

Predictive Values of the Ultrasound Parameters, CA-125 and Risk of Malignancy Index in Patients with Ovarian Cancer

Prediktivní hodnota ultrazvukových parametrů, CA-125 a indexu rizika malignity u pacientek s karcinomem ovarií

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Summary

Backgrounds: Assessment of predictive values for CA-125, ultrasound features (US) and risk of malignancy index (RMI) in ovarian malignancy. **Material and Methods:** 115 patients, divided into: 1) group-A (n = 41) – ovarian malignancy; group-B (n = 74) – benign ovarian tumor; 2) subgroup-CA(a) with low CA-125 (< 35 U/mL) (n = 64); subgroup-CA(b) with slightly elevated CA-125 (35–130 U/mL) (n = 26); subgroup-CA(c) with high CA-125 (> 130 U/mL) (n = 25). **Results:** 1) patients of group-A were older (p < 0.05); CA-125 < 35 U/mL predominated in group-B (p < 0.001); 2) CA-125 < 35 U/mL showed relatively high NPV, sensitivity and specificity (82.8%; 0.732; 0.716, respectively). Our proposed graduation of CA-125 into three grades: a) < 35 U/mL; b) 35–130 U/mL; c) > 130 U/mL increased the specificity for both parameters: CA125 = 35–130 U/mL up to 0.811, and for CA-125 > 130 U/mL up to 0.905, and PPV for the latter parameter up to 72.0%; 3) US: a) highest sensitivity, as indicator for best distinguishing of diseased patients, showed: rugged margins and presence of septum/vegetations (0.878; 0.897, respectively); b) highest specificity, as indicator for best distinguishing of healthy patients: clear distinguish ability of tumor from surrounding tissue and absence of ascites (0.811; 0.932, respectively); c) presence of ascites had highest PPP (100%) i.e. it was the best malignancy predictor; 4) RMI showed only relatively high NPV for RMI ≤ 200 (76.8%); 4) additional analysis of RMI in correlation with proposed CA-125 gradation increased the predictive values of RMI: a) subgroup-CA(a): NPV and sensitivity for RMI ≤ 200 (81.6%; 0.818, respectively) and NPV for RMI > 200 (86.7%); b) subgroup-CA(b): specificity for RMI ≤ 200, as good indicator for distinguishing healthy patients (0.929); c) subgroup-CA(c): sensitivity for RMI > 200, as good indicator for distinguishing diseased patients (0.944). **Conclusion:** CA-125 and US, as single criteria were not accurate. RMI is good indicator only in correlation with CA-125.

Key words

ovarian neoplasms – serum – tumor markers – CA-125 protein – ultrasonography – prognosis

Condesation

Serum-CA-125 and ultrasound findings as single criteria in malignancy diagnosis of ovarian tumors are not enough accurate. RMI increases the accuracy of the preoperative diagnosis.

The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.

Autoři deklarují, že v souvislosti s předmětem studie nemají žádné komerční zájmy.

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Souhrn

Výhodiska: Zhodnocení prediktivní hodnoty CA-125, ultrazvukových parametrů (US) a indexu rizika malignity (RMI) u karcinomu ovarií. **Materiál a metody:** 115 pacientek rozdělených do: 1) skupina A (n = 41) – ovariální malignita; skupina B (n = 74) – benigní ovariální nádor; 2) podskupina CA(a) s nízkým CA-125 (< 35 U/ml) (n = 64); podskupina CA(b) s mírně zvýšeným CA-125 (35–130 U/ml) (n = 26); podskupina CA(c) s vysokým CA-125 (> 130 U/ml) (n = 25). **Výsledky:** 1) pacienti ve skupině A byli starší ($p < 0,05$); ve skupině B převažoval CA-125 < 35 U/ml ($p < 0,001$); 2) CA-125 < 35 U/ml bylo charakterizováno poměrně vysokým NPV, senzitivitou a specifitou (82,8 %; 0,732, resp. 0,716). Námi navrhované uspořádání hladin CA-125 do tří stupňů: a) < 35 U/ml; b) 35–130 U/ml; c) > 130 U/ml zvýšilo specifitu obou parametrů: CA125 = 35–130 U/ml na 0,811, pro CA-125 > 130 U/ml až na 0,905 a PPV pro druhý z parametrů na 72,0 %; 3) US: a) nejvyšší senzitivitu, a tedy nejvyšší schopnost rozlišit pacientky postižené onemocněním prokázaly: nepravidelné ohraničení a přítomnost septa/růst (0,878, resp. 0,897); a) nejvyšší specifitu, a tedy nejvyšší schopnost rozlišit zdravé pacientky, prokázaly: jasné vyčlenění nádoru z okolní tkáně a nepřítomnost ascitu (0,811, resp. 0,932); c) přítomnost ascites měla nejvyšší PPP (100%), a byla tedy nejsilnějším prediktorem zhoubnosti; 4) RMI prokázalo poměrně vysoké NPV pouze pro RMI ≤ 200 (76,8 %); 4) následná analýza RMI v korelaci s navrhovaným odstupňováním CA-125 zvýšila prediktivní hodnotu RMI: a) podskupina CA(a): NPV a senzitivita pro RMI ≤ 200 (81,6 %, resp. 0,818) a NPV pro RMI > 200 (86,7 %); b) podskupina CA(b): specifita pro RMI ≤ 200 jako dobrý indikátor identifikace zdravých pacientek (0,929); c) podskupina CA(c): senzitivita pro RMI ≤ 200 jako dobrý indikátor identifikace nemocných pacientek (0,944). **Závěr:** CA-125 ani US jako jednotlivé indikátory nebyly přesné. RMI je dobrým indikátorem pouze v korelaci s CA-125.

