EJGO European Journal of Gynaecological Oncology

New method: are tumor markers in vaginal-washing fluid significant in the diagnosis of primary ovarian carcinoma?

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Summary

Objective: Ovarian cancer is the seventh most common cancer in women worldwide, with nearly a quarter of a million women diagnosed every year. The serum tumor markers cancer antigens CA 125, CA 19-9, and carcinoembryonic antigen (CEA) are potentially of clinical value for the diagnosis of ovarian cancer. A diagnostic tool that is noninvasive, simple to perform, and specific is needed to predict primary ovarian cancer. The purpose of this study was to evaluate the diagnostic sensivitity and specificity of vaginal-washing tumor markers CA 125, CA 19-9, and CEA for diagnosis of primary ovarian cancer. *Materials and Methods:* In the current prospective study, 30 patients with advanced primary ovarian cancer and 30 patients with benign ovarian cysts were enrolled. The vaginal-washing fluid samples were obtained the day before surgery and were immediately centrifuged and stored at -80 °C until analysis. Measurements of CA 125, CA 19-9, and CEA were determined using fully-automated chemiluminescent microparticle immunoassays. *Results:* The vaginal fluid concentrations of CA 125, CA 19-9, and CEA in patients with primary ovarian carcinoma were significantly higher ($p < 0.001$) compared to those in patients with benign adnexal masses ($p < 0.001$). In the ROC curve analysis, the optimal cut-off values for the detection of primary ovarian cancer were >295 for CA 125 ($p < 0.001$), > 101 for CA 19-9 ($p < 0.001$), and >135 for CEA ($p < 0.001$). *Conclusion:* Vaginal-washing tumor markers CA 125, CA 19-9, and CEA are simple, noninvasive, and reliable diagnostic tests for the detection of primary ovarian cancer.

Key words: Vaginal-washing tumor markers; CA 125; CA 19-9; CEA.

Introduction

Ovarian cancer is the seventh most common cancer in women worldwide, with nearly a quarter of a million women diagnosed every year. Despite its relatively low incidence rate, ovarian cancer is an extremely lethal disease. Most patients (75%) present with advanced-stage (III/IV) tumors, for which the five-year survival rate is 30% [1].

The detection of tumor markers has been shown to be an effective and noninvasive diagnostic tool for the diagnosis of ovarian cancer. The serum tumor marker cancer antigens CA 125, CA 19-9, and carcinoembryonic antigen (CEA) are potentially of clinical value for the diagnosis of ovarian cancer [2].

CA 125 is the most commonly-used biomarker for diagnosis and follow-up of ovarian cancer. It is a high-molecular-weight glycoprotein with an elevated serum level (>35 U/ml) in 50-90% of patients with ovarian cancer, depending on the cancer stage. However, the diagnostic performance of serum CA 125 for early-stage ovarian cancer is as low as 25% for Stage I and 61% for Stage II [3].

CEA is a protein that may be elevated in malignancies that produce it, particularly in mucinous cancers associated with the gastrointestinal tract or the ovary. However, some benign conditions have also been associated with an elevated CEA, including cholecystitis, liver cirrhosis, and pancreatitis [4].

CA 19-9 is a mucin protein that may be elevated in ovarian cancer, and it is also used in ovarian cancer management. CA 19-9 levels may be elevated in a variety of other malignant and benign conditions. CA 19-9 may be used as predictive test for the differentiation of ovarian cancer from benign adnexal masses [5].

However, in practice, there is no information available regarding the use of vaginal washings for tumor markers CA 125, CA 19-9, and CEA in the diagnosis of ovarian cancer. The present authors hypothesized that the use of vaginal-washing tumor markers CA 125, CA 19-9, and CEA may increase diagnostic sensitivity and/or specificity in ovarian cancer. Receiver operating characteristic (ROC) curves have been widely used as a standard approach for calculating the sensitivity and specificity of medical diagnostic tests. In this study, the authors aimed to investigate the diagnostic sensitivity and specificity of vaginal-washing tumor markers CA 125, CA 19-9, and CEA by ROC analysis.

