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Human papillomavirus combined with cytology and margin status identifies patients at risk for recurrence after conization for high-grade cervical intraepithelial neoplasia

Y. Ruano, M. Torrents, F.J. Ferrer
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Summary
Objective: To compare the ability of cytology, human papillomavirus (HPV) testing and co-testing to identify recurrence of patients treated by loop electrosurgical excision procedure (LEEP) for cervical intraepithelial neoplasia (CIN) 2-3. Materials and Methods: Retrospective analysis (R.A.): the medical records of 372 women treated for CIN 2-3 were reviewed. Resection margin, HPV typing, Pap smears, and biopsies post-LEEP were collected. Prospective analysis (P.A.): 97 women were followed post-LEEP by cytology, HPV test and colposcopy every six months. Results: Positive margins were found to be an independent risk factor for recurrent disease (OR 0.192; 95% CI 0.074-0.497 in R.A. and OR 0.096; 95% CI 0.023-0.392 in P.A.). HPV testing showed less sensitivity than cytology (69% vs 84%, respectively in R.A. and 80% vs 100% in P.A.). Co-testing predicted recurrent disease at a sensitivity of 90.6% in R.A. and 100% in P.A. Conclusion: Co-testing is the best option in follow-up protocols after treatment for CIN 2-3. If margins are free and co-testing is negative at six and 12 months, 18 months visit could be avoided.

Key words: Human papillomavirus; Conization; Cervical intraepithelial neoplasia; Surveillance; Recurrence.

Introduction
The relationship between high risk human papillomavirus (HR-HPV) and high-grade cervical intraepithelial neoplasia (CIN 2-3) or invasive cervical cancer has been clearly demonstrated [1].

The loop electrosurgical excision procedure (LEEP) is the standard procedure for conservative treatment of CIN 2-3. However, residual or recurrent disease occurs in 1.5% to 48% of patients [2-18].

The most important factors reported as being associated with recurrent CIN after conization include cone margin status and persistent HR-HPV infection [5, 18]. Other factors such as smoking, immunosuppression or age have been related to a higher risk in some series [15, 19] but not in others [2,3].

Cervical cytology has a relatively low sensitivity in the follow-up period (20-100% in different studies) [12, 18, 20, 21]. Due to this limitation of the Pap test, there is a growing interest in HPV-testing as a surveillance tool that, alone or in conjunction with cytology, may increase sensitivity and negative predictive value (NPV) for identifying women at high risk of recurrence.

Studies which investigate the potential role of HPV testing during the post-treatment period are profoundly heterogeneous, making it difficult to draw clear conclusions. Because of this, there is no uniform follow-up protocol.

The aim of this study was to compare the ability of cytology, HPV testing and co-testing to identify recurrence of patients treated by LEEP for CIN 2-3. The authors also investigated whether any factors can predict, prior to follow-up, the eventual development of recurrent disease.

Materials and Methods
The study was divided into two analyses: retrospective and prospective analysis.

RETROSPECTIVE ANALYSIS
Study population, variables and follow-up
The medical records of 939 women were reviewed. These subjects underwent LEEP at the Department of Gynecology of the Asturias University Hospital (HUCA), Spain, between 1995 and 2009. Inclusion criteria were histologically confirmed CIN 2-3 in the conization specimen or in the initial colposcopy-directed biopsy. Women were excluded if (a) CIN 2-3 was not diagnosed on histology, (b) HPV test results pre or post-conization and many epidemiological patient characteristics were not available, (c) no follow-up visits were available, and (d) hysterectomy post-LEEP was immediately performed because large/multifocal CIN 2-3 or invasion in the cone was described. The remaining 372 women comprised the analytic population (Figure 1).
Patient characteristics were collected: age at conization, menopausal status, parity, smoking habits, immunosuppression or chronic disease, other sexually transmitted infections (STI), age at first intercourse, and mode of contraception.

Clinical data reviewed were: resection margin, HPV test results pre- and post-conization, HPV typing, follow-up Pap smears, and punch biopsies.

Mean follow-up time was 66 months (range 4-181, median 57). The average number of follow-up Pap smears per patient was 11.6 (range 1-22, median 5) but HPV testing was not so common in the first years of this study, hence the average number of follow-up HPV tests was 2.6 (range 0-19, median 2). Histologically confirmed presence of CIN 2+ was considered as recurrent disease.

Loop excision

All procedures were performed in an outpatient setting by three experienced surgeons. The electrosurgical procedure was performed with a LEEP system which was set to 50-52 W for cut and coagulation and 60-62 W for cautery. Wire electrodes were used, cone biopsy excisor beginning at the 12 o’clock position or loop electrode for the “cowboy-hat” procedure: an ectocervical flat sweep followed by a deeper endocervical sweep using a smaller loop. The 12 o’clock position and margins were marked by ink. A ball electrode was used to cauterize the margins of the defect and achieve hemostasis after excision.

HPV testing

The real time polymerase chain reaction (PCR) method was used to detect viral DNA. In brief, after amplification, PCR products were analyzed by electrophoresis and hybridized with radio-labeled generic probes. HPV-DNA amplicons (L1 positive) were hybridized with type-specific oligonucleotide probes [6, 11, 16, 18, 31, 33, 45 and 58]. From 2002 HPV genotyping by direct DNA sequencing was used for those samples that could not be typed with hybridization.

PROSPECTIVE ANALYSIS

The authors collected 111 women with histologically confirmed CIN 2-3 who were treated by LEEP in the Department of Gynecology of the Asturias University Hospital between January and December 2010. Inclusion/exclusion criteria and variables were the same as in the retrospective analysis. The remaining 97 women comprised the analytic population (Figure 2). All women were prospectively followed-up and scheduled for visits every six months after treatment, until July 2012. On each visit, a Pap smear, HPV test, and colposcopy were performed. Mean follow-up time was 24 months (range 19-30, median 24 months). The average number of follow-up Pap smears and HPV tests per patient was 3.4 (range 1-5, median 3).

The study protocol was approved by the Regional Clinical Research Ethics Committee of Principado de Asturias and all patients signed the informed consent. LEEPs were performed by “cowboy-hat” procedure.

From May 2011, Cobas 4800 validated PCR was used for HPV testing. The Cobas 4800 system uses amplification of target DNA by PCR and subsequent nucleic acid hybridization for the detection of 14 HR-HPV types in a single analysis: 16, 18 or other HR-HPV [31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68].
Statistical analysis

Data were analyzed with the SPSS 17.0 software. The correlation between treatment failure with clinical factors (categorical variables) was determined by Fisher’s exact test using two-by-two tables. Chi-square testing for non-categorical variables was used. A p value of < 0.05 was statistically significant. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated by logistic regression at multivariate analysis. The diagnostic accuracy of cytology, HPV testing, and co-testing as detection tools after conization was determined by the sensitivity, specificity, positive predictive value (PPV) and NPV of the test.

Results

RETROSPECTIVE ANALYSIS

Characteristics of the study group

The mean age of the studied population (n=372) was 35.6 years (range 18-80.5). HPV testing pre-treatment was available in 351 women: it was negative in 101 (29%) and positive in 250 (71%). HPV type 16 was the most prevalent type at baseline (64%), followed by types 33 (6.4%) and 18 (5.6%). Among women with initial negative HPV test (101), 84 remained negative during follow-up after treatment (83%), but 17 (17%) again acquired an HPV infection (we defined this situation as “new infection”).

Among the 250 women with initial positive HPV test, 25 acquired “another infection” by an HPV type that was different from the type detected pre-LEEP, regardless of whether there was clearance of the initial infection or not.

Recurrent disease and HPV clearance

During follow-up, 32 patients of 372 (8.6%) developed recurrent disease. Most recurrences (68.7%) were identified within the first year after treatment (mean time 19 months, range 3-140 months, median 7).

Among the 238 women who were HPV positive before the procedure and for whom a post-treatment HPV test was available, 216 (91%) became negative during follow-up for the same type detected at baseline. HPV was cleared within the first year post-LEEP in 151 patients (151/216, 70%). In six women (3%), it was cleared beyond the first year of follow-up and in 59 (27%) clearance time could not be determined because of a lack of HPV tests available. There was no significant difference in clearance time depending on HPV type.

Among the 22 women (22/238, 9%) with persistent HPV infection post-LEEP, type 16 was identified most frequently (18/22, 82%), nevertheless no significant difference between the clearance rates of different HPV types was found.

In three of the 17 (17.5%) women with “new infection” the HPV was not cleared during follow-up. These three persistent “new infection” caused recurrent disease.

Among the 25 women with “another infection”, four remained positive during follow-up post-LEEP.

Margins and other predictors of recurrent disease

Surgical margins were positive in 36 cases (36/372, 9.7%). Of the overall group of women with negative margins, 23 developed recurrent disease (23/334, 6.8%). Nevertheless, among the 36 cases with incomplete resection of CIN, there were nine recurrences (25%), showing a significant difference (p = 0.00024, Figure 3).

Patient characteristics are listed in Table 1. Higher age at conization and menopausal status were associated with significantly higher risk of recurrent disease. Positive margins (OR 0.192; 95% CI 0.074 to 0.497) and higher age at conization (OR 1.061; 95% CI 1.019 to 1.104) resulted to be independent from other risk factors at multivariate analysis.

Identification of recurrent disease

Table 2 shows the sensitivity, specificity, PPV, and NPV for recurrent disease of HPV testing, cytology and co-testing.

- **HPV testing**: 32 women had persistent HPV infection post-LEEP (25 the same initial type, three “new infection” and four “another infection”) and 316 cleared baseline/other HPV types or remained negative during follow-up. Twenty-two of the 32 recurrences of this study were diagnosed among the women with persistent HPV infection.

- **Cytology**: 27 of 32 recurrences had some positive cytology (≥ ASCUS) during follow-up prior to the confirmatory biopsy (CIN2+). The remaining five women with residual disease had completely negative cytological surveillance.
Co-testing: 29 of 32 recurrences had positive cytology or HPV testing during follow-up (positive co-testing), but all pap smears and HPV tests post-LEEP were negative (negative co-testing) in the other three women with recurrent disease prior to the confirmatory biopsy.

PROSPECTIVE ANALYSIS
Characteristics of the study group

The mean age of the studied population (n=97) was 40 years (range 22-71). HPV tests pre-treatment were negative in 29 women (30%) and positive in 68 (70%). HPV type 16 was the most prevalent type at baseline (41%), followed by types 31 (13%) and 18 (7%). Among women with an initial negative HPV test, 21 remained negative during follow-up after treatment (72.4%), but 8 (27.6%) acquired a “new infection”. Among the 68 women with an initial positive HPV test, 12 acquired “another infection”.

Recurrent disease and HPV clearance

During follow-up, 15 patients of 97 (15.4%) developed recurrent disease. Most recurrences (60%) were identified within the first 6 months after treatment (mean time 10 months, range 4-24, median 6).

Among the 68 women who were HPV positive before the procedure, 56 (82%) became negative during follow-up for the same type detected at baseline. HPV was cleared within the first six months post-LEEP in 51 patients (51/56, 91%). In the remaining five women (9%) it was cleared between six and 12 months after treatment. There was no significant difference in clearance time depending on HPV type.

Among the 12 women with persistent HPV infection post-LEEP, type 16 was identified most frequent (67%), nevertheless no significant difference between the clearance rates of different HPV types was found.

In three of the eight (37.5%) women with “new infection” the HPV was not cleared during follow-up. These three persistent “new infection” caused recurrent disease. Among the 12 women with “another infection”, three remained positive during follow-up post-LEEP.

Margins and other predictors of recurrent disease

Surgical margins were positive in 30 cases (30/97, 31%). Of the overall group of women with negative margins, three developed recurrent disease (3/64, 4.7%). Nevertheless,
Table 3. — Patient characteristics related to recurrence rate. Prospective analysis.