Klíčová slova

nádory vaječníků – sérum – nádorové markery – CA-125 protein – ultrazvuk – prognóza

Backgrounds

Ovarian cancer is the most lethal gynecologic malignancy in adult women. The 5-year survival rate for stage III and IV is 31%, and for stage I is 95% [1]. Unfortunately, early diagnosis is difficult because the lack of specific symptoms in early disease due to inaccessible location of the ovaries. Common symptoms, such as abdominal bloating and early satiety, indicate more advanced disease, involving the upper abdomen and present in approximately 70% of patients at the time of diagnosis [2]. Because of the fact that currently available screening tests do not achieve high levels of sensitivity and specificity, screening is not recommended for the general population, but only for women at high risk (strongly family history of ovarian cancer and those with BRCA 1 or BRCA 2 mutations). Trans-vaginal ultrasound with its high resolution enables detailed scrutiny of ovarian lesions [3]. According to certain authors [4,5], the anechoic cysts with thin walls, without inner or outer nodularity, and associated with CA-125 levels within normal limits (< 35 U/ml) can be safely punctured. As some studies have proved [6], ovarian malignancy at its very early stage can be present in a small simple cyst or even in a normal-sized ovary. All subjects were given an explanation of the study and written informed consent was obtained.

The quantitative evaluation of some tumor markers, such as: CEA, CA-125,

CA-72-4 or even CA-19-9, since certain time has been considered as valid method which can indicate of the malignant potential of ovarian tumors. So, in 1994 Woolas et al [7] reported about a screening program of the ovarian cancer with CA-125 evaluation. CA-125 is a glycoprotein, which expression can be found in almost all cells of the coelomic epithelium. In 1% of healthy women, elevated values of this tumor marker can be found in the absence of some cancer process [8]. In about 80% of women with non-mucinous epithelial cancer the serum levels of CA-125 is higher than 35 U/ml, but it is not rare the situation when the CA-125 is into the normal levels in presence of ovarian cancer in earlier stages [9]. In a review of several studies only 44% of women with ovarian cancer stage I had increased levels of CA-125 [10]. This is the main disadvantage of this method, as well as its increase during some physiological or benign pelvic diseases, such as: pelvic endometriosis, pelvic inflammatory disease or first trimester pregnancy. The combination of the ultrasound evaluation and the estimation of the serum levels of CA-125 could be an effective method for screening of ovarian cancer. A simple algorithm called risk of malignancy index (RMI), reported by Jacobs et al [11] or RMI 1, which incorporated the serum CA-125 level, menopausal status and ultrasound morphological features gave sensitivity of 85.4% and specificity of

96.9%. Currently, the network guidelines recommend calculation of the RMI [12] as modified by Tingulstad et al [13] or RMI 2.

Material and Methods

Eligibility criteria

- the presence of ovarian tumor requiring surgical treatment

The setting, location and timing

- University Clinic of Gynecology and Obstetrics, Medical Faculty, Skopje, in the period from the 1st of January 2009 to the 1st of January 2010

Precise details of the interventions

The whole study group was consisted of patients with presence of ovarian tumor (n = 115). The experimental group: group A (n = 41) was consisted of patients with histological feature of ovarian malignancy, but control group: group B (n = 74) of patients with histological feature of benign ovarian tumor. In every group, the Serum CA-125 level, ultrasound evaluation and RMI were performed preoperatively. Additionally, the whole study group was divided into three subgroups regarding the serum CA-125 levels:

- 1) subgroup CA(a) with low serum levels of CA-125 (< 35 U/mL) (n = 64);
- 2) subgroup CA(b) with slightly elevated serum levels of CA-125 (35–130 U/ml) (n = 26);
- 3) subgroup CA(c) with high serum levels of CA-125 (> 130 U/ml) (n = 25).