Revised manuscript accepted for publication August 7, 2014

Table 1. — *Demographic characteristics.*

Variable	Primary ovarian cancer	Benign ovarian cyst	*p*
Number of cases	30	30	
Age	55 (48-64)	51(45-60)	0.097
Post-menopausal	25	8	
Body mass index, (kg/m2)	23.5 (21.0-27.2)	22.8 (20.9-27.2)	0.072
Gravidy	3.9±1.1	3.7±1.2	0.609
Parity	2.5±1.3	2.3±1.1	0.505
Histological type	Papillary serous cystadenocarcinoma	Serous cystadenoma	
FIGO Stage	III-IV	Benign	
Ovarian mass size	8.9±1.3	8.4±1.2	0.543

P<0.05 was considered to indicate a statistically significant difference.

Table 2. — *Vaginal washing concentrations of CA 125, CA19-9, and CEA in different patient groups.*

Groups (ml)	CA125 (ng/ml)	CA19-9 (ng/ml)	CEA (ng/ml)	*p*
Primary ovarian cancer	354.5 ± 94.3	128.5 ± 23.2	193.1 ± 29.0	< 0.001
Benign adnexial mass	252.6 ± 48.8	82.8 ± 34.8	137.1 ± 41.6	< 0.001

$p < 0.05$ was considered to indicate a statistically significant difference.

Materials and Methods

In the current prospective study, 30 patients with advanced primary ovarian cancer (Group 1) and 30 patients with benign ovarian cysts (Group 2), all treated at the Department of Gynecology's Oncology Unit at Dicle University and Department of Gynecology at Kocaeli Derince Education and Research Hospital between March 2008 and January 2014, were included in this study. All 60 of these pre- and post-menopausal women, aged 45 years or older, had presented to a gynecologist with a pelvic mass (defined as a simple, complex, or solid ovarian cyst/pelvic mass). None of the patients had history of surgery for tubal ligation. The set of primary ovarian cancer patients (n=30, Group 1) comprised the study group, while the remaining 30 patients with benign ovarian cysts comprised the control group (n=30, Group 2). All of the patients underwent pelvic ultrasonography (transabdominal + transvaginal) performed by an expert gynecological sonographer prior to surgery. All adnexal lesions were described according to the morphological and vascular features as suggested by the consensus opinion from the International Ovarian Tumor Analysis (IOTA) group [6].

The primary ovarian cancer patients were surgically staged and debulked to achieve minimal residual tumor volume via laparotomy. International Federation of Gynecology and Obstetrics (FIGO) criteria were used to stage the ovarian cancer patients [7]. The benign ovarian cysts were removed by laparoscopic surgery. All tissue pathologic analysis of the fallopian tubes, ovaries, and uteri was performed by a gynecologic pathologist. All patients gave written consent, and the study was approved by the local Ethics Committees.

Sampling of vaginal-washing fluid

The vaginal-washing fluid (VWF) samples were obtained one day before surgery. A sterile speculum examination was performed on each patient, during which ten ml of sterile normal saline was injected into the posterior fornix of the vagina and then aspirated from the posterior vaginal fornix with the same syringe. Each vaginal fluid sample was sent immediately to the laboratory, where it was immediately centrifuged at 3,000 rpm for ten minutes and the supernatant was stored at -80°C until analysis. All speculum examinations were performed by the same gynecologic oncologist.

Measurement of vaginal tumor markers

Vaginal-washing testing for CA 125, CA 19-9, and CEA was performed using a fully-automated chemiluminescent microparticle immunoassays (CMIA), according to manufacturer's instructions, and appropriate controls were included in each run.

Statistical analysis

The results are reported as means ± SD. A *t*-test was performed for demographic characteristics. The Mann-Whitney U test (SPSS 17.0 statistical software package for Windows) was applied to determine the differences in marker levels. The MedCalc statistical software package was utilized to assess the difference between different areas under the curve (AUC). To evaluate the diagnostic sensitivity and specificity, positive and negative predictive values were calculated at the optimal cut-off. A $p < 0.05$ was considered to indicate a statistically significant difference.

Results

The demographic parameters and histological types are shown in Table 1. The concentrations of CA125, CA19-9, and CEA in patients with primary ovarian carcinoma were significantly higher ($p < 0.001$) compared to patients with benign adnexal masses (Table 2). The diagnostic indices for the vaginal-washing tumor markers' cut-offs are presented in Table 3.