<table>
<thead>
<tr>
<th>Patients' characteristics (Retrospective analysis)</th>
<th>No recurrences (%)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52 smokers</td>
<td>9 (17.3%)</td>
<td>p = 0.59</td>
</tr>
<tr>
<td>45 non-smokers</td>
<td>6 (13.3%)</td>
<td>(NS)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 menopausal</td>
<td>3 (19%)</td>
<td>p = 0.7</td>
</tr>
<tr>
<td>81 non-menopausal</td>
<td>12 (15%)</td>
<td>(NS)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46 women ≥40 years</td>
<td>11 (24%)</td>
<td>p = 0.03</td>
</tr>
<tr>
<td>51 women &lt;40 years</td>
<td>4 (8%)</td>
<td>(NS)</td>
</tr>
<tr>
<td>14 women ≥50 years</td>
<td>2 (14.3%)</td>
<td>p = 0.9</td>
</tr>
<tr>
<td>83 women &lt;50 years</td>
<td>13 (16%)</td>
<td>(NS)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 women ≥3 V. deliveries</td>
<td>1 (10%)</td>
<td>p = 0.61</td>
</tr>
<tr>
<td>87 women &lt;3 V. deliveries</td>
<td>14 (16%)</td>
<td>(NS)</td>
</tr>
<tr>
<td>Other STI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 women YES</td>
<td>6 (37.5%)</td>
<td>p = 0.007</td>
</tr>
<tr>
<td>81 women NO</td>
<td>9 (11%)</td>
<td></td>
</tr>
<tr>
<td>Age at first intercourse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 women &lt;15 years</td>
<td>1 (33.3%)</td>
<td>p = 0.38</td>
</tr>
<tr>
<td>94 women &gt;15 years</td>
<td>14 (15%)</td>
<td>(NS)</td>
</tr>
<tr>
<td>73 women &lt;20 years</td>
<td>14 (19%)</td>
<td>p = 0.077</td>
</tr>
<tr>
<td>24 women ≥20 years</td>
<td>1 (4%)</td>
<td>(NS)</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 women yes</td>
<td>6 (20%)</td>
<td>p = 0.4</td>
</tr>
<tr>
<td>67 women no</td>
<td>9 (13.4%)</td>
<td>(NS)</td>
</tr>
<tr>
<td>Mode of contraception</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 women hormonal</td>
<td>5 (14.3%)</td>
<td>p = 0.826</td>
</tr>
<tr>
<td>26 women barrier</td>
<td>5 (19.2%)</td>
<td>(NS)</td>
</tr>
<tr>
<td>36 women no H-no B</td>
<td>5 (14%)</td>
<td></td>
</tr>
</tbody>
</table>

V. deliveries (vaginal deliveries), NS (not significant). No H-no B (no Hormonal-no Barrier).

Table 4. — Sensitivity, specificity, PPV and NPV of HPV test, cytology, and co-testing as predictors of recurrent disease. Prospective analysis.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV test</td>
<td>80</td>
<td>92.7</td>
<td>66.6</td>
<td>96.2</td>
</tr>
<tr>
<td>Cytology</td>
<td>100</td>
<td>81.7</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Co-testing</td>
<td>100</td>
<td>66</td>
<td>35</td>
<td>100</td>
</tr>
</tbody>
</table>

among the 30 cases with incomplete resection of CIN, there were 12 recurrences (40%), showing a significant difference (p = 0.000013, Figure 4).

Patient characteristics are listed in Table 3. Higher age at conization and other STI were associated with significantly higher risk of recurrent disease.

Positive margins (OR 0.096; 95% CI 0.023 to 0.392) and other STI (OR 0.252; 95% CI 0.064 to 0.998) resulted to be independent from other risk factors at multivariate analysis.

Identification of recurrent disease

Table 4 shows the sensitivity, specificity, PPV, and NPV for recurrent disease of HPV testing, cytology, and co-testing.

- HPV testing: 18 women had persistent HPV infection post-LEEP (12 the same initial type, three “new infection” and three “another infection”) and 79 cleared baseline/other HPV types or remained negative during follow-up. Twelve of the 15 recurrences of this study were diagnosed among the women with persistent HPV infection.

- Cytology: The 15 women with recurrent disease had some positive cytology yielding a sensitivity of 100%
- Co-testing: When both tests were combined, all women with recurrent disease were detected (sensitivity and NPV 100%).

Follow-up post-treatment

Among the 36 women with free margins that were followed for at least two years, 24 had negative co-testing in every visit: if co-test was negative at six and 12 months, then it was negative at 18-24 or 30 months visits. The remaining 12 patients, including the three women with free margins and recurrence, had positive cytology or HPV test at six to 12 months.

Nevertheless, one patient with positive margins and normal co-testing at six to 12 months developed high grade squamous intraepithelial lesion at 18 months post-LEEP.

Discussion

The present data showed 70-71% of women HPV-positive pre-LEEP, a slightly lower rate than described before, although the present authors agree with other authors in describing type 16 as the most prevalent [6, 10, 12, 13, 18].

The recurrence rate in the prospective analysis was much higher than in the retrospective one (15.4% vs. 8.6%). This is probably due to conization during the year 2010 being more conservative, above all in women with childbearing desires and to a higher mean age of patients in that period. Nonetheless, both rates are included in the range described by others (3-17%) considering only studies that define recurrence as CIN 2-3 histologically confirmed [8, 9, 12, 14-16]. Most reviewed studies found a peak incidence of recurrence within the first two years post-treatment, but some of them [9,14] have a precisely two year follow-up. In the few publications with long term follow-up [10, 15, 22, 23] recurrent cases are described beyond the first two years. In particular Chua et al. [23] found 9/26 recurrences two to eight years post-treatment. Eight of 32 recurrences in our retrospective analysis occurred between two and 11 years after LEEP.

HPV clearance rate (82-91%) and clearance time after treatment (most in first 6-12 months) in the present study are similar to previous investigations [3,13].

Age and positive surgical margins were found to be risk factors for recurrent disease, although age was not independent in prospective analysis. Women over 35-40-50 years had an increased recurrence rate in many studies, perhaps because of an abnormal immunity or higher HPV persistence [8, 18, 22, 24]. Incomplete excision of CIN is a risk factor but an inexact predictor for recurrence: most women with affected margins will never present recurrent disease and, on the other hand, a small but significant number of patients with free margins will develop recurrence [2, 5, 7, 16]. It is important to emphasize that the increased recurrence rate in the prospec-
tive analysis did not represent an increased invasive cervical cancer rate in the Department of Gynecology (HUCA), although follow-up time in this analysis was not very long.

Cytology showed very high sensitivity and NPV for recurrent disease in this study. In particular, both were calculated as 100% in the prospective analysis, although this could be due to a small n. Published cytology sensitivity ranges from 20 to 100% [2, 12, 18, 20, 21]. In contrast with most previous reports [6-8, 12, 16, 17, 23], HPV showed less sensitivity than pap smear in our hospital (69% vs 84% and 80% vs 100%), but NPV was similar, very high in both tests (97% vs 98% and 96% vs 100%). In spite of our HPV sensitivity being lower than described by others (83-100%) [2, 4, 5, 8, 9, 11, 12, 17, 21], in the large case-control study of Acladiouss et al. [25] HPV only showed 47% sensitivity six months post-conization. Nonetheless the present authors hope that the Cobas 4800 PCR will improve HPV predictive power in the near future in Asturias University Hospital.

In respect to co-testing, it is clear in the present study and others [6, 7, 9, 21, 25] that combining cytology and HPV increases sensitivity and NPV and, although specificity and PPV decrease, the former are more important in this context of a potentially lethal disease. In this way, it is possible to select a group of patients with very low risk of recurrent disease that could be returned early to routine screening.

However, Strander et al. [26] published that 76% of recurrences more than two years post-LEEP occurred in women that had cleared HPV after treatment, so most probably these patients were infected with HR-HPV at a later stage. The host conditions that once led to the initial CIN usually persist and these women have an increased risk for re-infection and persistence with HR-HPV. They concluded that this is the probable cause for the limited value of HPV for the design of long term follow-up and criticize other small prospective studies that publish a very high NPV for HPV but with a fairly short follow-up period and a minimal number of recurrences [12, 27].

**Proposed surveillance protocols**

Several reports [12, 15, 21, 27] conclude that risk of recurrent disease is so low in women with negative co-testing at six months that restesting at 12 months can be omitted and scheduled again at 24 months. The need for fewer tests would be a cost benefit and would be more convenient for the patient. Only when co-testing is negative during at least 24 months should women be referred to a routine screening program.

Kitchener et al. [28] recently published a large prospective study (n=917). They found that the risk of residual CIN 2-3 is negligible in women who were HPV/Pap negative at six months so these patients could be safely returned to three- or five-yearly recall.

Some authors advise caution and emphasize that more relaxed follow-up in HPV test-negative women still awaits further evaluation in larger studies with longer follow-up times [14, 29].

On the basis of the present prospective analysis, the authors recommend that patients with free margins and negative co-testing at six and 12 months could omit the 18 months visit and retest at 24 months. Nevertheless, if margins are positive, visits should be at 6-12-18-24 months with cytology and HPV testing. In the present retrospective analysis, tests were not done at fixed time intervals therefore the authors cannot conclude the same, but it should be noted that the three women with false negative co-testing that developed recurrent disease were diagnosed thanks to routine colposcopy in the Department of Gynecology (HUCA). Furthermore, the retrospective analysis had a large study population (n=372) and a long-term follow-up (up to 15 years), therefore the authors could confirm that eight of 32 recurrences (25%) developed more than two years post-LEEP. Among these eight long-term recurrences, three (37.5%) were caused by persistent “new/another infections”, results in concordance with Strander et al. [26]. Another three of these recurrences occurred, surprisingly, in women who tested negative for HPV pre-LEEP and every visit post-LEEP, and the remaining three long-term recurrences were caused by persistent same HPV-type infection present before treatment.

Thus, the present authors agree with several authors [22, 24, 30] that long-term risk of recurrent dysplasia or invasive cancer remains higher among women treated for CIN 2-3, hence it is important not to stop routine screening at age 65 as in the general population. More intensive follow-up is proposed, preferably for at least 20-25 years after conization, particularly for those women older than 35-40 years at treatment. Moreover, the authors consider that the available evidence is not strong enough to abbreviate surveillance at the Colposcopy Department to less than two years.

The Spanish Society for Colposcopy and Cervical Pathology maintains the follow-up protocol published in 2006 [31]: co-testing and colposcopy at three to six months followed by two annual Pap smears. If these are negative, women are returned to cervical screening.

The American Society for Colposcopy and Cervical Pathology has recently updated its guidelines [32]. In its 2006 recommendations, if a single HPV test or cytology at six and 12 months were negative, women were returned early to screening. Current guidelines suggest co-testing at 12 and 24 months and if both are negative, it is repeated three years later. Then, women are returned to screening for at least 20 years.

**Conclusion**

HR-HPV testing, in combination with cytology, is the best option in follow-up protocols after treatment for CIN 2-3. The authors also recommend routine colposcopy during the first two years post-conization. If margins are free and co-testing is negative at six and 12 months, 18 months visit could be avoided.
References


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Introduction

On a global level, cervical cancer is the second most common and fifth deadliest cancer in women [1, 2]. It occurs in approximately 16 per 100,000 women per year and accounts for nine per 100,000 deaths annually [3]. Approximately 80% of cervical cancers occur in developing countries [4]. In 2008, it was estimated that there were 473,000 cases of cervical cancer [5] and in 2010 225,000 deaths worldwide [6].

Cancer screening using the Papanicolaou (Pap) test smear can identify precancerous and potentially precancerous changes in cervical cells and tissue. Treatment of high-grade changes can prevent the development of cancer in many cases. In developed countries, the widespread use of cervical screening programs has dramatically reduced the incidence of invasive cervical cancer [7].

Infection with some types of human papillomavirus (HPV) is the greatest risk factor for cervical cancer, followed by smoking [8]. Although not all of the causes of cervical cancer are known, several other contributing factors have been implicated [9]. HPV infection appears to be a necessary factor in about 90% for the development of cervical cancer [10].

The global variability of treatment of cervical cancer is mostly due to large variances in disease burden in developed and developing nations, access to skilled surgeons in radical pelvic surgery, and the evolution of fertility-sparing options in developed countries. Because cervical cancers are radiosensitive, radiation may be used in all stages where surgical options do not exist.

Materials and Methods

In the present study, six provinces of the Republic of Panama, were covered which included medical tours, whose studies were published in a recent article and where the value of routine colposcopy was highlighted [11]. This value was reflected in recruitment of patients with OR which reached about 53%, defined as a result of health control, while the OR was confirmed in those patients referred for suspected uterine cervical cancer [12].

Between 1982 and 2012, we examined 12,679 women, applied 26,889 colposcopies, 26,251 colpocytologies, and 5,868 directed biopsies were applied to them. The Colpo-Pap methodology was applied, that is taking a Pap smear sample through a colposcopic approach. Through a direct line of vision of the lesion via colposcopy, a biopsy was taken (Table 1).