The study was approved by the local research ethics committee (LREC) of the Association of Gynecologists and Obstetricians of Macedonia.

How sample size was determined

Every patient who had ovarian tumor required operative treatment, assessed for eligibility (n = 121). Six patients were excluded because they refused to be operated. So, 115 patients were randomised.

Aim of the work

- 1) to estimate the predictive values of: Serum CA-125, ultrasound findings and RMI as a single criterion for malignancy;
- 2) to estimate the predictive values for RMI in cases with mildly elevated Serum CA-125 (35–130 U/ml), levels, which are doubtful for malignancy;
- 3) to establish the most accurate ultrasound parameter for malignancy;
- 4) to estimate whether our proposed graduation of serum CA-125 levels into three grades: less than 35 U/mL; between 35 and 130 U/mL; and more than 130 U/mL can improve the accuracy of the parameters: CA-125 and RMI in prediction of ovarian malignancy.

Specific hypotheses

- 1) serum CA-125, ultrasound findings and RMI as a single criterion are not enough reliable parameters for excluding ovarian malignancy;
- 2) RMI > 200 in cases with mildly elevated Serum CA-125 (35–130 U/ml), is not enough reliable parameter for ovarian malignancy;
- 3) presence of ascites is the most accurate ultrasound parameter for malignancy, but tumor size ≤ 6 cm and cystic structure for benign tumor nature;
- 4) our proposed graduation of serum CA-125 levels into three grades: less than 35 U/mL; between 35 and 130 U/mL; and more than 130 U/mL could improve the accuracy of RMI in prediction of ovarian malignancy.

Clearly defined primary and secondary outcome measures

The preoperative evaluation:

- 1) *Demographic data*;
- 2) *Ultrasound examination*, regarding: size, tumor structure, distinguish ability of the tumor from the surrounding tissue, spread of the tumor, presence of the ascites, feature of margins, thickness of the capsule if the tumor was cystic, as well as pre-

sence of septum/papillary vegetation or quality of liquor into the cystic tumor;

- 3) *Preoperative value of Serum CA-125* with ECI (enhanced chemiluminescence technique) with original CA-125 II tm kit (Johnson & Johnson);
- 4) *The modified RMI according to Tingulstad et al* [13] for each woman was calculated using the formula:

$$RMI = U \times M \times \text{serum CA-125}$$

Five ultrasound features suggestive of malignancy were sought to derive the ultrasound score (U): multilocular feature, presence of solid elements, bilateral appearance, presence of ascites, evidence of metastases. An ultrasound score (U) of 1 was given if none or one of these features was present, and a score of 3 was given if two or more of these features were detected. A menopausal score (M) of 1 or 3 was given to pre- and postmenopausal women, respectively. Referral to the Northern Gynaecological Oncology Centre [14] the value RMI > 200 was considered as sign of malignancy.

Histopathological examinations: The operative specimens were fixed in 10% neutral buffered formalin for 24 to 48 hours and routinely processed in pa-

Tab. 1. Demographic data: age, gynecological age, parity, familiar history for ovarian tumor or cancer, habits of smoking and alcohol consuming, body mass index.

Variable	Group A (with malignant ovarian tumor) (n = 41) column 1	Group B (with benign ovarian tumor) (n = 74) column 2	X ² (t) column 1–2
age (years) (mean ± SD) [^]	48.61 ± 4.09	37.43 ± 3.91	1.98*
gynecological age [†]			
• puberty/adolescence	0/41 (0.00%)	8/74 (10.8%)	6.58*
• generative age	19/41 (46.3%)	46/74 (62.2%)	0.17
• early postmenopause	7/41 (17.1%)	9/74 (12.2%)	0.20
• senium	15/41 (36.6%)	11/74 (14.9%)	5.93*
parity (mean ± SD) [*]	1.85 ± 0.8	2.1 ± 0.7	0.23
body mass index (BMI) (mean ± SD) [^]	24.85 ± 3.0	22.1 ± 3.2	0.63
smoker [†]	18/41 (43.9%)	28/74 (37.8%)	0.22
alcohol consumer [†]	6/41 (14.6%)	9/74 (12.2%)	0.008
family history for ovarian tumor [†]	4/41 (9.7%)	3/74 (4.1%)	0.02
family history for any kind of cancer [†]	10/41 (24.4%)	11/74 (14.8%)	1.03

([^]) = Student's t-test

([†]) = Mantel-Haenzel's X² test with df of 1

(*) p < 0.05

(**) p < 0.01

(***) p < 0.001

raffin wax. They were examined by light microscopy by the same pathologist, who was not informed of the patient group.

