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*Studies on angiogenesis in the benign and malignant ovarian neoplasms with the use of color and pulsed Doppler sonography and serum CA-125, CA-19.9, CA-72.4 and vascular endothelial growth factor measurements**

Ovarian cancer is the leading cause of death from malignant genital tract neoplasms in women living in industrialized countries (21). The accurate diagnosis of early stages of the disease is extremely difficult and until recently the detection of cancer confined to the ovaries has been made only in a relatively small proportion of patients (25). The main reason for this fact is that the most common of malignant adnexal tumors – epithelial ovarian cancers – do not produce any characteristic symptoms in early stages. The correct diagnosis is therefore made mostly in the late phase of the disease (5, 31, 32). This fact has a strong impact on poor survival rates obtained worldwide, despite introduction of modern and more aggressive treatment modalities. It has been well documented that the cure rate of this devastating disease can be improved and that the treatment is effective in 80-95% of women with cancer localized in one or both ovaries (stages IA to IC, according to FIGO classification). Epidemiological data also indicate that the mortality could be decreased by half if detection rates of clinical stage I ovarian cancer could be increased from currently reported 20% to those planned at least by 80% (28).

Since adnexal masses are frequently encountered in women during their reproductive years there is a clinical need for the introduction of diagnostic tests which could efficiently distinguish between probably benign and possibly malignant ovarian tumors. Most of the tumors are benign and do not require surgical intervention (25). In rare cases, however, a prompt and immediate surgical procedure is mandatory. The improvement in test specificity could help to avoid unnecessary operation (16). Moreover, in cases of benign tumors accurate diagnosis could allow to plan a less invasive surgical procedure such as laparoscopy (6). Historically, a palpable and persistent adnexal tumor was re-

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quired to raise a suspicion of ovarian cancer. In recent years, a number of biologic markers have been identified that may provide useful prognostic information in the early detection of ovarian malignancy. To date, the most widely studied ovarian cancer associated antigen has been CA-125 (4). A value of either 35 U/mL (10) or 65 U/mL (associated with a lower false positive rate and higher specificity) has been used to predict the presence of ovarian malignancy, but increased plasma levels of CA-125 have also been found in patients with endometriosis (15). Mucinous carcinomas which comprise 10-20% of epithelial ovarian cancer cases often do not produce elevated serum CA-125. In such cases complementary tumor markers such as CA-19.9 and CA-72.4 can be used (8, 11, 23).

Currently, the majority of clinicians make their treatment decisions using a panel of diagnostic procedures. These include bimanual examination, serum tumor markers assessment, computed tomography, magnetic resonance imaging and gynecological ultrasound (23). The latter has been extensively used since the introduction of transvaginal probes almost 20 years ago. The introduction of color and pulsed Doppler sonography in the late 80s allowed the detection of tumor blood supply and technical advances in spatial image resolution enabled studies on the development of neoplastic vessels (4). The process of new blood vessels formation is called angiogenesis and is required for tumor growth and progression. Vascularization of the tumor is accomplished through a series of sequential steps before or during the multistep progression to neoplasia (10). Several events occur during the formation of new vessels, including production of protease enzymes, upregulation of positive regulators of angiogenesis, and downregulation of negative factors (2). Recent knowledge of tumor angiogenesis may have implications in diagnosis and treatment. There is a clinical need for the development and evaluation of algorithms to distinguish benign tumors from malignant ovarian ones. The independent variables may include tumor markers, transvaginal ultrasonography with B-mode and color Doppler imaging with pulsed spectral analysis, computed tomography, and magnetic resonance imaging (17). In view of ever-increasing health care costs it is necessary to make optimal use of the limited resources and to avoid referring patients for multiple expensive imaging techniques or invasive procedures.

New ultrasound systems have capability to detect very small tumor vessels and a very slow blood flow velocities. The technical ability to indirect detection of angiogenesis has allowed to study blood supply in many tumors (Fig. 1). For instance, the lowest velocities typically detected in B&K Ultrasound Systems range from 0.8 cm/s in color Doppler flow mapping to 0.15 cm/s in "power" Doppler mode. In normal conditions angiogenesis is tightly regulated and appears in the female reproductive tract in the endometrium as well as in the ovarian follicle and *corpus luteum* formation (2). There are currently more than 30 known angiogenic stimulators and inhibitors. Most of malignant tumors cannot grow beyond 1-2mm^3 in size without new blood vessel formation. According to Folkman et al. (10) a critical point is the change of cells type from non-angiogenic to angiogenic with following uncontrolled vessel growth. This has been called an "angiogenic switch". A tumor starts to grow rapidly and eventually can be detected during the clinical examination.