The diagnosis was clinical, cytological, histological, and the patient was defined with OR as a simple HPV, pure dysplasia or associated with HPV, as well as cancers with or without HPV, and finally, pure OR, which meant that the lesion was without cytological and histological support.

The profile of patients evaluated corresponded to these parameters: two pregnancies, two births, no abortions, menarche between 13 and 15 years, the beginning of coitus between 15 and 20 years, two sexual partners throughout life, and almost all used the family planning method. The age range was from eight to 90 years [13].

Treatment was applied adequately to different pathologies and consisted in cryotherapy, and/or associated to cauterization, electro-
coagulation, and surgery where it was required. The patients treated conservatively were checked every three months, until the viral lesions disappeared, and then every six months or a year.

We achieved an initial diagnosis, that is to say, recruitment, whose value is of utmost importance when it comes to preventing cervical cancer, but also the conclusion or evolutionary diagnosis. Both diagnoses are important because they sustain the findings, and in the end, they are a true representation of Panama’s reality.

Results

Diagnosis led to 6,411 women with OR. HPV reached 87% with 5,498 cases in total; pure HPV cases were 4,257, those associated with dysplasia were 1,096 cases, and 145 cases associated with cancer. The OR cases corresponded to 50.5% of total women attended in the past 30 years of this study; a considerably elevated number, but the percentage of the reported annual OR corresponded to an average of 44%, and almost exclusive to new patients which entered the study (Table 1).

The preventive cancer study’s analytical diagnosis was achieved. The cytology contributed a risk diagnosis of 50%; most representative of the false negative was inflammatory alterations.

Concerning colposcopy, it reached a 79% OR, while the most frequent pathological pictures were atypical re-epithelization zone (ARZ) in 47%, leukoplakia in 22%, and HPV in 13%.

The directed biopsy with the colposcopic approach indicated 94% OR and false negatives were only 6%. While performing the analysis, we observed that by integrating the three diagnostic methodologies, we reduced false negatives to a mere 1%, confirming that concordance of the three methods is not the most common. Facing this discordance we worked with the most severe diagnosis.

We guided the majority of these patients, to local conservative treatments, such as cryotherapy, cauterization, caustic or chemical, and we emphasized the importance of successive controls, in order to assess their evolution, thus repeating, adding or adjusting the recommended treatments. The applied treatments were 3,780, distributed in 2,305 cryotherapies, 549 cauterizations, 702 mixed, and others in 224. Surgical treatments such as hysterectomy were few. We suspended cervical conizations in the early 1990s (Table 2) [14].

The evolution of the patients, which we carried out controls of every six months or every year, indicates that in total and in follow-up controls, that there were 210 cases in the year 2011, and 200 cases in 2012. Thus, we have the following results: healing reached 90%; improvement 3%; persistence 3%; recurrence 2%, and progression 2% (Table 3) [15].

In the most conservative aspect, and in the annual evolution, based on patient controls, it is shown that the values remain identical; though the patients have had different degrees of pathology, including cancer treated conservatively [16-18].

These clinical evaluations of lesions considered as OR eradication, contrast with findings of molecular biology studies, which began in December 2011 and also covered OR cured patients (patients with OR recently diagnosed and healthy ones). We managed to diagnose 90% of them with high-risk HPV. Within the same group, the most frequent subtypes were 31, 18, 35, and 16, while only ten cases were negatives [19-21].

HPV’s behavior, pathophysiology, numerical incidence, and oncogenic potential have been analyzed by many researchers. The many doubts regarding the genesis of cancer and its chronic action (innate or acquired response) has become clear and has led to the use of vaccines in a prophylactic or therapeutic sense [22-24].

In January 2012, we included the PCR studies in samples of fibroids or myomas due to high incidence and above all, rapid development. These samples were obtained in the myomectomies, or in the hysterectomies performed during
that year, and which results, surprisingly, reached a 100% of HPV. Among them, the most frequently subtypes found were 16 and 18.

Discussion
Panama is a small country with a high incidence of cancer. If we evaluated the actual precursor of cancer lesions incidence, we would have to consider that in the 30 years of study, we are failing to minimize this great illness, and above all, the death of many women because of late presentation, above all due to the bureaucracy in healthcare institutions, physicians, and ultimately, themselves.

Despite diagnosing 6,411 OR cases, evolutionary diagnosis is very important, because with it, we validate the application of conservative therapeutic measures. Thus our results are justified since invasive cancer had zero progression in the controlled patients up to 30 years, and in the last five years of studies, we had no cases of cancer at the time of concluding them. With these successes, there is nothing more to add.

Conclusions
The inspection of the different aspects of the HPV affected women’s profile, such as the planning methods, sexual experiences, the discipline in their own health management, good response to the recommendations of treatments, and care-taking, as well as compliance with medical appointments, and calmly receiving the PCR results, compared with the clinical pictures, healing was performed.

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c-Met and RON expression levels in endometrial adenocarcinoma tissue and their relationship with prognosis

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Summary

Objective: To investigate the potential relevance of c-Met and RON gene expression in patients with adenocarcinoma of the endometrium and analyze the relationships among the c-Met and RON expression, clinicopathological characteristics, and patient survival. Materials and Methods: The study included 60 cases diagnosed with endometrial adenocarcinoma with more than five-years follow-up. Total RNA from formalin-fixed paraffin-embedded tissues of 60 adenocarcinomas of the endometrium and normal endometrium tissues were isolated for c-Met and RON quantitative analysis by real-time real-time polymerase chain reaction (RT-PCR). Results: The c-Met and RON expression in endometrial adenocarcinoma was significantly higher than that in normal endometrial tissues (p < 0.01), with average up-regulated levels of 3.94 ± 1.88 and 2.74 ± 0.88, respectively. Moreover, high c-Met expression was significantly correlated with the histological stage (p = 0.017), and high RON expression was related to histological stage (p = 0.035), muscle invasion (p = 0.006), and lymph node metastasis (p = 0.018). Multivariate Cox regression analysis revealed that the co-expression of c-Met and RON was an independent prognostic factor for adenocarcinoma of the endometrium and was significantly associated with decreased overall survival (HR = 3.571, p = 0.014). Conclusion: The co-expression of c-Met and RON is associated with a poor prognosis in endometrial adenocarcinoma and is an independent prognostic marker for endometrioid adenocarcinoma.

Key words: c-Met; RON; Endometrioid adenocarcinoma; Prognosis.

Introduction

Studies of epithelial tumors have demonstrated that receptor tyrosine kinases (RTKs) of transmembrane proteins play a basic role in cell growth, differentiation, and survival, and are important in tumor occurrence and development. c-Met and RON (recepteur d’origine nantais) belong to the MET proto-oncogene family, a distinct subfamily of RTKs. c-Met protein can be activated by its ligand hepatocyte growth factor receptor. c-Met can be conformationally changed by their receptor intracellular tyrosine kinase domain, and participate in multiple cellular processes, including proliferation, differentiation, morphogenesis, and infiltration [1]. RON can induce cell differentiation and development of epithelial tumors [2, 3]. Recent studies have shown that c-Met and RON are both separately expressed and co-expressed in various tumors [4, 5] and interact in several epithelial tumors, leading to tumorigenesis. Therefore, understanding tyrosine kinase expression will help to define the role of c-Met and RON in carcinogenesis and their potential as molecular targets for tumor therapy.

Endometrial carcinoma is a common gynecological malignant tumor, accounting for 20–30% of all malignant tumors of the female genital tract. For all endometrial carcinomas, the incidence of endometrial adenocarcinoma is 80–90%. The present study evaluated the c-Met and RON expression levels in endometrial adenocarcinoma and normal endometrial tissue by real-time polymerase chain reaction (RT-PCR), and explored the relationship between c-Met and RON expression and clinicopathological features in endometrial adenocarcinoma.

Materials and Methods

Test specimens

Formalin-fixed paraffin-embedded (FFPE) archived samples of endometrial adenocarcinoma from 60 cases were collected from January 2005 to December 2006 in Wenzhou City, at the Traditional Chinese and Western Medicine Hospital Pathology Department, and FFPE archived samples from 20 cases with normal endometrium were used for paraffin tissue RNA extraction. Simultaneously, the authors collected from March 2010 to October 2011 in Wenzhou Hospital of Integrated Chinese and Western medicine for “endometrial adenocarcinoma” line operation of fresh specimens of 15 cases of endometrial cancer, because of “uterine leiomyoma” line of hysterectomy in ten cases of normal endometrium. Following resection, fresh tissue was immediately frozen in liquid nitrogen, and then stored at -80°C. Fresh specimens tissue for RNA extraction control with paraffin. All cases with FFPE tissue had complete clinical pathological data available and were followed up for more than five
years. The 60 FFPE cases of endometrial carcinoma underwent an operation for staging and pathological grading by two experienced pathologists using the 2000 International Federation of Gynecology and Obstetrics (FIGO) pathological staging criteria. Fifteen cases were classified as pathological Stage G1, 30 as Stage G2, and 15 as Stage G3. Twenty-eight cases were clinically classified as Stages 0–I, 14 cases as Stage II, and 18 cases as Stage III. Thirty-six cases had no myometrial invasion or depth of myometrial invasion ≤ 1/2; 24 cases had a depth of myometrial invasion >1/2; 47 cases had no lymph node metastasis, and 13 cases showed metastasis. Patients received no radiotherapy, chemotherapy, or hormone therapy. Clinical and pathological data, as well as follow-up data, for the 60 cases with endometrial adenocarcinoma are presented in Table 1.

Follow-up

All cases were followed up by telephone. Those who could not be contacted at the fourth attempt were followed up through local medical institutions or personnel inquiries. The deadline was July 1st, 2011. During the five-year follow-up period, six cases (10%) were lost and 14 died.

Real-time quantitative reverse transcription–PCR

FFPE RNA extraction was performed as follows: (1) FFPE samples were dewaxed by adding xylene on slices, incubating the samples for ten minutes at room temperature, followed by overturning and blending to melt the paraffin. Residual xylene was removed by washing twice with absolute ethyl alcohol, and then the lamellar precipitate was air-dried. (2) RNA was then extracted and purified using the RecoverAllTM total nucleic acid isolation kit for FFPE. (3) RNA was dissolved by adding 60 μL of elution solution to purified RNA. Of the purified RNA solution, 20 μl was reserved for detection of RNA quality and reverse transcription, and the remainder was stored at -80°C.

RNA was extracted by first liquid-nitrogen grinding fresh tissue into a powder, addition of TRIzol, and then extracting the RNA according to the TRIzol instruction manual.

Primers were designed using the Primer 3 software, accessed online. Primers were designed with a GC content of 40–60% and a product size of 89–203 bp. Primers were then evaluated using Oligo 6.0 software. Primers contained intron sequences to reduce interference from genomic DNA and were synthesized.

The RT–PCR primer pairs used for c-Met and RON expression were as follows:

c-Met (212 bp): upstream, 5′-CAGGCAGTGAGCATG- TAGT-3′/downstream, 5′-GATGATTCCCTCGGTCAGAA-3′; RON (292 bp): upstream, 5′-TGGGGACCACCTACTCTTG- 3′/downstream, 5′-GAGCCAGGACACTCTTCTG-3′; GAPDH (314 bp): upstream, 5′-GGTCGGAGTCAACGGATTTG- 3′/downstream, 5′-ATGAGCCCCAGCCTCTCCAT-3′. The 25-μL PCR reaction contained 12.5 μL of 2× QuantiTect SYBR Green PCR buffer, 10 μM of primers and 1 μL or 2 μL of cDNA sample (300 ng), RNA enzyme inactivation 8.5 μL. Thermal cycle conditions were as follows: 94°C for five minutes; 40 cycles of 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 60 seconds, and 72°C for ten minutes.

After selecting the appropriate baseline, the crossover point of the fluorescence curves and baseline was termed the Ct (cycle threshold) value. The smaller the Ct value under the same baseline, the earlier the fluorescence signal detected, reflecting a higher number of gene copies. The Ct values of the target and reference genes were calculated by the 2−ΔΔ Ct method [6], and GAPDH was applied as control. Compared with normal endometrial tissue, relative c-Met mRNA expression levels were calculated using the following formula: folds = 2−ΔΔ Ct, while Δ Ct = (Ct Δ which gene − Ct endogenous reference gene) study group − (Ct Δ which gene − Ct endogenous reference gene) control group.