Statistical methods: The Student's paired test was used to compare: demographic data. The predictive values, as well as sensitivity and specificity, as indicators of how well those patients with disease or non-disease were correctly classified, were analyzed according to the standard formulae [15].

- Positive predictive value (PPV) = individuals with ovarian malignancy and a positive test / all those with a positive test
- Negative predictive value (NPV) = individuals without ovarian malignancy and a negative test / all those with a negative test
- Sensitivity = individuals with ovarian malignancy and a positive test / all those with ovarian malignancy
- Specificity = individuals without ovarian malignancy and negative test / all those without ovarian malignancy

The Mantel-Haenzel's X^2 test was used to compare: demographic data, Serum CA-125 and ultrasound characteristics, according to the formula:

$$X^2 = n \frac{([AD-BC]-n/2)^2}{(A+B)(C+D)(A+C)(B+D)}$$

Results

There were some significant differences in demographic data between the groups. Namely, puberty and adolescence age was most frequent among the patients in group B ($p < 0.05$), but senium in group A ($p < 0.05$). In total, the patients with ovarian malignancy were significantly older than those with benign ovarian tumors ($p < 0.05$) (Tab. 1).

On the Tab. 2 we represent the differences between the groups regarding the preoperative Serum CA-125. The subjects with Serum CA-125 < 35 U/ml were more frequent in group B vs group A ($p < 0.001$), but those with Serum CA-125 ≥ 35 U/ml were more frequent in group A vs group B ($p < 0.001$). Nevertheless, there were no differences in prevalence of patients with mildly elevated CA-125 (35–130 U/ml) between the groups. The value of Serum CA-125 < 35 U/ml showed a relatively high NPV for malignancy, as well as sensitivity and specificity (82.8%; 0.732; 0.716 respectively). The value of Serum CA-125 U/ml ≥ 35 U/ml showed the same high sensitivity and specificity as previous parameter. Nevertheless, our proposed graduation of serum CA-125 levels into three grades: less than

35 U/mL; between 35 and 130 U/mL; and more than 130 U/mL increased the specificity for both parameters: CA125=35–130 U/mL up to 0.811, and for CA-125 > 130 U/mL up to 0.905, as well as PPV for the latter parameter up to 72.0% (Tab. 2).

The Tab. 3 represents the differences between the groups regarding the ultrasound features of the ovarian tumors, as well as the predictive values of each particular one. Almost all ultrasound features showed very significant differences between the groups. So, the ultrasound characteristics such as: tumor size > 6 cm, structure of the tumor (solid/cystic), unclear distinguish ability of the tumor, presence of the ascites into the abdominal cavity, rugged margins, thickness of the capsule > 2 mm, presence of septum/papillary vegetation into the cyst thicker than 2 mm, multi-locular feature and presence of intra-cystic liquor with high density were more frequent in patients with malignant ovarian tumor ($p < 0.001$, all of them). Regarding the PPV $> 70.0\%$, as a likelihood of the ultrasound feature for that the individual has a malignant disease, only the presence of ascites into the abdominal cavity showed the high one. The NPV

Tab. 2. Differences among the both study groups regarding the preoperative CA-125 serum level (our proposed graduation of the serum CA-125 levels).

Variable	Group A (with malignant ovarian tumor) (n = 41)	Group B (with benign ovarian tumor) (n = 74)	Differences between group A and group B column 1–2	Positive predictive value for malignancy	Negative predictive value for malignancy	Sensitivity	Specificity
CA 125 < 35 U/mL subgroup CA(a) (n = 64/115)	11/41 (26.8%)	53/74 (71.6%)	23.30***	17.2%	82.8%	0.732	0.716
CA 125 = 35–130 U/mL subgroup CA(b) (n = 26/115)	12/41 (29.3%)	14/74 (18.9%)	0.07	46.2%	53.8%	0.293	0.811
CA 125 > 130 U/mL subgroup CA(c) (n = 25/115)	18/41 (43.9%)	7/74 (9.5%)	16.43***	72.0%	28.0%	0.439	0.905
CA 125 elevated (≥ 35 U/mL)	30/41 (73.2%)	21/74 (28.4%)	19.67***	58.8%	41.1%	0.732	0.716
Mantel-Haenzel's X^2 test with df of 1:							
(*) $p < 0.05$							
(**) $p < 0.01$							
(***) $p < 0.001$							

Tab. 3. Ultrasound characteristics of the ovarian tumors.

