. 29th-30th of November, London, England.
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19. R.Mačuks, I.Baidekalna, S.Doniņa.Serumu HE4 un CA125 salīdzinoša analīze olnīcu vēža diagnostikā saistībā ar ultrasonogrāfiskajām pazīmēm un menopauzālo stāvokli. Oral presentation ar Scientific conference of Riga Stradins University. 29th-30th of March, 2012, Riga.

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