In the ROC curve analysis, the optimal cut-off values for the detection of primary ovarian cancer were > 295 for CA 125 with an AUC equal to 0.81 ($p < 0.001$) (Figure 1). In the ROC curve analysis, the optimal cut-off values for the detection of primary ovarian cancer were > 101 for CA 19-9 with an AUC equal to 0.87 ($p < 0.001$) (Figure 2). In the

Table 3. — *The diagnostic indices for the vaginal-washing tumor markers' cut-offs are presented.*

Markers	ROC area (%)	95% CI	Sensitivity (%)	Specificity (%)	PPV (%) (95% CI)	NPV (%) (95% CI)
CA 125 (ng/ml)	81.5[a]	(69.4 - 90.4)	63.3	90.0	86.3	71.0
CA19-9 (ng/ml)	87.8[a]	(76.8 - 94.8)	86.7	83.3	83.8	86.2
CEA (ng/ml)	84.2[a]	(72.5 - 92.4)	100	66.7	75	100

[a]$p < 0.001$ compared with CA125, CA19-9, and CEA.ROC: receiver operating characteristic. CI: confidence interval.

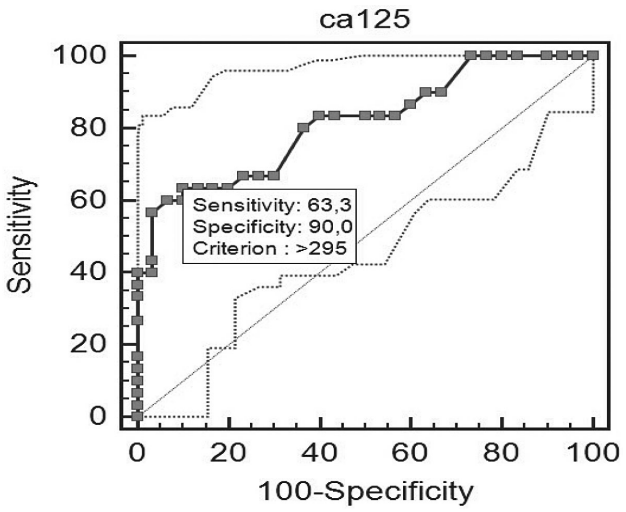

Figure 1. — Receiver operating characteristic (ROC) curve of CA 125 in distinguishing primary ovarian cancer from benign adnexal mass.

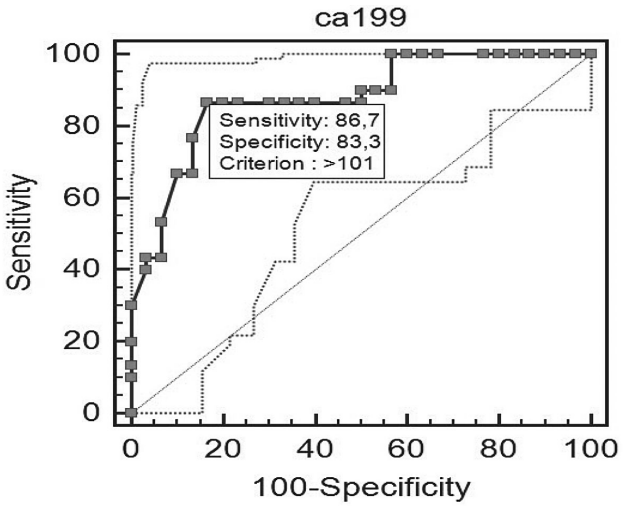

Figure 2. — Receiver operating characteristic (ROC) curve of CA 19-9 in distinguishing primary ovarian cancer from benign adnexal mass.

ROC curve analysis, the optimal cut-off values for the detection of primary ovarian cancer were > 135 for CEA with an AUC equal to 0.84 ($p < 0.001$) (Figure 3).