Variable	Group A (with malignant ovarian tumor) (n = 41)	Group B (with benign ovarian tumor) (n = 74)	X ² differences between group A and B column 1–2	Positive predictive value for malignancy	Negative predictive value for malignancy	Sensitivity	Specificity
size							
• ≤ 6 cm	11/41 (26.8%)	52/74 (70.3%)	21.89***	17.5%	82.5%	0.268	0.703
• > 6 cm	30/41 (73.2%)	22/74 (29.7%)	18.38***	57.7%	42.3%	0.487	0.297
tumor structure							
• solid	2/41 (4.9%)	10/74 (13.5%)	3.13	16.7%	83.3%	0.487	0.135
• solid and cystic	20/41 (48.8%)	12/74 (16.2%)	12.36***	62.5%	37.5%	0.487	0.162
• cystic	19/41 (46.3%)	52/74 (70.3%)	7.45**	26.8%	73.2%	0.463	0.703
distinguish ability of tumor from the surrounding tissue							
• clear	19/41 (46.3%)	60/74 (81.1%)	16.46***	24.1%	75.9%	0.466	0.811
• unclear	22/41 (53.7%)	14/74 (18.9%)	13.23***	61.1%	38.9%	0.536	0.189
spread of the tumor							
• unilateral tumor	30/41 (73.2%)	46/74 (62.2%)	0.98	39.5%	60.5%	0.732	0.378
• bilateral tumor	11/41 (26.8%)	28/74 (37.8%)	1.96	28.2%	60.5%	0.268	0.622
ascites							
• absence	28/41 (68.3%)	69/74 (93.2%)	14.40***	28.9%	71.1%	0.683	0.932
• around the adnexa	2/41 (4.9%)	5/74 (6.8%)	0.66	28.6%	71.4%	0.488	0.067
• into the abdominal cavity	11/41 (26.8%)	0/74 (0.00%)	18.96***	100.0%	0.00%	0.268	0.00
feature of margins							
• smooth	5/41 (12.2%)	53/74 (71.6%)	39.69***	8.6%	91.4%	0.122	0.716
• rugged	36/41 (87.8%)	21/74 (28.4%)	34.93***	63.1%	36.8%	0.878	0.284
thickness of the capsule							
• ≤ 2 mm	9/39 (23.1%)	47/74 (63.5%)	16.18***	16.1%	83.9%	0.231	0.635
• > 2 mm	30/39 (76.9%)	27/74 (36.5%)	14.61***	52.6%	47.4%	0.769	0.365
presence of septum/papillary vegetation into the tumor:							
• none	1/39 (2.6%)	29/74 (39.2%)	18.91***	3.3%	96.7%	0.025	0.392
• ≤ 2 mm	3/39 (4.7%)	30/74 (40.5%)	14.52***	9.1%	90.9%	0.077	0.405
• > 2 mm	35/39 (89.7%)	15/74 (20.3%)	45.64***	70.0%	30.0%	0.897	0.203
• uni-locular cyst	14/39 (35.9%)	56/74 (75.7%)	20.71***	20.0%	80.0%	0.359	0.757
• multi-locular cyst	25/39 (64.1%)	18/74 (24.3%)	14.97***	58.1%	41.9%	0.641	0.243
Quality of the liquor into the cyst:							
• with high density	29/39 (74.4%)	32/74 (43.2%)	8.44**	47.5%	52.4%	0.743	0.432
• with low density	10/39 (25.6%)	42/74 (56.8%)	10.91***	19.2%	80.8%	0.256	0.568

Mantel-Haenzel's X² test with df of 1:

(*) p < 0.05

(**) p < 0.01

(***) p < 0.001

> 70.0% as a likelihood that the individual is free from malignant disease, was found for the following ultrasound features: tumor size ≤ 6 cm, solid tumor structure, cystic tumor structure, clear

distinguish ability of the tumor from the surround tissue, unilateral tumor, absence of the ascites, smooth margins, capsule ≤ 2 mm, absence of septum/papillary vegetation into the cysts ≤ 2 mm,

uni-locular cyst, intra-locular liquor with low density. The sensitivity > 0.700, as an indicator of how well those patients with disease are correctly classified according to the particular ultrasound fea-

ture, was noted only for: uni-laterality of the tumor, rugged margins, thicker than 2 mm capsule, septum/papillary vegetation thicker than 2 mm, and intra-locular liquor with high density. The specificity > 0.700, as an indicator of how well those without malignant disease are correctly classified according to particular ultrasound feature, was noted for the following features: tumor size ≤ 6 cm, cystic structure, clear distinguish ability of the tumor from the surround tissue, absence of the ascites, smooth margins and uni-locular feature.