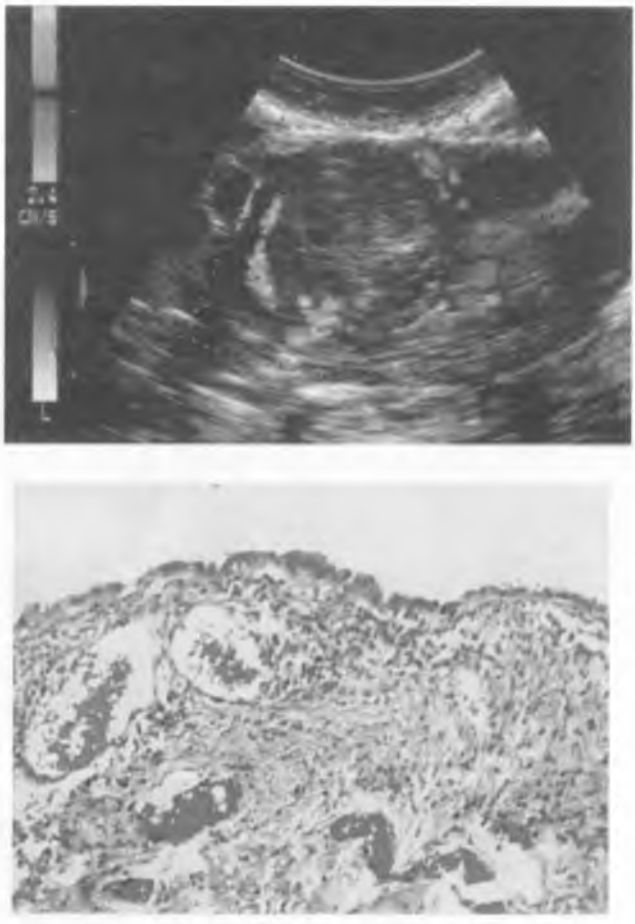

Fig. 1 Histology demonstrating ovarian blood vessels (upper) and an example of angiogenesis detection with color Doppler flow mapping within the corpus luteum of a normal ovary (lower)

Moreover, the highest probability of metastatic spread is in the tumor regions with high microvessel density. There are several lines of evidence to support the observation that angiogenesis assessement could be a prognostic factor related to the tumor growth and metastases (1, 2). During the last decade the correlation has been found among others in melanoma, breast, prostate, endometrial and cervical cancer (25). Vascular endothelial growth factor (VEGF) is a member of heparin-binding growth factors and one of the most potent and specific endothelial cells stimulators. This polypeptide has been also initially known as a "vascular permeability factor" (VPF), since based on molecular basis it can increase the vascular permeability more than 50,000 stronger than histamine (24).

VEGF is a 34- to 50-kd dimer composed of two identical disulfide-linked subunits that arise from differential splicing of a single gene. It is induced under hypoxic conditions in a wide variety of tissue types and binds to the receptor tyrosine kinases Flt-1 and KDR/Flk-1 (1, 30). Because fast growing hypoxic tumors display increased angiogenesis, VEGF protein levels may serve as a surrogate marker for rapid and uncontrolled new blood

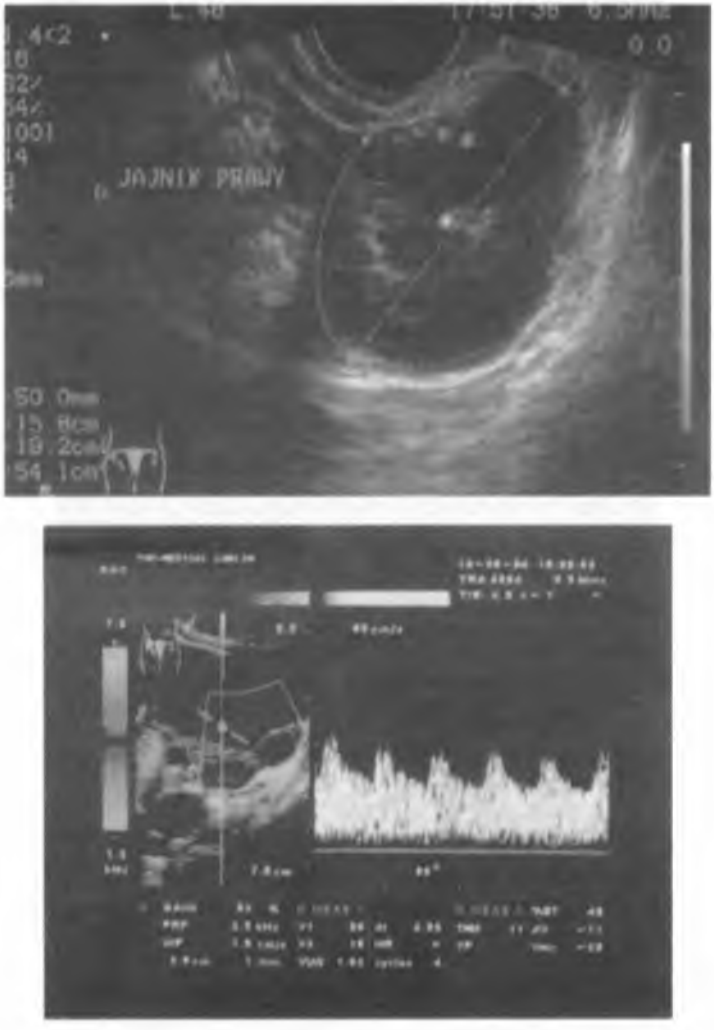

Fig. 2. Spectral and color Doppler blood flow mapping in a septum of a complex but benign ovarian cyst (upper) and "power" Doppler detection of angiogenesis in the solid part of the small malignant ovarian tumor (lower)

vessel formation. The cytokine promotes tumor angiogenesis in many malignant tumor types by stimulating endothelial-cell proliferation and promoting vascular permeability. Several recent clinical studies indicated that VEGF serum concentrations were elevated in patients with early and advanced ovarian cancers (1, 30). Therefore, it has been postulated that VEGF could be a potential marker discriminating benign tumors from malignant adnexal ones.