Figure 1. — Comparison of c-Met and RON expression using total RNA extracted from matched samples of formalin-fixed paraffin-embedded tissue (FFPET) and frozen tissue. Comparison of theΔCt values for paired FFPET and frozen tissue for (A) c-Met, (B) RON, and (C) GAPDH.
c-Met and RON expression levels in endometrial adenocarcinoma tissue and their relationship with prognosis

(Ct target gene− Ct endogenous reference gene) control group indicates the expression of c-Met mRNA in endometrioid adenocarcinoma relative to that in normal endometrial tissue. The relative quantity of RON mRNA was calculated in the same way.

Statistical analysis

Data were analyzed using the SPSS software, version 15. The results are expressed as X ± s, and group comparisons were performed using independent sample tests. A value of \( p < 0.05 \) was deemed to indicate statistical significance. Relationships between c-Met and RON expression and clinicopathological features of endometrioid adenocarcinoma were assessed by the χ2 test. The Kaplan–Meier method and Cox regression analysis were used to evaluate relationships among c-Met and RON expression, clinicopathological features of endometrioid adenocarcinoma, and prognosis.

Results

Reliability of RT-PCR for detection of c-Met and RON expression in paraffin-embedded tissues

The ratios of the absorbances at 260 and 280 nm (A260/A280) of overall RNA extracted from paraffin-embedded endometrial carcinoma tissue were 1.75–1.95; there was no significant difference between the two groups (\( p > 0.05 \)). Levels of c-Met and RON expression were correlated (R2 = 0.898 and 0.88, respectively; \( p < 0.01 \), linear regression; Figures 1A and 1B). In paraffin-embedded endometrial adenocarcinoma tissue, \( \Delta \text{Ct c-Met} = 5.34 \pm 1.13 \) whereas \( \Delta \text{Ct c-Met} = 5.23 \pm 1.01 \) in the corresponding fresh tissue. In paraffin-embedded endometrial adenocarcinoma tissue, \( \Delta \text{Ct RON} = 5.70 \pm 1.85 \) whereas \( \Delta \text{Ct RON} = 6.21 \pm 1.53 \) in the corresponding fresh tissue.

GAPDH RNA expression levels in the two types of tissues were also related (Figure 1C).

Relationship between relative c-Met and RON expression levels in endometrial carcinoma and clinicopathological features

Relative mRNA expression levels of c-Met and RON in endometrial adenocarcinoma tissue were calculated to be 3.94 ± 1.88 and 2.74 ± 0.88, respectively. These levels followed a normal distribution (\( p > 0.05 \)) and were significantly higher than those in normal endometrial tissue (0.672 ± 0.098 for endometrial adenocarcinoma tissue; 0.512 ± 0.109 for normal endometrial tissue; \( p < 0.01 \)). c-Met mRNA expression levels in endometrial adenocarcinoma was related to pathological staging (\( p = 0.017 \)). RON expression levels in patients were related to pathological staging (\( p = 0.035 \), depth of myometrial invasion (\( p = 0.006 \)), and lymph node metastasis (\( p = 0.018 \); Table 1).

Table 1. — Distributions of c-Met and RON gene expression and biological indicators.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Number of cases</th>
<th>c-Met mRNA</th>
<th>RON mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( x \pm s )</td>
<td>( t )</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;50</td>
<td>38</td>
<td>3.457 ± 0.736</td>
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<tr>
<td>≥50</td>
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<td>Menopause</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>15</td>
<td>3.639 ± 0.924</td>
<td>1.003</td>
</tr>
<tr>
<td>No</td>
<td>45</td>
<td>3.411 ± 0.689</td>
<td></td>
</tr>
<tr>
<td>Clinical stage</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I, II</td>
<td>42</td>
<td>3.698 ± 0.892</td>
<td>1.361</td>
</tr>
<tr>
<td>III*</td>
<td>18</td>
<td>3.397 ± 0.664</td>
<td></td>
</tr>
<tr>
<td>Muscle invasion</td>
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<td></td>
<td></td>
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<tr>
<td>&lt;1/2</td>
<td>36</td>
<td>3.546 ± 0.841</td>
<td>0.697</td>
</tr>
<tr>
<td>≥1/2</td>
<td>24</td>
<td>3.397 ± 0.665</td>
<td></td>
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<tr>
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<td>I</td>
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<td>Metastasis</td>
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<tr>
<td>No metastasis</td>
<td>47</td>
<td>3.397 ± 0.665</td>
<td></td>
</tr>
</tbody>
</table>

*No Stage IV patients were identified.

Figure 2. — The prognostic significance of c-Met and RON expression in endometrial carcinoma patients. Patients who co-expressed c-Met and RON had a significantly worse survival compared with patients who had single-receptor-positive tumors or no receptor expression.
The present study detected the expression of c-Met and RON by RT-PCR. The results showed there were significant correlations between the two types of tissue. (R² = 0.898 and 0.88, p < 0.01), which signifies that the detection of c-Met and RON expression by RT-PCR is sufficiently reliable. The results also showed that routine paraffin-embedded archived tissue may be used for mRNA retrospective studies, so that the resources of the Department of Pathology are utilized fully.

Research has shown that c-Met positive cancer cells have higher ability of invasion and remote metastasis, and RON has the same impact as c-Met does. Additionally, the role of the interaction between c-Met and RON in tumorigenesis and progression is supported by the results of an in vitro tumor-related study [9]. The relationship between c-Met and endometrial adenocarcinoma is usually investigated by immunohistochemistry in a semiquantitative manner; however, few such studies have been performed. To-date, no study has reported the relationship between the RON gene and endometrial adenocarcinoma. Additionally, the role of both genes in development of endometrial adenocarcinoma is unclear.

The present study detected the expressions of c-Met and RON in FFPE of 60 cases of endometrial adenocarcinoma and 20 cases of normal endometrium. The present results showed c-Met and RON expression levels in endometrial adenocarcinoma tissues was significantly higher than those in normal endometrial tissue (p < 0.01). Significant correlations were found between abnormal RON expression and histological stage, depth of myometrial invasion, and lymph node metastasis in the first consultation. Abnormal c-Met expression was related to pathological staging. There were significant correlations between c-Met and RON expression and pathological stages and histological grades. The later the pathological staging, the lower the differentiation and the higher the expression of c-Met and RON. The
two expression levels were significantly correlated with the depth of myometrial invasion and the status of metastasis when initial diagnosis is established ($p < 0.05$), indirectly confirming that these genes may promote development of endometrial adenocarcinoma. High expression of the tyrosine kinases c-Met and RON may be a relatively late event in endometrial adenocarcinoma and may be related to invasion and metastasis [4]. Therefore, assessment of c-Met and RON expression in endometrial adenocarcinoma provides a basis for further treatment. The present results are consistent with the findings that different subtypes of the same ErbB receptor family interact and are related to poor prognosis [3]. Cheng et al. [5] reported co-expression of c-Met and RON to be an important indicator of poor prognosis in bladder cancer and related to tumor recurrence. Through Cox multiple regression analysis, the present study indicated that high expression of c-Met and RON ($p = 0.014$) and pathological grade ($p = 0.016$) were indicators of poor prognosis in endometrial adenocarcinoma. High level of co-expression of c-Met and RON may thus represent a “molecular prognostic index” in endometrial adenocarcinoma.

In summary, up-regulated c-Met and RON expression levels were closely related to the occurrence, development, and prognosis of endometrial adenocarcinoma. Simultaneous detection of the expression of both genes provides diagnostic and prognostic information regarding endometrial adenocarcinoma. However, further research is needed to elucidate the mechanism underlying the role of c-Met and RON in the development of endometrial adenocarcinoma. In terms of their role in carcinogenesis, c-Met and RON may represent novel target molecules for gene therapy as a future treatment option for endometrial adenocarcinoma.

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Prognostic value of INPP4B protein immunohistochemistry in ovarian cancer

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Summary

Purpose of investigation: Ovarian cancer is associated with poor prognosis and altered protein expression patterns may be useful for identifying patients likely to have poor disease outcomes. The impact of altered INPP4B protein expression on prognosis is unclear. The aim of this study was to evaluate the implication of INPP4B expression changes in a large series of ovarian cancer tissue samples. Materials and Methods: Tissue microarrays were constructed from 599 epithelial ovarian tumors and stained with antibodies for INPP4B, p53, and PTEN. Proportional hazard models were used to estimate survival hazard ratios (HRs) associated with altered protein expression. Results: Seventy-nine percent of the ovarian cancers demonstrated loss of INPP4B, whereas 53% showed aberrant p53 expression (i.e., complete loss of p53 or over-expression of p53) and 8% showed loss of PTEN. INPP4B was frequently lost in serous and endometrioid cancer subtypes, aberrant p53 expression was most common among serous subtype, and loss of PTEN was most common among endometrioid tumors ($p$ for all three proteins across histologic subtypes ≤0.0001). INPP4B loss or aberrant p53 expression were both associated with increased mortality (HR = 1.84; 95% CI 1.27 - 2.68 and HR = 3.10; 95% CI 2.33 - 4.11, respectively); however, in multivariate models, only the relationship with p53 achieved statistical significance (HR = 1.20; 95% CI 0.82 - 1.76 for INPP4B and HR = 1.73; 95% CI 1.28 - 2.34 for p53). Conclusion: The INPP4B protein is frequently lost in serous and endometrioid subtypes of ovarian cancer. A possible prognostic role of INPP4B for endometrioid ovarian tumors requires further evaluation.

Key words: Ovarian cancer; Prognosis; INPP4B; PTEN; p53; Survival.

Introduction

Ovarian cancer is the most fatal gynecologic malignancy (case fatality approaching 70%) due to typically advanced stage at clinical presentation and poor prognosis [1, 2]. Chemotherapy for ovarian cancer consists of platinum compounds in combination with taxanes [2]. Poor survival rates follow, in part, resistance to chemotherapy [2]. Currently, ovarian cancers are classified based on tumor clinical stage and histologic features, but neither individual response to chemotherapy nor prognosis relate to these features alone [1]. Identification of new molecular markers, such as altered expression of key proteins, is critical for the development of targeted treatments. The deregulation of proteins in pathways involved in cell-cycle progression, apoptosis, and angiogenesis is likely to contribute to poor prognosis and to platinum resistance [3].

The phosphoinositide 3-kinase (PI3K)/AKT signaling pathway is important for the regulation of cell growth, proliferation, differentiation, apoptosis, and intracellular trafficking [4]. Studies of ovarian cancer cell lines and animal models reveal that activation of these pathways may lead to chemotheraphy resistance [3]. PI3K signaling is altered in up to 45% of ovarian cancers [5]. Somatic mutations of genes in the PI3K/AKT pathway are rare, whereas gene amplifications are more common [5]. Mutations in the class I PI3K gene (PIK3CA) and PTEN, a negative regulator of PI3K/AKT signaling have been reported in ovarian cancers, however the frequency of alterations is modest compared to in other epithelial cancers [6]. In this study, we investigated the role of a recently identified tumor suppressor gene in the PI3K pathway, inositol polyphosphate 4-phosphatase (INPP4B), in ovarian cancer prognosis in a large series of unselected 599 epithelial ovarian tumors originating from patients in Ontario, Canada.
Materials and Methods

Study population
In the province of Ontario, Canada, all residents newly diagnosed with primary invasive epithelial ovarian cancer from January 1995 through December 1999 and from January 2002 through December 2004 were identified by checking case notifications at the Ontario Cancer Registry. Pathology reports were reviewed by the investigators for each case, to determine study eligibility, and tumor histologic type. Patients were between 20 and 79 years of age at the time of diagnosis. Through a standardized-script risk-factor questionnaire, information on known and suspected ovarian cancer risk factors and demographic information was collected by telephone. Details of clinical staging, treatments received, and response to treatment were obtained from medical record review. The study was approved by the institutional review boards of the University of Toronto and Yale University (see [7] for further detail on the study population and BRCA1 mutation analyses).

Ovarian cancer case confirmation and tumor block collection
Diagnostic confirmation and coding of tumor characteristics (invasive vs. borderline, histologic type, and stage) was determined through pathology reports and other medical records. Paraffin-embedded tissue blocks representative of the ovarian carcinoma were requested for each confirmed ovarian cancer case. Medical record information was requested from treating hospitals a minimum of one year after diagnoses, to ensure that primary surgery and chemotherapy had been completed.