Nevertheless, the highest sensitivity, as an indicator for best distinguishing the diseased patients, showed the following parameters: rugged margins and presence of septum/vegetations, (0.878; 0.897, respectively). The following ultrasound parameters showed the highest specificity, as an indicator for best distinguishing the healthy patients: clear di-

stinguish ability of tumor from the surrounding tissue and absence of ascites (0.811; 0.932, respectively). The parameter: presence of the ascites into the abdominal cavity showed the highest PPP (100%) i.e. it was the best ultrasound predictor for ovarian malignancy (Tab. 3).

The histological features in the first study group-A (n = 41) consisted of patients with malignant ovarian tumor was as follows: serous adenocarcinoma (12/41), mucinous cystadenocarcinoma (7/41), endometrioid adenocarcinoma (12/41) clear cell ovarian carcinoma (5/41), others ovarian carcinomas (5/41). The stage of spreading was: borderline (IA) (10/41); stage IC (13/41); stage II (2/41); IIIA (4/41); IIIB (3/41); III C (9/41). The nuclear grade in the same group was as follows: G1 (10/41), G2 (14/41), G3 (17/41).

The histological features in the first study group-B (n = 74), consisted of patients with benign ovarian tumor was

as follows: serous ovarian cystadenoma (12/74), mucinous ovarian cystadenoma (13/74), benign teratoma (12/74) and simplex cysts (11/74) and endometrioma (26/74).

In order to estimate whether our proposed graduation of serum CA-125 levels into abovementioned three grades could improve the accuracy of RMI in prediction of ovarian malignancy, we made:

- 1) the predictive values for RMI for entire study group;
- 2) the predictive values for the three particular parts of RMI: menopausal status, ultrasound features and Ca-125 levels, as a single criterion;
- 3) the predictive values for RMI for the three abovementioned subgroups according to our proposed graduation of CA-125.

So:

- 1) the pre-menopausal status as a single criterion, noted with point 1 in

Tab. 4. Risk of malignancy index (RMI), its particular parts as a single criterion, such as menopausal status and ultrasound features, and the correlation between RMI and serum CA-125 levels.

Variable	Group A (with malignant ovarian tumor) (n = 41)	Group B (with benign ovarian tumor) (n = 74)	X ² differences between group A and B column 1–2	Positive predictive value for malignancy	Negative predictive value for malignancy	Sensitivity	Specificity
menopausal status (M)							
M = 1	19/41 (46.3%)	54/74 (73.0%)	9.26**	26.0%	74.0%	0.463	0.270
M = 3	22/41 (53.7%)	20/74 (27.0%)	6.96**	52.4%	47.6%	0.536	0.729
ultrasound features (U)							
U = 1	12/41 (29.3%)	52/74 (70.3%)	19.67***	18.7%	81.3%	0.293	0.297
U = 3	29/41 (70.7%)	22/74 (29.7%)	16.34***	56.9%	56.9%	0.707	0.703
CA 125 < 35 U/ml							
• RMI ≤ 200	9/11 (81.8%)	40/53 (75.5%)	0.005	18.4%	81.6%	0.818	0.245
• RMI > 200	2/11 (18.2%)	13/53 (24.5%)	0.71	13.3%	86.7%	0.182	0.755
CA125 = 35–130 U/ml							
• RMI ≤ 200	3/12 (25.0%)	1/14 (7.1%)	0.60	75.0%	25.0%	0.250	0.929
• RMI > 200	9/12 (75.0%)	13/14 (92.9%)	3.24	40.9%	59.1%	0.750	0.071
CA125 ≥ 130 U/ml							
• RMI ≤ 200	1/18 (5.5%)	2/7 (2.86%)	5.17*	33.3%	66.7%	0.055	0.714
• RMI > 200	17/18 (94.4%)	5/7 (71.4%)	0.82	77.3%	22.7%	0.944	0.286
RMI in entirely group (independent of CA125)							
• RMI ≤ 200	13/41 (31.7%)	43/74 (58.1%)	8.45**	23.2%	76.8%	0.317	0.419
• RMI > 200	28/41 (68.3%)	31/74 (41.9%)	6.34*	47.5%	52.5%	0.683	0.582

Mantel-Haenzel's X² test with df of 1:

(*) p < 0.05

(**) p < 0.01

(***) p < 0.001

RMI, was more frequent in group B ($p < 0.01$) and showed relatively high NPV for malignancy (74.0%). The post-menopausal status, noted with point 3 in RMI, was more frequent in group A ($p < 0.01$), and showed relatively significant specificity for malignancy (0.729);