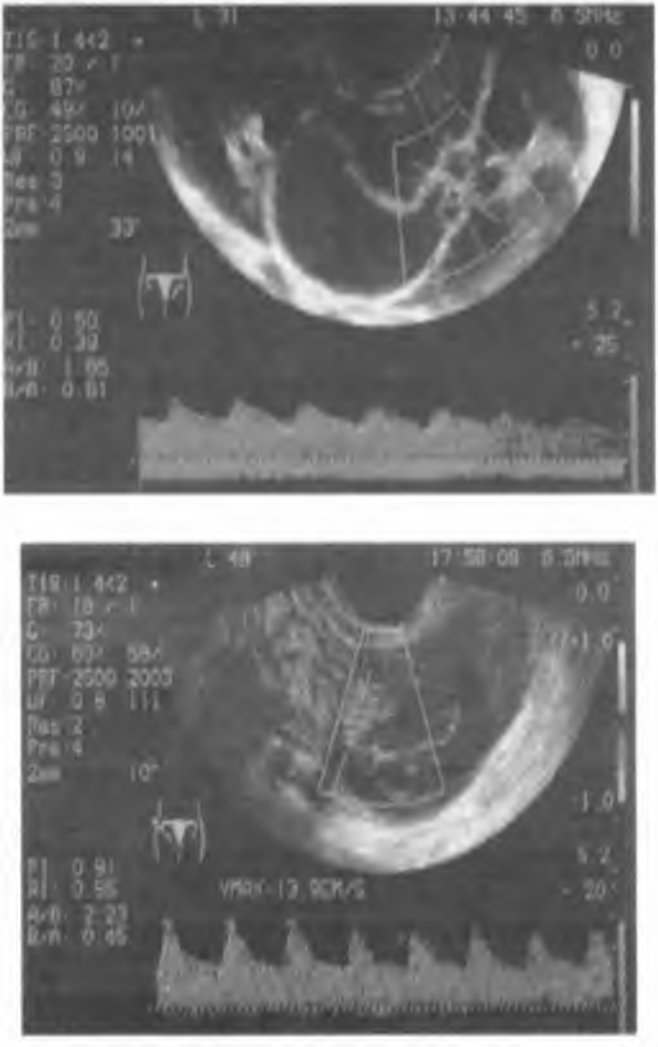

Fig. 3. Spectral analysis of high resistance (RI>0.5) to blood flow in a benign tumor (upper) and low-resistance (RI<0.4) flow in ovarian carcinoma (lower)

More than 10 years' experience with color Doppler sonography in the early discrimination between female pelvic masses indicates that this method is still controversial (5). The studies are based on the assumption that all fast growing tumors have to demonstrate new blood vessels formation and these vessels are detectable with color Doppler (Fig. 2). However, the limitations of sonographic assessment are substantial. First, new blood vessels are formed in several benign conditions such as pelvic inflammatory disease and endometriosis. Second, persistent angiogenesis is encountered in complex luteal cysts. Finally, areas of tumor necrosis especially in large masses often do not present detectable flow, however, the tumor can still be malignant (29). Low resistance to flow does not neccesarily indicate malignancy (Fig. 3). According to the current knowledge universal cut-off values of PI and RI cannot be accepted in screening for ovarian cancer (25). The main reason for this is too low predictive value, especially in premenopausal women.

The aims of this study were as follows: 1) to compare the diagnostic performance of several established ultrasound criteria along with tumor markers and VEGF, a potent angiogenesis stimulator in the initial assessment of adnexal masses; 2) to generate a predictive model that would permit the calculation of the risk of malignancy for individual patients and assist in the preoperative discrimination between malignant and benign ovarian tumors; 3) to determine if a combination of a patient's age, her menopausal status and measurements of serum CA-125, CA-19.9, CA-72.4 and VEGF could improve predictive values of single tests in early detection of ovarian cancer.

METHODS

The population comprised 4,876 women from the Lublin district who had transvaginal ultrasound examination of pelvic structures performed by one investigator during 1994-1999 study period. Ultrasound scanners type: 3535 and 2002 ADI (B&K Medical, Denmark) with transvaginal probes 5-6.5-7.5MHz were used in all studies. In rare cases of large tumors transabdominal 3.5MHz probe was also used. Final analysis included 451 patients with adnexal masses which persisted beyond 2-3 months of follow-up and their presence has been documented with transvaginal sonography. The following clinical data were recorded: age, day of the cycle, menopausal status. In all postmenopausal patients the years elapsed since menopause were also noted. Patients on medical therapy or current smokers were excluded. Tumor was identified if a suspected adnexal mass had a volume >20ml in premenopausal and >10ml in postmenopausal women (25). Following gray-scale examination the whole tumor area was searched for blood flow with the use of color flow mapping (CFM). Localization of detected blood vessels within tumor solid parts, papillae and septa was noted. To measure color representing blood flow a semi-quantitative subjective 4-points scale flow recently proposed by Timmermann et al.