Immunohistochemistry
Tissue microarrays (TMAs) were constructed, with duplicate 0.6 mm cores from each of the 599 patient tumors included in this study. INPP4B-specific monoclonal antibodies were produced by immunizing mice with purified recombinant human His-INPP4B and were characterized as described [8]; P53 (NCL-p53-DO7) and PTEN (M362729) protein expression were assessed by immunohistochemistry using commercial antibodies and standard techniques. All stained TMA slides were digitized using a slide scanner at 40X magnification. INPP4B expression was scored based on percent positive tumor cells within the following categories: completely negative, >0 but ≤5%, >5 but ≤25%, >25 but ≤50%, and >50% positive nuclear staining. p53 protein expression was also scored based on percent positive tumor cells: completely negative, >0 but ≤50% positive staining, and >50% positive nuclear staining. Negative and >50% positive staining patterns are indicative of p53 aberrations. PTEN staining showed both nuclear and cytoplasmic localization and was scored based on both expression patterns. Staining was quantified by intensity, with negative expression representing undetected staining in tumor cells and positive expression in stromal cells; focal positive expression representing weaker staining in tumor compared to stromal cells; and positive expression representing equal staining intensity in tumor and stromal cells. Figure 1 shows representative images for each of the stains.

Ascertainment of outcomes
Survival status was determined both by computerized linkage of subject identifying information to death certificate data maintained by the Ontario Cancer Registry, and by chart review at local hospitals. This linkage provided information on date and cause of death. The Ontario Cancer Registry, which began in 1964, compiles information on cancer incidence, mortality, and survival in Ontario. Previous evaluation of the Ontario Cancer Registry record-linkage approach for determination of vital status from death certificates has shown that it is more accurate than manual approaches.

![Figure 1. — Representative IHC images for antibody stains.](image-url)
Table 1. — Characteristics of 599 epithelial ovarian cancer cases, Ontario, Canada.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total, n (%)</th>
<th>INPP4B positive</th>
<th>INPP4B absent</th>
<th>PTEN positive</th>
<th>PTEN absent</th>
<th>p53</th>
<th>p53 over-expression</th>
<th>p53 absent</th>
<th>BRCA1 mutation, n (%)</th>
<th>BRCA2 mutation, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology, n (%)</strong></td>
<td>n = 566</td>
<td>n = 117</td>
<td>n = 449</td>
<td>n = 588</td>
<td>n = 277</td>
<td>n = 79</td>
<td>n = 311</td>
<td>n = 450</td>
<td>41 (8%)</td>
<td>25 (5%)</td>
</tr>
<tr>
<td>Serous</td>
<td>315 (53%)</td>
<td>39 (12%)</td>
<td>276 (47%)</td>
<td>162 (28%)</td>
<td>150 (26%)</td>
<td>64</td>
<td>32 (51%)</td>
<td>307 (49%)</td>
<td>8 (19%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>40 (7%)</td>
<td>3 (10%)</td>
<td>37 (93%)</td>
<td>15 (25%)</td>
<td>25 (48%)</td>
<td>5</td>
<td>3 (5%)</td>
<td>32 (53%)</td>
<td>7 (17%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>139 (23%)</td>
<td>32 (23%)</td>
<td>107 (77%)</td>
<td>129 (83%)</td>
<td>100 (72%)</td>
<td>19</td>
<td>11 (22%)</td>
<td>118 (69%)</td>
<td>12 (29%)</td>
<td>10 (25%)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>43 (7%)</td>
<td>2 (5%)</td>
<td>41 (95%)</td>
<td>18 (25%)</td>
<td>25 (48%)</td>
<td>8</td>
<td>2 (4%)</td>
<td>16 (26%)</td>
<td>7 (17%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td><strong>Stage, n (%)</strong></td>
<td>n = 540</td>
<td>n = 45</td>
<td>n = 495</td>
<td>n = 530</td>
<td>n = 280</td>
<td>n = 79</td>
<td>n = 311</td>
<td>n = 450</td>
<td>41 (8%)</td>
<td>25 (5%)</td>
</tr>
<tr>
<td>I</td>
<td>124 (21%)</td>
<td>16 (13%)</td>
<td>108 (87%)</td>
<td>108 (21%)</td>
<td>96 (34%)</td>
<td>30</td>
<td>20 (26%)</td>
<td>88 (24%)</td>
<td>7 (17%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td>II</td>
<td>106 (18%)</td>
<td>20 (19%)</td>
<td>86 (81%)</td>
<td>96 (18%)</td>
<td>90 (86%)</td>
<td>16</td>
<td>14 (15%)</td>
<td>82 (16%)</td>
<td>7 (17%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td>III</td>
<td>283 (47%)</td>
<td>44 (16%)</td>
<td>239 (84%)</td>
<td>235 (83%)</td>
<td>200 (70%)</td>
<td>27</td>
<td>27 (10%)</td>
<td>208 (74%)</td>
<td>12 (15%)</td>
<td>7 (10%)</td>
</tr>
<tr>
<td>IV</td>
<td>84 (14%)</td>
<td>10 (12%)</td>
<td>74 (88%)</td>
<td>71 (88%)</td>
<td>64 (79%)</td>
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<td>11 (13%)</td>
<td>60 (74%)</td>
<td>7 (10%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td><strong>BRCA1 mutation, n (%)</strong></td>
<td>n = 450</td>
<td>n = 45</td>
<td>n = 405</td>
<td>n = 435</td>
<td>n = 250</td>
<td>n = 79</td>
<td>n = 311</td>
<td>n = 450</td>
<td>41 (8%)</td>
<td>25 (5%)</td>
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<tr>
<td>1</td>
<td>126 (28%)</td>
<td>16 (13%)</td>
<td>110 (87%)</td>
<td>114 (26%)</td>
<td>98 (85%)</td>
<td>30</td>
<td>20 (26%)</td>
<td>84 (20%)</td>
<td>7 (17%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td>II</td>
<td>108 (24%)</td>
<td>20 (19%)</td>
<td>88 (81%)</td>
<td>92 (18%)</td>
<td>82 (86%)</td>
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<td>14 (15%)</td>
<td>78 (18%)</td>
<td>7 (17%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td>III</td>
<td>279 (60%)</td>
<td>44 (16%)</td>
<td>235 (84%)</td>
<td>235 (83%)</td>
<td>200 (70%)</td>
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<td>7 (10%)</td>
</tr>
<tr>
<td>IV</td>
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<td>10 (12%)</td>
<td>71 (88%)</td>
<td>71 (88%)</td>
<td>64 (79%)</td>
<td>11</td>
<td>11 (13%)</td>
<td>60 (74%)</td>
<td>7 (10%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td><strong>BRCA2 mutation, n (%)</strong></td>
<td>n = 450</td>
<td>n = 45</td>
<td>n = 405</td>
<td>n = 435</td>
<td>n = 250</td>
<td>n = 79</td>
<td>n = 311</td>
<td>n = 450</td>
<td>41 (8%)</td>
<td>25 (5%)</td>
</tr>
<tr>
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<td>110 (87%)</td>
<td>114 (26%)</td>
<td>98 (85%)</td>
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<td>20 (26%)</td>
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<td>5 (12%)</td>
</tr>
<tr>
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<td>88 (81%)</td>
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<td>82 (86%)</td>
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<td>78 (18%)</td>
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<td>5 (12%)</td>
</tr>
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<td>208 (74%)</td>
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<td>7 (10%)</td>
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<td>71 (88%)</td>
<td>64 (79%)</td>
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<td>11 (13%)</td>
<td>60 (74%)</td>
<td>7 (10%)</td>
<td>5 (12%)</td>
</tr>
</tbody>
</table>

1 Other includes the following tumor types: mixed histology, epithelial not otherwise specified, and adenocarcinoma not otherwise specified.
Figure 2. — Ten-year survival among ovarian cancer patients by INPP4B expression among – a) all subtypes, b) endometrioid subtype only, and c) serous subtype only.

Figure 3. — Ten-year survival among ovarian cancer patients by p53 expression among – a) all subtypes, b) serous subtype only, and c) endometrioid subtype only.
Prognostic value of INPP4B protein immunohistochemistry in ovarian cancer

among the endometrioid subtype (17%). Approximately one-half of the tumors had normal p53 expression (47%), while 39% showed over-expression, and 13% had complete loss of p53 expression. Sixty-nine percent of serous tumors showed aberrant p53 expression (i.e., over-expression or complete loss of expression). Tumors from women with a BRCA1 or BRCA2 mutation were more likely to show loss of INPP4B and aberrant p53 expression.

Overall, loss of INPP4B expression was associated with significantly worse survival (HR = 1.84; 95% CI 1.27 - 2.68; p = 0.0001) (Figure 2a). Among the histological groups, the effect was strongest for women with endometrioid cancers (HR = 5.15; 95% CI 1.23-21.6; p = 0.03) (Figure 2b). INPP4B loss was not associated with survival among the other subtypes (p ≥ 0.70)(Figure 2c for serous subtype). Women with tumors with normal p53 expression experienced significantly better survival than women with aberrant p53 expression (i.e., either complete loss or over-expression) (HR = 3.10; 95% CI 2.33 - 4.11; p < 0.0001) (Figure 3a). After stratification by histologic subtype, aberrant p53 expression was associated with significantly worse prognosis for both serous (HR = 1.85; 95% CI 1.30 - 2.62; p = 0.0006) (Figure 3b) and endometrioid subtypes (HR = 4.81; 95% CI 2.35 - 9.84; p < 0.00001) (Figure 3c). Loss of PTEN expression was associated with better overall survival (HR = 0.56; 95% CI 0.31 - 1.00; p = 0.05); however, the sample size was too small to evaluate this relationship according to histologic subtype (Figure 4). Loss of INPP4B was strongly correlated with aberrant p53 expression: 61% of INPP4B-negative tumors also had aberrant p53 expression compared to 26% of INPP4B-expressing tumors (Cochran-Mantel-Haenszel test p = 0.003; Table 3).

Univariate and multivariate hazard ratios and 95% CIs for ovarian cancer-specific mortality associated with INPP4B, PTEN, and p53 expression are presented in Table 4. Loss of INPP4B expression was associated with a two-fold increased mortality risk (HR = 1.84; 95% CI 1.27 - 2.68; p = 0.001) in the univariate model; however, after adjusting for age, histology and stage, the relationship was weaker and did not achieve statistical significance (HR = 1.20; 95% CI 0.82 - 1.76; p = 0.36). This effect was limited to the endometrioid subtype (HR = 4.04; 95% CI 0.89 - 18.4) compared to 1.0; 95% CI 0.64 - 1.58 for the serous tumors)(data not shown). In the univariate analysis, both p53 over-expression and complete loss of expression were strongly associated with

**Table 3. — Co-expression of INPP4B and p53.**

<table>
<thead>
<tr>
<th>Protein</th>
<th>INPP4B, n (%)</th>
<th>p53, n (%)</th>
<th>Positive</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>85 (33%)</td>
<td>176 (67%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over-expression</td>
<td>12 (16%)</td>
<td>65 (84%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>18 (8%)</td>
<td>205 (92%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4. — Ovarian cancer-specific mortality associated with p53, PTEN and INPP4B expression.**

<table>
<thead>
<tr>
<th>Protein</th>
<th>INPP4B</th>
<th>p53</th>
<th>PTEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate HR</td>
<td>Multivariate HR</td>
<td>Multivariate HR</td>
</tr>
<tr>
<td></td>
<td>(95%CI) p</td>
<td>(95%CI) p</td>
<td>(95%CI) p</td>
</tr>
<tr>
<td>INPP4B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Negative</td>
<td>1.84 (1.27-2.68) 0.001</td>
<td>1.28 (0.87-1.88) 0.21</td>
<td>1.20 (0.82-1.76) 0.36</td>
</tr>
<tr>
<td>p53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Overexpression</td>
<td>3.33 (2.48-4.47) &lt;0.00001</td>
<td>2.27 (1.65-3.12) &lt;0.00001</td>
<td>1.79 (1.31-2.44) 0.0003</td>
</tr>
<tr>
<td>Absence of p53</td>
<td>2.44 (1.62-3.67) &lt;0.00001</td>
<td>1.63 (1.07-2.49) 0.02</td>
<td>1.56 (1.02-2.38) 0.04</td>
</tr>
<tr>
<td>Aberrant1</td>
<td>3.10 (2.33-4.11) &lt;0.00001</td>
<td>2.10 (1.54-2.85) &lt;0.00001</td>
<td>1.73 (1.28-2.34) 0.0004</td>
</tr>
<tr>
<td>PTEN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Negative</td>
<td>0.56 (0.31-1.00) 0.05</td>
<td>0.80 (0.44-1.44) 0.46</td>
<td>0.75 (0.41-1.37) 0.35</td>
</tr>
</tbody>
</table>

1HR = hazard ratio; CI = confidence interval. 2Multivariate HR adjusted for age at diagnosis (continuous) and histologic subtype (serous, mucinous, endometrioid, clear cell, other). 3Multivariate HR adjusted as in column 2, plus stage (I, II, III, IV). 4Multivariate HR adjusted as in column 3, and additionally for all three proteins (i.e., INPP4B, p53 and PTEN expression). 5Aberrant includes tumors with no p53 expression or those over-expressing p53.
an increased risk of death (HR = 3.33; 95% CI 2.48 - 4.47; \( p < 0.00001 \)) and 2.44; 95% CI 1.62 - 3.67; \( p < 0.00001 \), respectively); these values were reduced but remained significant in multivariate analyses (HR = 1.79; 95% CI 1.31 - 2.44; \( p = 0.0003 \) and HR = 1.56; 95% CI 1.02 - 2.38; \( p = 0.04 \), respectively). This relationship did not vary by histologic subtype (HR = 1.82; 95% CI 0.25 - 2.65 vs. 2.46; 95% CI 1.05 - 5.75 for serous and endometrioid subtypes, respectively)(data not shown). The association between PTEN expression and ovarian cancer-specific mortality present in univariate analysis (HR = 0.56; 95% CI 0.31 - 1.00; \( p = 0.05 \)) became non-significant in multivariate analysis (HR = 0.75; 95% CI 0.41 -1.37; \( p = 0.35 \)).