- 2) the ultrasound criteria, noted with point 1 in RMI, as an indicator for benign tumor nature, predominated in group A ($p < 0.001$) and showed high NPV of 81.3%. On the contrary, the ultrasound criteria, noted with point 3 in RMI, as an indicator for malignant tumor nature, predominated in group B ($p < 0.001$), but their predictive values were low. Nevertheless, they showed relatively high sensitivity and specificity (0.707 and 0.703, respectively);
- 3) RMI for entire study group, as an indicator for risk of tumor malignancy showed only relatively high NPV for $RMI \leq 200$ (76.8%);
- 4) when we additionally analyzed RMI in correlation with CA-125 levels, we found that its predictive values and sensitivity/specificity increased. So, in subgroup CA(a) with $CA-125 < 35$ U/ml we noted this situation: high NPV and sensitivity (81.6% and 0.818, respectively) for $RMI \leq 200$; but also high NPV (86.7%) for $RMI > 200$. In subgroup CA(b) with CA-125 between 35 and 130 U/ml, we noted very high specificity, as an indicator for good distinguishing of healthy patients (0.929) for $RMI \leq 200$. In patients with $CA-125 > 130$ U/mL, $RMI > 200$ showed very high sensitivity, as an indicator for good distinguishing of diseased patients (0.944) (Tab. 4).

Discussion

Estimating the predictive values of different parameters for tumor malignancy: such as: serum CA-125, ultrasound findings and RMI, as a single criterion, we noted them relatively insignificant, that pertain to the uncertain diagnosis for ovarian malignancy. These findings confirmed our first specific hypothesis.

Nevertheless, when we divided all patients into three subgroups, according to our new proposed graduation of serum CA-125 levels into three gra-

des: less than 35 U/mL; between 35 and 130 U/mL; and more than 130 U/mL, we noted:

- 1) increased specificity of $RMI \leq 200$ up to 0.929 for patients with mildly elevated CA-125 (35–130 U/mL), as an indicator for good distinguishing of healthy patients;
- 2) increased sensitivity of $RMI > 200$ up to 0.944 for patients with highly elevated CA-125 (> 130 U/mL), as an indicator for good distinguishing of diseased patients. These findings confirmed our second specific hypothesis that the $RMI > 200$ in cases with mildly elevated Serum CA-125 (35–130 U/ml), is not enough reliable parameter for ovarian malignancy; These results also confirmed our forth hypothesis that our proposed graduation of serum CA-125 levels into three grades: less than 35 U/mL; between 35 and 130 U/mL; and more than 130 U/mL could improve the accuracy of RMI in prediction of ovarian malignancy. Therefore, our opinion is that patients with mildly elevated CA-125 (35–130 U/mL) and $RMI > 200$ necessarily should be undergone additional diagnostic methods, including operation and histological verification. Tsukishiro et al [16], using an serum secretory leukocyte protease inhibitor levels (SLPI) cut-off of 50 ng/ml and a CA-125 cut-off of 30 U/mL, found that with both markers elevated, the sensitivity was 95%, the specificity was 100%, the PPV was 100%, and the NPV 89% between the malignant and benign cysts. Rzym-ski et al [17], evaluating the serum concentrations of soluble intracellular adhesion molecule-1 (sICAM-1), as well as CA-125 in 45 women with benign ovarian tumors, observed higher sICAM-1 concentrations in fibrothecomas and lower in endometrial and dermoid cysts. Serum ICAM-1 concentrations correlate with some histological types of benign tumors, but not with tumor volume. Levels of CA-125 were more effective than ICAM-1 in ovarian tumors differentiation.

Regarding the ultrasound features, in our study:

- 1) presence of ascites into the abdominal cavity showed highest PPV (100%) i.e. it was the best ultrasound predictor for ovarian malignancy;
- 2) the parameters: clear distinguish ability of tumor from the surrounding tissue and absence of ascites showed highest specificity, i.e. they were the best parameters for good distinguishing of healthy patients (0.811; 0.932, respectively). These findings only partially confirmed our third specific hypothesis.

Sagiv et al [18], in their study of 21 patients with extremely large cystic/complex ovarian cysts, reached the umbilicus or higher, and were not associated with ascites or enlarged pelvic or para-aortic lymph nodes on computed tomography scan, found Serum CA-125 levels within the normal range or mildly elevated (< 130 U/mL). In one of them (1/21, 4.8%) they found ovarian malignancy. Geomini et al [19], estimating 181 women with ovarian masses with three-dimensional ultrasonography and three-dimensional power Doppler, found that: central vessels were present in 15% of the benign masses, 69% of the malignant masses and 27% of the masses of borderline malignancy; and the likelihood ratios for presence of central vessels for a mass being malignant and/or borderline was 4.9 (95%, CI 2.1–12).