(27) was applied. Briefly, a score of 1 was given if no flow was detected, a score of 2 represented minimal color presence, a score of 3 moderate flow and a score of 4 for abundant flow (>50% of vessels) within the studied part. Scores 1, 2 and 3 were compared (sensitivity, specificity, positive and negative predictive values) with the score of 4 treated as a positive test result. Spectral Doppler analysis followed color flow mapping. For each blood vessel detected a peak systolic velocity (PSV), pulsatility (PI) and resistive (RI) indices were recorded from good quality frozen image of at least 3 consecutive flow velocity waveforms. For further statistical assessment highest peak systolic velocity and corresponding PI and RI were used. Blood samples were collected by venipuncture from women in whom surgical procedures were planned within 7 days before the operation. Following centrifugation at 1500 g for 15 minutes serum was separated, divided in 3-5 aliquots and frozen in -20^0C until assay procedure. CA-125 serum measurements were recorded in 294 of the examined patients. Concentrations of CA-19.9 and CA-72.4 were assayed in 109 and 91 women, respectively. VEGF was assayed in 116 women of whom 16 had ovarian cancer confirmed by histology. Serum CA-125, CA-19.9 and CA-72.4 were assayed with AXIA 2 apparatus (BioMerieux, France). CA-125 and CA-19.9 antigens were measured with ELISA Cobas Core EIA II (Roche, Swiss) assay kits. Immunoenzymatic assay of serum CA 72.4 concentrations was made with the use of Centocor (Malvern, Pennsylvania, USA) assay kit. VEGF concentrations were assayed on microplates with the use of Quantikine Human VEGF Immunoassay (R&D Diagnostics, Minnesota, USA). Sensitivity of the assay was 9 pg/ml, and interassay variablity was less than 10%.

The statistical analysis was performed with the use of Statistica for Windows v.5.0 software (StatSoft, USA). Sensitivity, specificity, positive and negative predictive values were calculated for all ultrasound and biochemical variables. Receiver-Operator characteristics (ROC) curves (13) were plotted to determine which cut-off levels of CA-125, CA-19.9, CA-72.4 or Doppler variables: color score, PI, RI and PSV could best discriminate between benign and malignant ovarian masses. Receiver-Operator characteristics (ROC) curves were plotted with GraphROC (Turku, Finland). A non-parametric comparison of areas under the constructed curves allows an objective comparison of the tests (13). Because the main goal of this work was to characterize angiogenesis in ovarian tumors, further analysis included 116 consecutive patients in whom Doppler, preoperative serum CA-125 and VEGF data were available. Menopausal status of each patient was also included. Stepwise forward multiple regression analysis was then used to assess which independent variable had statistically significant influence on regression coefficient "Z" used for a discrimination between benign and malignant tumors. The coefficient has a numeric value which can further be log transformed for a true probability of malignancy estimation. In all calculations $p<0.05$ was considered statistically significant.

RESULTS

The final analysis included 451 women aged 13 to 76 years (mean 38 years, median 39 years) in whom adnexal tumors were sonographically confirmed. There were 47 cases of adnexal malignancy and 404 tumors were benign (Table 1). Postmenopausal women with

Table 1. Final diagnosis of detected adnexal tumors

Benign tumors	N (%)
Serous cystadenoma	177 (43.8%)
Luteal cyst	77 (19 %)
Endometrial cyst	66 (16.3%)
Mature teratoma	31 (7.7%)
Tuboovarian abscess	27 (6.7%)
Mucinous cyst	23 (5.7%)
Myoma	3 (0.7%)
Malignant tumors	**N (%)**
Serous adenocarcinoma	19 (40.4%)
Malignant germ cell tumor	6 (12.7%)
Mucinous adenocarcinoma	5 (10.6%)
Endometrioid carcinoma	5 (10.6%)
Borderline malignancy	5 (10.6%)
Mixed epithelial tumor	4 (8.5%)
Clear cell carcinoma	1 (2.1%)
Metastases to the ovary	2 (2.1%)

benign tumors comprised 16.3% (66 of 404) and women with malignant tumors 46.8% (22 of 47%). Malignant tumors were confirmed by pathological examination in all cases. There were 286 women operated because of non-malignant adnexal tumor. In 118 initially detected simple or luteal cysts regressed spontaneously and were not detected during control ultrasound performed within 2-3 months. Only 7 women with malignant neoplasms had FIGO stage I disease, one had stage II and the rest had stage III or IV of ovarian cancer. Analysis of sonomorphologic features of the tumors revealed that the most frequent tumors were unilocular cysts, serous or mucinous. Solid tumors were the least frequently detected and half of them were malignant. Multilocular cysts or cysts with solid elements were in most cases benign. Medians of benign and malignant tumors

diameters were 61 and 86 mm, respectively. The differences were statistically significant (Mann-Whithey U test, $p<0.05$).

The accuracy of Doppler sonography was as follows: RI and PI had their best predictive values at cut-off levels 0.63 and 0.87 respectively. PSV optimal discrimination level was 21 cm/s. The sensitivity was between 85% (RI), 67% (PSV) and 66% (PI). For the subjective color assessment sensitivity was 87%. 294 women had their CA-125 serum assays available. For CA-19.9, CA-72.4 and VEGF the corresponding numbers were 109, 92 and 116 patients, respectively. Optimal cut-off level for CA-125 was 67.5 U/ml and this produced diagnostic accuracy of 69.4%. For CA-19.9 the best predictive value was 13.4 U/ml (accuracy of 74%) and for CA-72.4 (4.1 U/ml) the accuracy was 77.8%. The highest diagnostic accuracy of 75% was found for the COLOR value of (1). VEGF con-

Table 2. Prognostic values of various VEGF serum concentrations in 116 women with adnexal tumors