The relationship between loss of INPP4B or aberrant p53 expression and survival did not vary by stage (data not shown). Survival analysis by stage for PTEN expression was not possible since there were no Stage I/II tumors showing loss of PTEN.

**Discussion**

The goal of the current study was to evaluate whether tumor INPP4B protein expression conveys better or worse prognosis of invasive epithelial ovarian cancer. In this study of 599 women with such disease, aberrant p53 expression was associated with significantly increased ovarian cancer mortality. Loss of INPP4B expression was associated with worse survival for the endometrioid subtype; however, this association did not achieve statistical significance in the multivariate model. In contrast, loss of PTEN was weakly associated with improved survival in the univariate model; however, the number of cases in this category was small (n = 45). Expression of all three proteins varied across histologic subtypes and stage. Loss of INPP4B was common among serous and endometrioid cancers. Aberrant p53 expression was also common among serous tumors, more so than among the other histologic types. In contrast, loss of PTEN was most common among the endometrioid subtype.

INPP4B was originally identified as an enzyme that hydrolyzes the 4-position phosphate of PI(3,4)P2, in vitro [9]. The INPP4B gene resides at 4q31.21, a chromosomal locus frequently disrupted in breast cancer cell lines and basal-like, high-grade breast tumors [10]. Importantly, INPP4B was identified in an RNAi-based genetic screen for genes that suppress transformation of human mammary epithelial cells [11]. Together, these data suggest that INPP4B is a tumor suppressor protein, functioning through its regulation of PI3K signaling [12]. In support of this notion, downregulation of INPP4B in malignant proerythroblasts has been shown to be associated with increased phospho-AKT levels, correctable by re-expression of INPP4B [13]. Allelic losses of INPP4B, loss of INPP4B transcript and loss of INPP4B protein expression have been reported to occur in a majority of BRCA1 mutant [14] and basal-like breast cancer subtypes [8, 14]. Loss of INPP4B expression also correlates with shortened patient survival [14]. Therefore, like PTEN, INPP4B is a candidate tumor suppressor lipid-phosphatase that interferes with the PI3K/AKT pathway, and its substrate, PI(3,4)P2, plays an important role in AKT activation in vivo. As a result, this protein is being investigated as a biomarker and as a potential therapeutic target in breast cancer. There is increasing interest in the prospect that inhibition of the PI3K/AKT signaling pathway represents a potential treatment regimen. Interestingly, the present authors found that most of the tumors from women with a germline BRCA1 or BRCA2 mutation showed loss of INPP4B (as well as aberrant p53 expression).

To our knowledge, only one study (n = 50) to-date has evaluated whether INPP4B expression is associated with prognosis [14]. Gewinner et al., reported that absence of INPP4B detectability by IHC (26%) was associated with significantly reduced overall survival compared to patients with low (12%) or high (62%) expression (\( p < 0.0001 \)). Loss of INPP4B expression was also associated with increased prevalence of lymph node metastases at diagnosis (\( p = 0.04 \)). The authors did not report which histologic subtypes were included. In the present study, a high proportion of the ovarian tumors lacked INPP4B expression (79%), and low expression was associated with poor survival; however, with adjustment for clinical stage, histologic subtype or p53 status, INPP4B was not an independent marker of ovarian cancer prognosis. Similarly, when results were stratified by histologic subtype, the prognostic role of INPP4B was limited to the endometrioid subtype (\( p_{multivariate} = 0.07 \)), although this finding requires confirmation in a larger sample of endometrioid tumors.

**P53** is the most commonly mis-expressed or mutated tumor suppressor gene in human cancers [15]. Normally, p53 promotes cell-cycle arrest and initiation of repair mechanisms or shunting of cells to apoptosis [15]. This gene is also involved in the transcriptional regulation of PTEN and PI3K/AKT which are required for p53-mediated apoptosis [16]. The impact of p53 expression on ovarian cancer survival remains equivocal (reviewed in [17]). In a meta-analysis of 53 studies, aberrant p53 expression was associated with poor survival (pooled HR = 1.47; 95% CI 1.33 - 1.64) with significant heterogeneity between the studies and by histologic subtype [17].

Between 30-80% of high-grade invasive ovarian carcinomas carry P53 mutations [5, 18, 19], although rates as high as 98% have also been reported [20]. p53 mutations result in aberrant protein expression: either complete loss of expression or over-expression and is a common feature of high-grade serous carcinomas (compared with borderline serous, clear cell and endometrioid cancers) [21]. In the present study, aberrant p53 expression was more common in the serous subtype than in the others (69% of serous vs. 9% clear cell tumors and 30% of endometrioid). We found that both complete loss of expression and over-expression of p53 were associated with increased ovarian-cancer specific mortality (HR for aberrant p53 expression = 1.73; 95% CI 1.28 - 2.34). Further, the present data suggest that loss of INPP4B is
strongly correlated with aberrant p53 expression: 61% of INPP4B-negative tumors also had aberrant p53 expression compared to 26% of INPP4B-expressing tumors with aberrant p53 expression.

After p53, PTEN is the second most frequently mutated tumor suppressor gene in human cancers [22]. PTEN mutations occur in a wide range of tumor types [23]. PTEN is a lipid phosphatase that interferes with the PI3K pathway by dephosphorylating the 3-phosphate on PI(3,4,5)P3 to generate PI(4,5)P2 [22]. Thus, PTEN functions as a tumor suppressor through its ability to turn off the PI3K pathway. PTEN is frequently mutated in endometrioid cancers but not in other ovarian cancer subtypes [5, 24]. In the present study, only 8% of ovarian tumors showed loss of PTEN expression, though loss was more common in endometrioid subtypes (17%) in accordance with the published literature [5, 24]. Endometriosis or retrograde menstruation implants on the ovary or transformation of ovarian surface epithelium to endometrioid-like cells can lead to the development of clear cell or endometrioid tumors, some of which characterized by PTEN or MYC mutations, leading to loss of or over-expression of these proteins [25, 26]. PTEN mutations are commonly observed in normal epithelium of the endometrium as well as in endometriosis and in endometrioid adenocarcinomas, suggesting that loss of PTEN expression may be a step in the progression from endometriosis to cancer [27-31].

Results of studies evaluating the influence of PTEN expression on ovarian cancer recurrence have been mixed. Several studies reported no relationship between loss of PTEN and survival [32-35] whereas two observed that reduced PTEN expression was associated with shortened relapse-free interval [36] or decreased disease-free survival [37]. These analyses have been limited by somewhat small sample sizes (range 20-232), by the inclusion of only a subset of histologic subtypes or of only early- or late-stage tumors, along with large variability in proportions of ovarian tumors staining negative for PTEN [33, 35-39]. Results from the largest study (n = 232) showed that lack of PTEN staining was associated with earlier stage disease, non-serous subtypes, and improved progression-free survival but not overall survival [38]. We found no significant relationship between PTEN expression and risk of ovarian cancer-specific mortality. Improved endometrial cancer survival has been seen with loss of PTEN [40], perhaps due to enhanced sensitivity to chemotherapy. Collectively, the data support a possible role for PTEN in the development of endometroid tumors, and possibly clear cell tumors as well [24, 38].

To our knowledge, this is the largest study to-date that has evaluated the prognostic role of INPP4B expression for ovarian cancer. A major strength of this study is the large sample size, allowing stratified analyses by histologic subtype. Moreover, the use of TMA allowed for simultaneous staining of many tumor samples. However, possible weaknesses of this analysis include the resultant smaller sample size despite the large number of women initially eligible for inclusion in the TMA. In addition, we cannot exclude a possible effect of neoadjuvant chemotherapy on protein expression; however, we did not have access to this information.

Conclusion
In summary, we observed here that aberrant p53 expression is a frequent event in serous ovarian cancer and that it is associated with relatively poorer survival. Although not an independent marker of prognosis, loss of PTEN was more often found in endometrioid than in serous tumors. They have also shown that loss of INPP4B expression may be a prognostic marker for ovarian cancer, in particular for those of the endometrioid subtype. This data support a possible role of multiple pathways of development for the various ovarian cancer subtypes. Integrating tailored treatment options based on tumor protein expression may ultimately lead to improved outcomes following the diagnosis of ovarian cancer.

Acknowledgements
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mutations in endometrioid but not serous or mucinous epithelial


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Correlation between transcription factor activator protein-2β (TFAP-2β) and endometrial carcinoma

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Summary

Aims: To investigate the correlation between transcription factor activator protein-2β (TFAP-2β) and endometrial carcinoma (EC).

Materials and Methods: The study comprised 60 randomly selected patients diagnosed and treated at the 2nd Affiliated Hospital of Harbin Medical University from November 2011 to June 2012 for endometrial carcinoma (n = 30) and myoma of uterus (n = 30). The expression of TFAP-2β mRNA in endometrial carcinoma was analyzed by real-time reverse transcription polymerase chain reaction (RT-PCR). Body mass index (BMI), waist circumference, and venous blood samples were obtained before abdominal surgery clinically.

Results: The expression of TFAP-2β mRNA in endometrial tissue of patients with EC was higher than that of normal endometrium (p < 0.05). The expression of TFAP-2β mRNA in endometrial tissue of patients with metabolism syndrome was higher than that of lean ones (p < 0.05). There was no significant difference in the expression of TFAP-2β mRNA in endometrial tissue between patients with both EC and metabolism syndrome and in those with EC only. The expression levels of TFAP-2β mRNA had positive correlation with triglyceride (r = 0.271, p < 0.05) and high-density lipoprotein (HDL) (r = 0.314, p < 0.05). There was no significant correlation between the expression of TFAP-2β mRNA and CA125, fasting plasma glucose, low-density lipoprotein (LDL), waist circumference, total cholesterol, and BMI.

Conclusions: TFAP-2β constituted promoter activity in EC and also contributed to the development of the metabolic syndrome. TFAP-2β may influence the occurrence and development of EC through regulating the expression of various adipokines and lipoprotein metabolism. Probably TFAP-2β can be a candidate tumor marker for EC.

Key words: Adipokines; Endometrial carcinoma; Metabolism syndrome; Real-time RT-PCR; TFAP-2β.

Introduction

Endometrial carcinoma (EC) is one of the three female reproductive tract malignant tumors. Its incidence has been accounted for gynecological malignant tumor first and the second most common cause of gynecologic cancer death in Europe and the United States. The incidence of EC is lower in developing countries, but the ratio of mortality to incidence is higher [1]. In recent years, economic development rapidly of many developing countries, including China has led to the increase of its incidence. People’s living habits and diet structure has been westernized, hence the incidence of obesity has also increased. Furthermore, the incidence of EC has obviously risen by informal hormone replacement therapy and other factors, including obesity, diabetes, hypertension, infertility, and menopause delay.