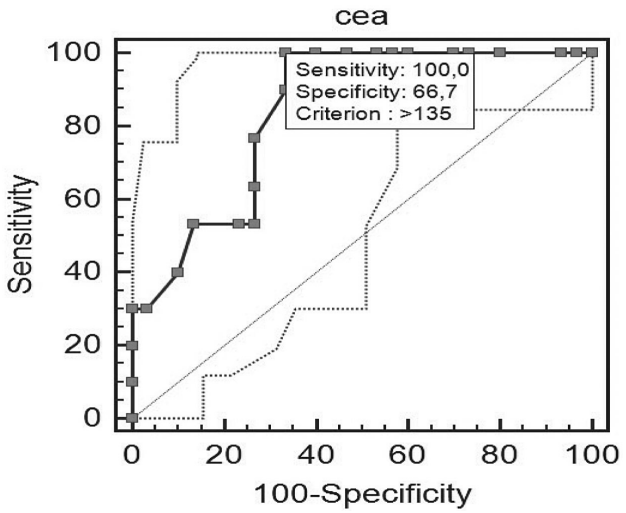

Figure 3. — Receiver operating characteristic (ROC) curve of CEA in distinguishing primary ovarian cancer from benign adnexal mass.

Discussion

Tumor markers are substances measured in blood or other bodily fluids; they are found in normal tissue but may be produced in large amounts when tissue undergoes neoplastic change. They may be products of normal, benign, or malignant tissue on cell surfaces [1-6].

As previously described, serum biomarkers are widely used in ovarian cancer screening and diagnosis, as well as for monitoring treatment response and recurrent disease status in ovarian cancer patients. CA 125 has a sensitivity of 73.2% and a specificity of 79.2% in predicting ovarian malignancy. However, CA 125 is increased not only in cases of ovarian cancer but also in some benign conditions. Isolated serum CA 125 values lack adequate sensitivity or specificity, and a false-positive CA125 value may result in unnecessary diagnostic work-up or surgery [8].

CEA has been used to monitor colorectal cancer for decades and is reported to be elevated in 30-65% of ovarian epithelial cancers. Tumors of the ovary contain a population of intestinal-like cells that resemble those present in colonic adenomas. Serum concentrations of CEA exceeding five ng/ml are often found in patients with ovarian cancer. Serum CEA elevation occurs more often in mucinous tumors than in serous tumors of the ovary [9].

CA 19-9 is a sialylated antigen, which is expressed in gastrointestinal adenocarcinomas and ovarian cancers. CA 19-9 is also used to monitor disease response to therapy or to detect recurrence in patients with ovarian cancer [10].

The combination of CA 125, CA 19-9 and CEA provides a higher level of discriminatory power than any of these markers alone for distinguishing benign from malignant ovarian masses [11]. Thus, we need simple, reliable, and noninvasive tests for the diagnosis of ovarian cancer. There is no unique and noninvasive gold-standard test applicable in ovarian cancer patients with high accuracy. The purpose of the present study was to determine the effectiveness of utilizing vaginal washings for analysis of tumor markers CA 125, CA 19-9, and CEA in the diagnosis of ovarian cancer, since there is no current literature data on this subject.

The present authors hypothesized that tumor markers excreted from tumor cells, due to regurgitation of tumor cells through the fallopian tube, may be detected in vaginal washing fluid. The present results showed that vaginal-washing concentrations of these three markers were significantly higher in primary ovarian carcinoma than in benign adnexal masses. The optimal cut-off value of 295 ng/ml for the detection of primary ovarian cancer was proposed for CA 125. The sensitivity, specificity, positive predictive value, and negative predictive value of CA 125 were 63%, 90%, 86%, and 71%, respectively. The optimal cut-off value of 101 ng/ml for the detection of primary ovarian cancer was proposed for CA 19-9. The sensitivity, specificity, positive predictive value, and negative predictive value of CA 19-9 were 86%, 83%, 83%, and 86%, respectively. The optimal cut-off value of 135 ng/ml for the detection of primary ovarian cancer was proposed for CEA. The sensitivity, specificity, positive predictive value, and negative predictive value of CEA were 100%, 66%, 75%, and 100%, respectively.

The present authors propose that the sampling of vaginal washings for the tumor markers CA 125, CA 19-9, and CEA is a simple, noninvasive, and reliable diagnostic test for the detection of primary ovarian cancer.

In conclusion, vaginal-washing tumor markers CA 125, CA 19-9, and CEA were found to have high sensitivity, specificity, and positive and negative predictive values in the diagnosis of primary ovarian cancer. The present study demonstrates that the measurement of vaginal-washing tumor markers is a better strategy than utilizing blood-sampling methods.

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