VEGF (optimal cut-off value)	Sensitivity	95% CI	Specificity	95% CI	Accuracy
100 pg/ml	80.0	59.0-93.4	18.7	11.1-28.3	49.3
150 pg/ml	76.0	54.6-90.8	29.7	20.5-40.2	52.8
200 pg/ml	72.0	50.4-88.1	35.2	25.4-45.9	53.6
300 pg/ml	60.0	38.5-79.0	50.5	39.8-61.2	55.3
350 pg/ml	60.0	38.5-79.0	54.9	44.1-65.4	57.5
450 pg/ml	44.0	24.2-65.2	68.1	57.5-77.5	56.1
600 pg/ml	36.0	17.8-57.7	78.0	68.1-86.1	57.0

centration of 350 pg/ml had the best accuracy of 57% in women without their menopausal status use (Table 2). When menopausal status was also considered, the best prognostic values of this test were found at 350 pg/ml before menopause and 450 pg/ml after menopause. The calculated accuracy was 56.1% and 68.2%, respectively. As shown in Table 3, the accuracy of other markers assessment ranged from 69.4% (CA125=67.5 U/ml) to 77.8% (CA-72.4 = 4. 1 U/ml). The highest area under the ROC curve in women without distinguishing their menopausal status was found for CA-72.4 and subjective COLOR assessment. Most useful biochemical tests for postmenopausal patients were found for CA-72.4 and CA-125 whereas COLOR was the most useful Doppler sonography related test (Fig. 4). Only slightly lower values for areas under ROC curves were found for pulsatility and resistive indices. However, all theoretically calculated values of single tests

Table 3. Prognostic values for all applied tests in the studied population of women with adnexal tumors

Test (optimal cut-off value)	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV	NPV
COLOR (1)	87.0	63.0	75.0	0.70	0.83
PI (0.87)	66.0	79.9	72.9	0.77	0.70
RI (0.63)	85.1	60.8	73.0	0.68	0.80
PSV (21.0)	68.1	44.5	56.3	0.55	0.58
CA-125 (67.5)	59.5	79.4	69.4	0.74	0.66
CA-19.9 (13.4)	97.5	50.7	74.1	0.66	0.95
CA-72.4 (4.1)	85.0	70.6	77.8	0.74	0.82
VEGF (731)	32.0	89.0	60.5	0.74	0.57

PPV – positive predictive value; NPV – negative predictive value.

Table 4. Comparison of areas under the ROC curve for all diagnostic tests in the studied population

Test	AUROC*	SE	95% CI
COLOR score	0.8029	0.0351	0.7327-0.8731
PI	0.7770	0.0375	0.7020-0.8520
RI	0.7899	0.0335	0.7229-0.8569
PSV	0.5701	0.0373	0.4955-0.6447
CA-125	0.6626	0.0505	0.5616-0.7636
CA-19.9	0.6906	0.0502	0.5902-0.7910
CA-72.4	0.8270	0.0458	0.7354-0.9186
VEGF	0.5895	0.0695	0.4505-0.7285

* AUROC – area under ROC curve.