Obesity is associated with multiple diseases, such as breast cancer, ovarian cancer, polycystic ovary syndrome and EC [2]. Obesity plays important role in EC, but the mechanisms of action remain unclear. Adipose tissue secretes leptin and adiponectin that reportedly participates in carcinogenic processes, such as cell proliferation, angiogenesis, and insulin regulation. Metabolic syndrome, a combination of medical disorders included obesity and diabetes et al., and is associated with increased endometrial cancer risk [3].

As a nuclear transcription factor, transcription factor activator protein-2 (TFAP-2) control target gene expression through closing, opening, increasing or decreasing signal transduction. It is also involved in vertebrate growth regulation, apoptosis, the occurrence and development of tumor in pathological conditions [4,5]. TFAP-2 was first purified and cloned from HeLa cells in 1987 [6]. TFAP-2 affects bidirectional regulation in tumor genesis and evolution through the regulation of tumor associated gene expression. TFAP-2 family members include TFAP-2α, TFAP-2β, TFAP-2γ, TFAP-2δ, and TFAP-2ε. TFAP2α can improve the patients with advanced bladder cancer sensitivity to cisplatin [7]. TFAP-2γ has been reported to have an effect on different target genes from Her2 breast cancer phenotype to regulate the hormone response breast cancer [8]. TFAP-2β has been associated to metabolic syndrome through playing an important role in the regulation of adipokines expression in vivo [9]. TFAP-2β is also known as a molecular marker to detect tumor and a therapeutic target for anticancer therapy [10]. The purpose of current study was to assess the association between TFAP-2β and endometrial carcinoma.
The authors hypothesized that TFAP-2β may have a high correlation with endometrial carcinoma.

**Materials and Methods**

**Subjects**

The present study including 60 patients (of the 60 patients, 30 were diagnosed and treated for EC and 30 were diagnosed and treated for myoma of uterus) were randomly selected at the 2nd Affiliated Hospital of Harbin Medical University from November 2011 to June 2012. The patients fulfilled the following inclusion criteria: (I) scheduled for surgical treatment, (II) all patients were not receiving any chemotherapy, radiotherapy, and prior targeted tumor treatment, and (III) free of severe acute illness. The endometrial tissues of patients with myoma of uterus were tested histopathologically as normal endometrium.

Body mass index (BMI) and abdominal circumference were measured in each subject. Venous blood samples were obtained before abdominal surgery. Abdominal obesity was determined by the definition criteria of the metabolic syndrome proposed by the International Diabetes Federation. *p < 0.05.

**Endometrial tissue**

Endometrial tissue biopsies were obtained during panhysterectomy, which was either laparoscopic or open-abdominal surgery. Each specimen was divided into several sections and all were immediately frozen and stored in liquid nitrogen.

**Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR)**

Total cellular RNA was extracted from the frozen endometrial tissues using Trizol reagent and 1,000 ng of total RNA was reverse transcribed with a reagent kit. The primers for TFAP-2β were commercially designed and synthesized. The specific primers used of TFAP-2β were: 5’-TAAAGCGGGGAGATGGGATG-3’ and 5’-GGAGAAGTGAG-GGAGGGAGAA-3’. The β-Actin mRNA was quantified for sample normalization. The thermal profile of reverse transcription consisted of 15 minutes at 37°C, five seconds at 85°C. The thermal profile of RT-PCR consisted of 30 seconds at 95°C, followed by 40 cycles of five seconds at 95°C, 30 seconds at 58°C, and 30 seconds at 72°C for human TFAP-2β. 2-ΔΔCT method to compare the relative expression of TFAP-2β mRNA was used. All PCRs were performed in duplicate and repeated twice.

**Statistical analysis**

Data were expressed as mean ± standard deviation. The Mann-Whitney Rank Sum Test was used for comparison of mean values. Pearson’s correlation coefficient was used to determine the relationship between TFAP-2β gene expression and other quantitative variables. A p value of < 0.05 was considered statistically significant. Analyses were conducted using the SigmaPlot 11.0 system.

**Results**

Previous studies have documented that certain adipokines have been involved in tumor growth, apoptosis, and cachexia [12, 13]. In order to minimize these influences, the patients with EC who were free of acute illness or cachexia were chosen. The subject characteristics are summarized in Tables 1 and 2.

The metabolic syndrome group had a statistically higher mean age (p = 0.042), BMI (p = 0.003), waist circumference (p < 0.001), triglycerides (p = 0.001), and fasting plasma glucose (p < 0.001) level than the lean group. However, there were no statistically significant differences between the mean values of the lean cases and metabolism syndrome cases in terms of the total cholesterol and low-density lipoprotein (LDL).

There were no statistically significant differences between the mean values of the normal endometrium cases and EC cases in terms of the age, BMI, waist circumference, triglycerides, total cholesterol, LDL, and high-density lipoprotein (HDL). However, the EC group had a statistically higher mean of fasting plasma glucose (p = 0.015) and CA125 (p = 0.003) level than the normal endometrium group.

**Expression of TFAP-2β mRNA in endometrial tissue**

To date, many studies had examined the expression of AP-2 in human different tumors such as breast cancer, malignant melanoma, cervical cancer, and ovarian cancer [14,
Correlation between transcription factor activator protein-2β (TFAP-2β) and endometrial carcinoma

15], but their opinions are conflicting; it seems AP-2 plays a two-way regulatory role in tumorigenesis. The present study examined the role of TFAP-2β mRNA expression in EC for the first time. To test the role of TFAP-2β in EC and metabolic syndrome, the expression of TFAP-2β mRNA in endometrial tissue was examined by relative quantitative real-time PCR technology. The expression of TFAP-2β mRNA in endometrial tissue of patients with EC was higher than that of patients with normal endometrium ($p < 0.05$, Figure 1). This result demonstrates that TFAP-2β constituted promoter activity in EC. The expression of TFAP-2β mRNA in endometrial tissue of patients with metabolic syndrome is higher than that of lean ones ($p < 0.05$, Figure 2). There was no significant difference in the expression of TFAP-2β mRNA in endometrial tissue between patients with both EC and metabolic syndrome and that with EC only (Figure 3).

Figure 1. — Expression of TFAP-2β mRNA in endometrial tissue between EC and normal endometrium ($p < 0.05$).

Figure 2. — Expression of TFAP-2β mRNA in endometrial tissue between metabolic syndrome and lean patients ($p < 0.05$).

Figure 3. — Expression of TFAP-2β mRNA in endometrial tissue between the patients with both EC and metabolic syndrome and those with only EC ($p = 0.155$).

Figure 4. — Relationship between TFAP-2β mRNA expression levels in endometrial tissues and triglyceride levels and HDL levels.
Correlation between the TFAP-2β mRNA levels and clinical data

The authors next investigated the correlation between expression of TFAP-2β mRNA in endometrial tissue and other clinical datas. TFAP-2β mRNA levels in endometrial tissue correlated positively with triglycerides ($r = 0.271$, $p < 0.05$, Figure 4a) and HDL ($r = 0.314$, $p < 0.05$, Figure 4b). CA125 had been recognized as a tumor maker and it has been associated with poor prognosis of EC [16]. It has also been verified that the EC group had a statistically higher mean CA125 ($p = 0.003$) level than the normal endometrium group in this study. Nevertheless, no significant correlation was observed between the expression of TFAP-2β mRNA and CA125 (Figure 5), fasting plasma glucose, LDL, waist circumference, total cholesterol, and BMI (Figure 6) in this experiment.

Discussion

EC is a hormone-dependent neoplasm and obesity is a well-known risk factor for it. Metabolism syndrome is used to evaluate risk factors of EC. TFAP-2β has been reported to be associated with several obesity related phenotypes [17]. The present results showed that the expression of TFAP-2β mRNA in endometrial tissue of patients with EC was higher than that of patients with normal endometrium. It indicated that TFAP-2β might be involved in the development of EC. The expression of TFAP-2β mRNA in endometrial tissue of the patients with metabolic syndrome is higher than that of lean ones was also observed in the present experiment. TFAP-2β had a high correlation with metabolic syndrome as well. In order to eliminate the effect of metabolic syndrome that may lead to high-expression of TFAP-2β mRNA of the patients with EC, the expression of TFAP-2β mRNA in endometrial tissue was contrasted between the patients with both EC and metabolic syndrome and those with EC only. The results showed no significant difference between them. It clarified that high-expression of TFAP-2β mRNA of the patients with EC was not simply...
due to a metabolic syndrome. It demonstrated that TFAP-2β may not only play an advanced role in EC but may also contribute to the development of the metabolic syndrome.

Systemic metabolic derangement is closely related to fat distribution [17]. Numerous studies have shown the adipokines level in endometrial carcinoma, especially leptin and adiponectin. Leptin was involved in proliferative processes of the endometrium and positively associated with EC [18, 19]. Low serum levels of adiponectin were independently associated with EC [20, 21]. Ugi et al. comprised 81 individuals and then suggested that TFAP-2β played a major role in the regulation of various adipokines and TFAP-2β correlated negatively with adiponectin and leptin [9]. Therefore, low serum levels of adiponectin should accompany high-expression of TFAP-2β. It corresponded to the present results that high-expression of TFAP-2β promoted tumorigenesis of EC. It can be reasonably inferred that TFAP-2β may effect the development of EC through regulating the expression of various adipokines such as leptin and adiponectin.

Detailed analysis of lipid components showed that there is a consistent relation between TG levels and EC risk [22]. Meanwhile, the metabolic pathway and its relation to EC risk are still unclear. The present study showed that the TFAP-2β mRNA levels in endometrial tissue correlated positively with serum triglycerides. This result indicated that TFAP-2β might lead to elevated triglycerides. The present results also showed that TFAP-2β mRNA levels in endometrial tissue correlated positively with HDL, which no study has yet reported. It indicates that TFAP-2β may regulate the lipoprotein metabolism, such as triglycerides and HDL. Therefore, TFAP-2β may influence the tumorigenesis of EC through regulating the expression of triglycerides and HDL.

No reliable tumor marker has been approved for EC. Serum CA125 was introduced as a circulating antigen in epithelial ovarian cancer first and it has been clinically widely used in EC [23]. The key tumor markers of EC are remaining debated. The value of CA125 in EC has been investigated in many studies; however, results have been conflicting. Sood et al. [16] have shown that preoperatively elevated CA125 would contribute to poor survival and several authors have also found that higher serum CA-125 levels correlated with stage, histopathology, and LN metastasis factors [24, 25]. Other reports have shown that CA125 levels and development of disease are not correlated [26]. Therefore, CA125 has been considered as a predictor of worse prognosis but not a reliable tumor marker for endometrial carcinoma. Our study has found that the endometrial carcinoma group had a statistically higher mean CA125 (p=0.003) level than the normal endometrium group as Sood et al. showed. Meanwhile, our result also showed that CA125 was not related to TFAP-2β mRNA expression in EC. TFAP-2β may predict the occurrence of endometrial carcinoma.

In conclusion, this study demonstrated that TFAP-2β constituted promoter activity in EC and also contributed to the development of the metabolic syndrome. TFAP-2β may influence the occurrence and development of EC through regulating the expression of various adipokines and lipoprotein metabolism. Most likely TFAP-2β can be a candidate tumor marker for EC. Future research work will focus on upstream regulation gene of induced HDL and triglycerides to study biologic effect of TFAP-2β on endometrial carcinogenesis.

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Evaluation of residual tumor locations in advanced ovarian cancer patients after incomplete primary cytoreduction

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4 Department of Perinatology and Gynecology, Poznan University of Medical Sciences, Poznan (Poland)

Summary

Background: Nowadays complete primary cytoreduction can be achieved in a large number of patients suffering from advanced ovarian cancer. However, there is a group of patients in whom complete tumor resection remains impossible. The authors analyzed the intraoperative limiting factors in patients with residual tumor after primary surgery treated in the present institution. Materials and Methods: Patients with advanced epithelial ovarian cancer (FIGO Stage IIIB-IV), who underwent primary incomplete surgery in the present institution between 2006 and 2008 were included in this study. Patients’ records were evaluated regarding to intraoperative findings and final surgical results. Results: The authors identified 39 eligible patients in their registry. Twenty-six (66.7%) patients underwent surgery with residual tumor < 1 cm and 13 (33.3%) ≥ 1 cm. The most frequent location of residual tumor limiting complete surgery was disseminated bowel carcinomatosis in 34 (87.2%) patients. Moreover significant differences in tumor residuals locations and operative time between patients with residuals < 1 cm and ≥ 1 cm were reported (p < 0.05). Conclusions: The most frequent reason for incomplete primary cytoreduction remains disseminated carcinomatosis. However, in patients with residuals under one cm, its frequency is significantly higher. The complication rate is comparable in patients independently of residual tumor < 1 cm and ≥ 1 cm. Therefore the cytoreductive efforts should be made even in primarily not completely operated patients in order to achieve residuals under one cm.