In order to realize our second aim of the work, we analyzed the predictive values for RMI in cases with mildly elevated Serum CA-125 (35–130 U/ml), levels, which are doubtful for malignancy and found very high specificity (0.929) for $RMI \leq 200$; and high sensitivity (0.750) for $RMI > 200$; but also high PPV for $RMI \leq 200$ (75.0%), the fact which confirmed our suspicion that this group of patients could be the most confounding regarding the nature of the ovarian tumor, and the decision for operative treatment. On the other hand, we found high NPV (86.7%) for $RMI > 200$ in subgroup CA(a) with $CA-125 < 35$ U/ml. These above-mentioned findings: high PPV for $RMI \leq 200$ in subgroup CA(b) with slightly elevated CA-125 and also high NPV for $RMI > 200$ in subgroup CA(A) with low CA-125 were in favor of the parame-

ter CA-125 vs the parameter RMI in patients with Ca-125 lower than 130 U/mL regarding the prediction of ovarian malignancy.

Ulusoy et al [20], assessing the ability of the RMI in 296 women with adnexal masses found that the RMI with cut-off level of 153 identified malignant cases more accurately than any individual criterion (PPV of 65.9%; NPV of 85.5%; sensitivity of 0.764 and specificity of 0.779). Bailey et al [21] in their series of 182 patients with a pelvic mass found that 24% patients had benign tumors, 6% had tumors of borderline malignancy, and 70% had invasive tumors. An RMI > 200 had a sensitivity of 88.5% for diagnosing invasive lesions. The overall sensitivity of this algorithm for diagnosing all borderline, invasive ovarian tumors, or primary peritoneal lesions was 87.4% and the PPV was 86.8%. Therefore, they recommend the RMI for continued use. Szperek et al [22] assessing the usefulness of their artificial neural network computer model, which included: age, menopausal status, BMI, grayscale and Doppler ultrasonographic features, as well as levels of CA-125 and tissue polypeptide specific antigen, in series of 686 women, found very high sensitivity and specificity of this method in prediction of ovarian tumor malignancy (96.0% and 97.7%, respectively). Roupá et al [23] proposed the combination of their Transvaginal Ultra Sonography score ≥ 35 points and CA-125 ≥ 30 U/ml as an accurate screening procedure for ovarian malignancy. In their retrospective case-control pilot study of 120 women with ovarian neoplasia, they found a sensitivity of 81.7% and specificity of 100.0% of this combination. Moolthiya et al [24] compared the ability of two risk of malignancy indices to discriminate between benign and borderline/malignant ovarian tumor in 209 women with pelvic masses and found that cut-off 200 gave sensitivity of 70.6%, specificity of 83.9%, PPV of 75% and NPV of 80.6% for RMI 1, and sensitivity of 80%, specificity of 78.2%, PPV of 71.6% and NPV of 85.1% for RMI 2. In our study of 115 women with ovarian masses, the Tingulstad' RMI gave sensitivity of only 47.5%, specificity of 52.5%, PPV of 68.3% and NPV of 58.2% for RMI > 200

and CA-125 > 35 U/ml, but sensitivity of 94.4% and PPV of 77.3% for RMI > 200 and CA-125 > 130 U/ml. Van den Akker et al [25] in series of 548 women with ovarian masses found PPV of 48% and NPP of 96% for RMI > 200 in detection of ovarian cancer. Montagnana et al [26] evaluated the predictive values of their ROMA (Risk of malignancy algorithm) as separate logistic regression algorithm in 104 women with a pelvic mass (55 with epithelial ovarian cancer and 49 benign cases) and found that ROMA had high predictive values (84.6% specificity and 82.5% sensitivity) only for postmenopausal women, but not in pre-menopausal women.

Conclusion

We found Serum CA-125, ultrasound findings and RMI, as single criteria for malignancy not enough accurate. In the subgroup of patients with CA-125 between 35 and 130 U/ml we noted high PPV and specificity (75.0% and 0.929, respectively) for RMI < 200; and high sensitivity (0.750) for RMI > 200. These results for RMI confirmed our suspicion that this subgroup of patients could be the most confounding regarding the nature of the ovarian tumor, and the decision for operative treatment. We recommend that the RMI should be interpreted carefully and only in correlation with serum levels of CA-125, for every case individually. For that reason we propose a graduation of serum CA-125 levels into three grades: less than 35 U/mL; between 35 and 130 U/mL; and more than 130 U/mL and analyzing the RMI in light of this graduation. This procedure could improve the accuracy of RMI in prediction of ovarian malignancy.

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