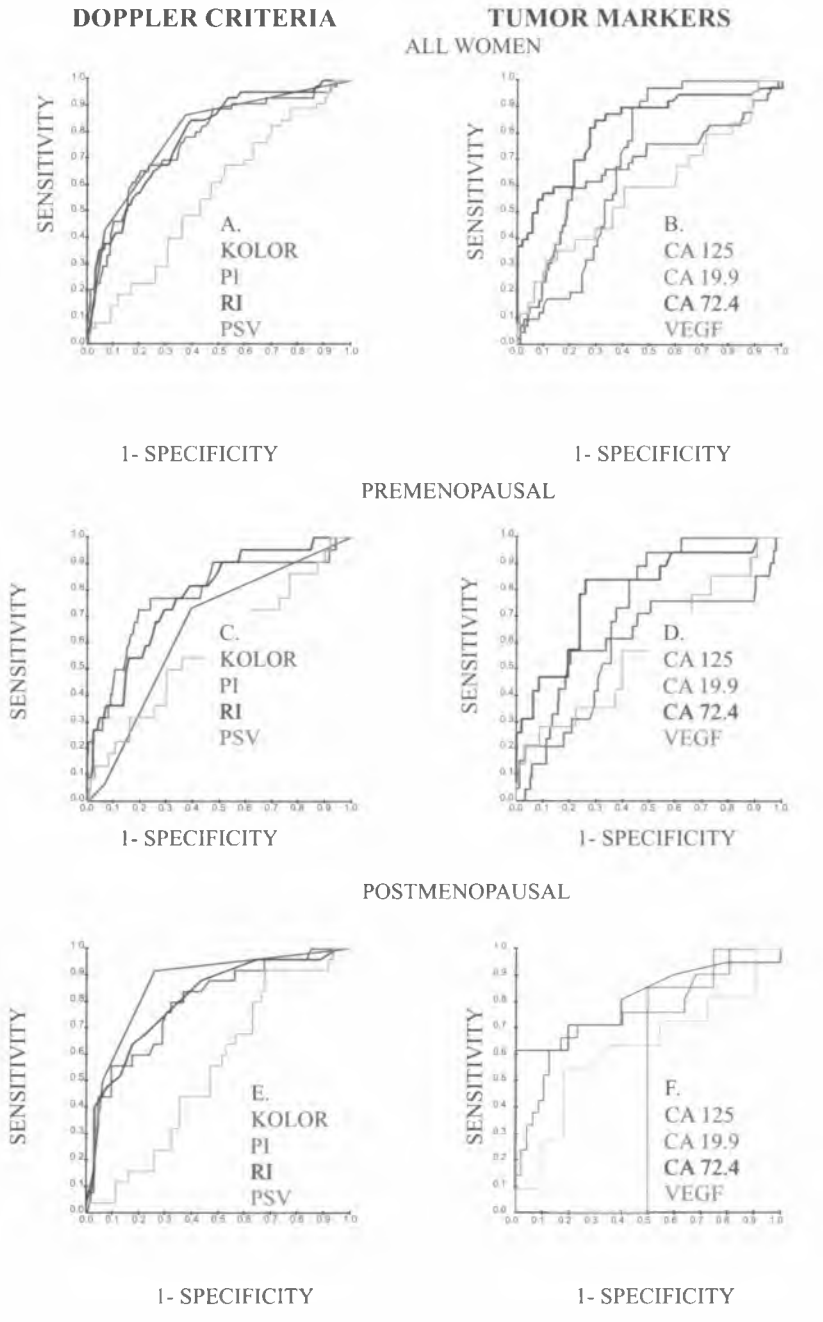

Fig. 4. ROC curves of Doppler and biochemical diagnostic tests used to characterize benign and malignant tumors in all studied women (A and B) and in the subgroups of premenopausal (C and D) and postmenopausal patients (E and F)

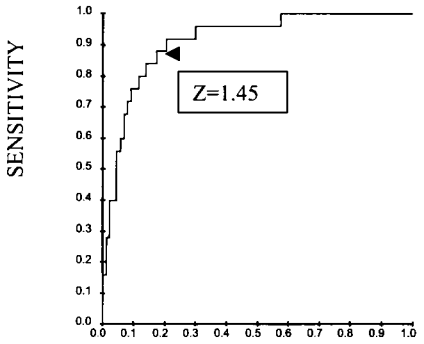

Fig. 5. ROC curve constructed for the regression coefficient "Z" in the studied population of 451 women with adnexal tumors. The best cut-off value is 1.45.

were too low to be clinically applicable for the discrimination between benign and malignant masses. Stepwise forward multiple regression analysis used for the assessment of several Doppler criteria (PI, RI, PSV), tumor marker CA-125 and VEGF as a potential angiogenesis marker has produced the following equation:
Z=1,3106+0,332(COLOR)*+0,01(PI)–0,38 (RI)*+0,103(PSV)+0,033(CA125)+0,079 (VEGF)
where "*" indicates statistical significance with $p<0.005$ (Chi-square likelihood-ratio test).

The statistical analysis revealed that only subjective COLOR content of the tumor and resistive index value had a significant contribution to the regression coefficient "Z". Subsequent analysis based on the area under the ROC curve measurements revealed that the best cut-off "Z" value was 1.45 (Fig. 5). Based on this value patients with benign and malignant tumors could be separated with a sensitivity of 72.0% (95% CI: 53.5% – 86.0%) and specificity of 91.9% (95% CI: 85.3%-96.1%). Calculated positive predictive value was 72%, negative predictive value was 92% and diagnostic accuracy was 87.5%.

DISCUSSION

Most ovarian cancers are detected when the tumor has already spread beyond pelvic structures (25). Importantly, a small size of the tumor does not preclude advanced stage of the disease (32). This in turn is the main reason of low survival rates of affected women (28). In the studied population the median size of detected malignant tumors was 86 mm and the median size of benign tumors was 61 mm. Despite significant differences in medians, the substantial proportion of malignant tumors had relatively small maximal

diameter and volumes which overlapped with benign tumors. Similar inconclusive results have been found for the tumor volumes. The data from this study confirm that bimanual gynecological examination may detect large masses but apparently is not sufficient to differentiate functional cysts from early and potentially curable ovarian cancer. Therefore, it is not suitable for screening for pelvic malignancies (23). It is important to note that if malignant tumor is suspected, the operation is mandatory. Therefore, the diagnosis has to be accurate in each affected woman. In order to increase survival rates all potentially curable cancers should be detected early and – if required – treated with maximal possible cytoreduction. Despite introduction of new diagnostic tools, a still existing problem appears that inexperienced surgeons especially in small hospitals may not be prepared to perform radical operation if unexpected ovarian cancer is found (16). These facts imply that a test or a combination of tests with very high specificity should be applied in all women with adnexal masses (12, 18, 22). An analysis of simple sonographic criteria such as papillary projections, septa and solid parts used in the analyzed population reveals that their specificity was insufficient with relatively high positive rates. Also, best cut-off values of serum CA-125, CA-19.9 and CA-72.4 especially in premenopausal women were not sufficient for an accurate discrimination between benign and malignant masses. The results are in line with the data published previously (11, 23)

The use of transvaginal and color Doppler sonography in differentiation of adnexal masses has now been used for more than a decade (5). One of the most important problems with color flow mapping has been its poor reproducibility. While early results of several investigators suggested a high accuracy for this technique (7, 17), more recent studies have documented a presence of low resistance flow in a substantial proportion of benign masses (3, 26, 27). In our preliminary study (8) we have found similar results which are in line with those of Tekay and Jouppila who were the first to prove that there is real overlap in both color Doppler and pulsed Doppler measurements between benign and malignant ovarian masses (26). This overlap is most frequently encountered in premenopausal women (29). This is not surprising because of physiologic neoangiogenesis in dominant follicles and *corpora lutea*, which can persist during the first week of the next cycle. The specificity of the examination remains insufficient. This has become especially important with an introduction of a very sensitive method of ultrasound slow flow detection, namely "power" Doppler (12). Therefore, it is not surprising that extensive research is undertaken to combine values of Doppler and transvaginal sonography with selected serum markers that might have diagnostic and prognostic relevance to ovarian cancer.

Support for the relationship between tumor angiogenesis and VEGF levels has been derived from two recent studies in ovarian cancer patients that reported a significant correlation between VEGF protein levels and poor prognosis in a multivariate model. Currently, the literature linking preoperative VEGF serum levels to early detection and prognosis in ovarian cancer is less complete. Two studies conducted on cohorts of 134 (1) and 112 (30) women reported an association between VEGF protein levels and ovarian

malignant tumors, but one study (19) with a cohort of 256 women found no relationship between VEGF levels in malignant and benign ovarian tumors. Own results indicate that measurements of serum concentration of this cytokine are not useful in preoperative discrimination of adnexal tumors.

To date serum CA 125 is the tumor marker with the highest association with ovarian cancer. Tests that are based on the measurement of this tumor marker will detect nearly 80% of advanced (stage III or further) ovarian cancers but only 40% to 50% of stage I disease. The specificity is low when the test is applied to premenopausal patients because false-positive results are frequently encountered during menstruation or pregnancy and in a wide variety of benign conditions, such as endometriosis, pelvic inflammatory disease, uterine fibroids, early pregnancy and Meigs syndrome. To increase the reliability of serum CA 125 levels in the preoperative differentiation between malignant and benign adnexal masses, Jacobs et al. combined the CA 125 values with the ultrasonographic morphologic findings and the menopausal status of the patient to calculate their Risk of Malignancy Index (15). Many ultrasonographic morphologic scoring systems for the assessment of adnexal masses have been proposed (18, 22). However, most of these systems are either rather complex or not sufficiently reproducible. Furthermore, these scoring systems cannot be used to determine the probability of malignancy for an individual patient. The new possibility is to use artificial neural networks (ANN) for an analysis of the possible non-linear dependency between clinical and ultrasound data. ANN's are computer programs capable to find complex relationship between the preoperative data and, as recently suggested (27), may be more efficient than advanced regression analysis in discriminating adnexal tumors (3).

The results of this study indicate that the most important factors in differential diagnosis of ovarian masses were: menopausal status, gray-scale morphology, resistive index value and subjective COLOR assessment. Calculated regression coefficients can be further log transformed to the values between (0, 1) which in turn may be used to estimate true probability of cancer in individual patients. The probability can be calculated with the use of the following formula: $p = 1 / (1+e^{-Z})$, where "p" is probability, "e" is the base of natural logarithm and "Z" is the calculated regression coefficient (14). To the best of my knowledge this is the first study comparing the usefulness of color Doppler sonography with preoperative serum VEGF measurements in women with adnexal tumors. Low predictive values of VEGF concentration measurements indicate that probably this test may not be helpful in distinguishing between malignant and benign masses. It is important to note that the proposed new approach to angiogenesis detection and subjective quantification may be an additional and valuable tool in an initial diagnosis of adnexal tumors. The results indicate that the analysis of color Doppler content and blood flow indices along with known morphological criteria increase diagnostic accuracy of traditionally used diagnostic modalities such as bimanual examination, CA-125 serum levels and gray-scale sonography. However, clinical validity of these methods must be further prospectively tested in a new group of patients with adnexal tumors.

CONCLUSIONS

1. Color and pulsed Doppler sonography can increase diagnostic accuracy of transvaginal ultrasound and serum tumor markers in differentiation between benign and malignant adnexal masses.

2. Semiquantitative color assessment (COLOR) of tumor vascularity can improve the sensitivity and specificity of gray-scale sonography by increasing sensitivity and specificity of the test. Diagnostic accuracy of sonoangiography is, however, higher in postmenopausal than in premenopausal women.

3. Pretreatment serum VEGF measurement does not appear to add prognostic value to the established sonographic criteria of malignancy of adnexal tumors. Combining of VEGF and other tests does not increase prognostic value of the constructed predictive model.

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SUMMARY

The aim of the study was to investigate whether a combination of serum CA-125, CA-72.4; CA-19.9 and VEGF levels along with several currently used gray-scale criteria and pulsed and semiquantitative color Doppler blood flow assessment can be useful in preoperative discrimination of ovarian tumors.

Ultrasound examinations were performed between 1994 and 1999 with the use of transvaginal probes 5-6.5-7.0 MHz and transabdominal 3.5 MHz probe (B&K Medical 3535 and 2002 ADI, Denmark). Preoperative serum CA-125 and CA-19.9 (Roche, Switzerland) and CA-72.4 (Centocor, USA) were measured with EIA II immunoassays. VEGF (R&D Systems, USA) serum levels were measured by microplate immunoenzymatic method. Retrospective study included 4,876 women referred for sonography of pelvic structures. For final analysis 451 patients with persistent adnexal tumors were available. In all these women age, menopausal status, tumor size, volume and morphology as well as blood flow indices (PI, RI, PSV) and semiquantitative color assessment were noted. In women who were not operated the cysts were followed for 10-12 weeks until resolution which was confirmed by repeated sonography. Multiple regression analysis was used to determine which of the independent variables had prognostic significance in the constructed predictive model. ROC curves were plotted and areas under ROC for each test were calculated and compared.

286 women were operated and in 118 patients their tumors regressed. The sensitivity for Doppler indices was between 85% (RI), 67% (PSV) and 66% (PI). For the subjective color assessment sensitivity was 87%. 294 women had their CA-125 serum levels avail-

able. For CA-19.9, CA-72.4 and VEGF the corresponding numbers were 109, 92 and 116 patients, respectively. Optimal cutoff level for CA-125 was 67,5 U/ml and this produced diagnostic accuracy of 69.4%. For CA-19.9 the best predictive value was 13.4 U/ml (accuracy of 74%) and for the cut-off CA-72.4 level of 4.1 U/ml the accuracy of the test was 77.8%. Multiple regression analysis revealed that only RI measurements and subjective color assessment had significant influence on the constructed predictive model. The best cut-off value of regression index "Z" was obtained following ROC curves construction for sensitivity (true positive rate) and 1-specificity (false positive rate). In all 116 patients who had Doppler indices with both CA-125 and VEGF levels measured the highest accuracy was associated with "Z" = 1.45 in postmenopausal women. The prognostic model proposed in this study can be log transformed and further used in the estimation of the true probability of malignancy of a given mass in the new set of prospectively studied patients with ovarian tumors.

Color and pulsed Doppler can improve preoperative diagnosis of adnexal tumors when compared to transvaginal sonography alone or tumor markers assessment. The proposed semiquantitative evaluation of tumor vascularity increases the predictive value in terms of sensitivity and specificity. VEGF serum concentration was not useful in the preoperative discrimination of malignant and benign ovarian tumors.

Badania nad angiogenezą w łagodnych i złośliwych guzach jajnika przy zastosowaniu ultrasonografii z kolorowym Dopplerem oraz oceny stężeń śródbłonkowego czynnika wzrostu i wybranych markerów nowotworowych: CA-125, CA-19.9, CA-72.4 i VEGF

Celem pracy było zbadanie, czy zastosowanie techniki kolorowego kodowania przepływu w ocenie angiogenezy jest przydatne w różnicowaniu charakteru guzów jajnika oraz sprawdzenie czy kombinacja dwóch lub więcej z wymienionych parametrów: wieku pacjentki, badania ultrasonograficznego sondą dopochwową z kolorowym Dopplerem oraz oceny stężenia CA-125, CA-19.9, CA-72.4 i VEGF może podwyższyć wartości pojedynczego testu stosowanego w różnicowaniu łagodnych i złośliwych guzów przydatków macicy. Grupę badaną stanowiło 4876 kobiet z regionu lubelskiego, a do analizy zakwalifikowano ostatecznie 451 pacjentek z potwierdzonymi w USG guzami przydatków macicy. Analizowano wiek, status menopauzalny, maksymalny rozmiar i objętość guza oraz PI, RI, PSV i wartości cechy KOLOR. Badano stężenia antygenów CA-125, CA-19.9 i CA-72.4 oraz VEGF jako potencjalny marker neoangiogenezy. W przeprowadzonych obliczeniach zmiany czułości i swoistości testów przy zmianach wartości granicznej przedstawiono w formie krzywych ROC (GraphROC, Turku, Finlandia).W celu oceny, czy i które z badanych parametrów (zmienne niezależne) mają istotny statystycznie wpływ na prawdopodobieństwo istnienia nowotworu złośliwego lub łagodnego (zmienne zależne), zastosowano analizę regresji wielokrotnej. W badanej grupie wśród 451 kobiet w wieku od 13 do 76 lat (średnia 39 lat, mediana 38 lat) były 404 kobiety z łagodnymi guzami przydatkowymi oraz 47 ko-

biet z nowotworami złośliwymi. Odsetek pacjentek po menopauzie w grupie chorych z guzami łagodnymi wynosił 16,3% (66 z 404) oraz 46,8% (22 z 47) w grupie kobiet z nowotworami złośliwymi. Optymalnymi wartościami różnicującymi guzy złośliwe i łagodne były: PI = 0,87; RI = 0,63; PSV = 21 cm/s. Dla tych wartości dokładność diagnostyczna testu wynosiła odpowiednio: 72,9%; 73% i 56,3%. Dla pozostałych markerów najwyższe dokładności diagnostyczne zawierały się w przedziale od 57% (VEGF) przez 69,4% (CA125=67,5 U/ml) do 77,8% (CA-72.4 = 4,1 U/ml). Zastosowanie analizy regresji wielokrotnej do oceny wpływu wybranych zmiennych: parametrów dopplerowskich (PI, RI, PSV) oraz CA-125 jako najczęściej oznaczanego markera raka jajnika i VEGF jako potencjalnego markera angiogenezy wykazało, że tylko wartość cechy KOLOR oraz pomiar indeksu oporu RI miały statystycznie istotny wpływ na wartość współczynnika regresji. Analiza krzywej ROC dla różnych wartości progowych tego współczynnika wykazała, że najlepszą wartością graniczną różnicującą guzy łagodne i złośliwe w tej grupie kobiet było Z_t=1,45. Obliczone wartości prognostyczne wynosiły odpowiednio: czułość 72,0 % (95% CI: 53,5% - 86,0%), specyficzność 91,9% (95% CI: 85,3% - 96,1%). Dodatnia wartość predykcyjna PPV wynosiła 0,72, a ujemna wartość predykcyjna NPV była równa 0,92. Dokładność testu w tej grupie kobiet wynosiła 87,5%. Uzyskane wyniki wskazują na to, że w analizowanej grupie pacjentek z guzami przydatkowymi najbardziej przydatnym sposobem różnicowania nowotworów złośliwych i łagodnych było uwzględnienie statusu menopauzalnego kobiety, obrazu ultrasonograficznego guza, indeksu oporu RI i oceny unaczynienia przy pomocy techniki kolorowego Dopplera. Przydatność kliniczna proponowanych testów zostanie zweryfikowana w badaniach prospektywnych przy zastosowaniu wymienionych zaawansowanych metod analizy statystycznej różnych zmiennych prognostycznych.