Key words: Primary ovarian cancer; Advanced ovarian cancer; Incomplete cytoreduction.
Gynecological examination, vaginal ultrasound, and additional radiological imaging were standard diagnostic procedures prior to planned surgery. The patients’ individual records were reviewed and the following information was abstracted: age at the time of surgery, operative findings, residual disease at the completion of the procedure, blood loss, final pathologic diagnosis, and postoperative complications. The primary surgeon in all cases was a gynecologist oncologist.

End point of the present study was the evaluation the residual disease locations after primary cytoreduction in advanced epithelial ovarian cancer patients. All other statistical analyses were performed using SPSS 18.0.

### Results

Ninety-six patients treated at the Department of Oncology, Division of Gynecology, Poznan University of Medical Sciences were considered for inclusion in the present study. Twenty-six early ovarian cancer cases FIGO Stage I-IIIA were directly excluded. Among 70 patients with advanced ovarian cancer FIGO Stage IIIB-IV, five women received neo-adjuvant chemotherapy initiated by an outside institution and therefore were excluded. Complete primary cytoreduction could be achieved in 26 (37%) of those patients inclusive all FIGO Stage IIIB patients. Finally, 39 patients (aged 39 - 86, median 65 years) met the aforementioned criteria and were enrolled in the study. Twenty-six (67%) of studied patients underwent the surgery with residuals < 1 cm and 13 (33%) ≥ 1 cm in diameter.

#### Table 1. — Perioperative findings in studied patients.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Number</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIC</td>
<td>37</td>
<td>94.9</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>Histological type</td>
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<tr>
<td>Serous</td>
<td>36</td>
<td>92.3</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>Clear-cell</td>
<td>1</td>
<td>2.6</td>
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<tr>
<td>Grading</td>
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<td></td>
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<tr>
<td>G1</td>
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</tr>
<tr>
<td>G2</td>
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</tr>
<tr>
<td>G3</td>
<td>23</td>
<td>59.0</td>
</tr>
<tr>
<td>n/a</td>
<td>2</td>
<td>5.1</td>
</tr>
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</table>

#### Table 2. — Outcome of surgery performed in this center.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Number</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 cm in diameter</td>
<td>26</td>
<td>67</td>
</tr>
<tr>
<td>≥ 1 cm in diameter</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>Location of residual tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestines/mesentery</td>
<td>34</td>
<td>87.2</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>20</td>
<td>51.3</td>
</tr>
<tr>
<td>para-aortic</td>
<td>14</td>
<td>35.9</td>
</tr>
<tr>
<td>pelvis</td>
<td>6</td>
<td>15.4</td>
</tr>
<tr>
<td>Liver (capsule, hilus)</td>
<td>10</td>
<td>25.6</td>
</tr>
<tr>
<td>Spleen (capsule, hilus)</td>
<td>8</td>
<td>20.5</td>
</tr>
<tr>
<td>Others (stomach, pancreas)</td>
<td>6</td>
<td>15.4</td>
</tr>
</tbody>
</table>

#### Table 3. — Outcome of surgery with regards to postoperative tumor residuals.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Residuals &lt; 1 cm (%)</th>
<th>Residuals ≥ 1 cm (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of residual tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestines/mesentery</td>
<td>24 (92.0 %)</td>
<td>10 (77.0 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>9 (34.6 %)</td>
<td>11 (84.6 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>para-aortal</td>
<td>7 (26.9 %)</td>
<td>7 (53.8 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>pelvis</td>
<td>2 (7.7 %)</td>
<td>4 (30.8 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>4 (15.4 %)</td>
<td>6 (46.1 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Spleen</td>
<td>3 (11.5 %)</td>
<td>5 (38.5 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Others (stomach, pancreas)</td>
<td>3 (11.5 %)</td>
<td>3 (23.1 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Complications</td>
<td>3 (11.5 %)</td>
<td>1 (7.7 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Postoperative bleeding</td>
<td>1 (3.8 %)</td>
<td>0 (0.0 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Re-laparotomy</td>
<td>1 (3.8 %)</td>
<td>0 (0.0 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Infection</td>
<td>1 (3.8 %)</td>
<td>1 (7.7 %)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Median blood loss (range) in ml</td>
<td>182 (100 – 300)</td>
<td>93 (20 – 200)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Median duration of surgery (range) in minutes</td>
<td>110 (90 – 165)</td>
<td>70 (35 – 120)</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Thirty-four (87.2%) patients were in good performance status Eastern Cooperative Oncology Group (ECOG) 0 or 1 when five (12.8%) women were classified with ECOG 2.

The majority of patients (37 women) had FIGO Stage IIIC ovarian cancer and two women were classified as FIGO Stage IV due to malignant pleural effusion. However in both cases, no radiologic signs of pleural carcinomatosis were described (Table 1). Thirty-six (92.3%) patients had serous pathology, followed by mucinous – two (5.1%) and clear cell – one (2.6%). Grade was determined in 37 (95%) patients; among these, four (10.3%) had G1, ten (25.6%) had G2, and 23 (51.3%) had G3 (Table 1).

The most frequent localization of residual disease was bowel, followed by peritoneum, and para-aortic lymph nodes. Detailed localizations of residual tumor are presented in Table 2. Furthermore, the authors analyzed the residual tumor localization according to residual disease < 1 cm versus ≥ 1 cm. According to this subanalysis, they found differences in residual tumor localizations between both groups (Table 2). Although intestines remained the most frequent localization, in patients with residuals < 1 cm its frequency was significantly higher (p < 0.05). Involvement of para-aortic lymph nodes, liver and stomach was significantly higher in patients with residuals ≥ 1 cm (p < 0.05).

In five (12.8%) cases, patients’ performance status limited complete surgery. Two (7.7%) and three (23.1%) cases in < 1 cm and ≥ 1 cm groups, respectively.

Statistically significant differences were identified between surgeries with regards to operative time. In patients with postoperative residuals ≥ 1 cm, surgery was significantly shorter in comparison to women with residuals < 1 cm (p < 0.05) (Table 3). Major postoperative complications
occurred in four (10.3%) patients (Table 3). The authors observed significantly higher incidence of postoperative complications in cases with residual tumor < 1 cm versus ≥ 1 cm, respectively, three (11.5%) and one (7.7%) (p < 0.05). No significant difference in hospital stay was reported. Patients with residuals under one cm required 8.1 (range 6 – 12) days hospitalization and patients with residuals > one cm, 7.3 (range 5-10) days.

Discussion

Postoperative tumor-mass remains the most important prognostic factor in ovarian cancer patients. The current data shows that patients treated in specialized gynecological oncology departments more frequently receive treatment according to guidelines [11]. Furthermore, recent studies showed significantly higher incidence rate of residual tumor in non-specialized departments, which could have been removed during reoperation in gynecological oncology centers [16, 17]. Nevertheless, in substantial number of patients, complete cytoreduction remains not possible, even in specialized units.

At the time of the present study, primary cytoreduction with residuals under one cm was an established optimal operative treatment in advanced ovarian cancer cases. Even thought complete tumor resection constituted substantial number of operated cases [6, 8, 18]. Altogether, the optimal surgery in ovarian cancer patients reaches 80% [18]. Results from the present center correspond with international data in regard of the surgery completeness [6, 8, 18].

In the present study the authors analyzed only not pre-treated patients with advanced ovarian cancer. Thirty-nine patients had residual tumor after primary surgery and among those, majority (74%) with residuals under one cm. Analogically to available data, a disseminated bowel and/or mesenteric carcinomatosis was the most frequent limiting factor for complete cytoreduction in studied patients [18]. Aletti et al. [18] reported that patients’ performance status, carcinomatosis, and surgeon are independent factors associated with optimal residual disease. In the present study, five patients due to performance status could not be completely operated. Furthermore, bowel involvement was the most frequent localization of residual tumor, as well as the main reason for incomplete tumor resection indicated by the surgeons.

Subsequently, the authors performed a sub-analysis to evaluate the localizations of residuals regarding to postoperative size of the rest tumor, accordingly below and over one cm in diameter. Significant differences in frequency of residual tumor localization were observed. In the group of patients with residuals < 1 cm, a disseminated intestinal/mesentry tumor spread was significantly higher. Other localizations were significantly more frequent in the group with residuals ≥ 1 cm. Similarly, the operative times were significantly different. The authors presume that the differences result from intraoperative tumor spread and therefore estimated operability. Patients were classified as inoperable according to the assessment of abdominal cavity more quickly in those with residuals over one cm. Respectively in subgroup with residuals up to one cm in diameter, the main tumor mass appeared resectable and therefore the surgical efforts was reasonable. It is in accordance to patients’ surgical documentation. Therefore, surgeon tendency to employ radical procedures differ with regards to intraoperative findings. Accordingly, the duration of surgery differed between both subgroups. Fotopoulou et al. [19] introduced in 2010 an intraoperative Mapping of Ovarian Cancer (IMO) based on evaluation of quadrants involved with carcinomatosis in order to evaluate the predictability of tumor resection. Tumor dissemination pattern and maximal tumor load were significantly different between complete and incomplete operated patients, with less extrapelvic tumor involvement in the first group. Tumor spread involving multiple abdominal quadrants was the major negative predictor for complete tumor resection in primary ovarian cancer patients. The most frequent residual tumor localization reported by Fotopoulou et al. was bowel/mesentry. This observation complies with postoperative findings in the present patients.

The present authors observed postoperative complications in 10.2% of patients, which is comparable with other authors [19 - 22]. Moreover, no difference in complication rate between patients with residuals < 1 cm and ≥ 1 cm were noticed. Although blood loss was significantly higher in patients that underwent optimal cytoreduction, median value of 182 ml remained acceptable for this kind of operation.

Although the frequency of complete cytoreduction in advanced ovarian cancer increases (10, 11), certain tumor localizations remain inoperable even by gynecologists oncologists. As a consequence, the prognosis of those patients is significantly worse. The exact assessment of abdominal cavity in specialized center results in a cytoreduction with residuals < 1 cm in majority of cases. This approach combined with acceptable complication rate has a positive impact on prognosis. Nevertheless, further investigation in a larger group of patients is needed.

References


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A comparative study of intensity-modulated radiotherapy and standard radiation field with concurrent chemotherapy for local advanced cervical cancer

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Summary
Objective: This study aimed to compare three-dimensional conformal radiotherapy (3D CRT) and intensity-modulated radiotherapy (IMRT) combined with concurrent chemotherapy for cervical cancer. Materials and Methods: A total of 72 patients with Grades IIa–IIb cervical cancer were randomly divided into two groups, namely, the IMRT group for IMRT plan (primary lesion, 45 Gy/22; the pelvic wall lymphatic drainage area, 50 Gy/22), and the 3D CRT group (conformal pelvic radiotherapy, 45 Gy/22; subsequent supplement of pelvic wall, 6.0 Gy/3). Both groups received concurrent chemotherapy of nedaplatin 30 mg/m² weekly for six cycles, with an after-loading therapy of 6 Gy/6 each time. Results: In the IMRT group, the grade III diarrhea rate was 5.6% and the rate in the 3D CRT group was 30.6%; both groups significantly differed. No significant difference was observed between the overall survival and disease-free survival in first, second, and third years in both groups. Conclusion: Cervical cancer IMRT can significantly reduce the incidence of acute enteritis. For standard 3D CRT, no significant difference was observed in overall survival and disease-free survival.

Key words: Cervical cancer; Three-dimensional conformal radiotherapy; Intensity-modulated radiotherapy; Efficacy.

Introduction
With the advancements in computer technology, three-dimensional conformal radiotherapy (3D CRT) and intensity-modulated radiotherapy (IMRT) are gaining increased attention because of their dosimetry advantages. Regarding such advantage found by Portelance et al. [1] is the reduced prescription dose (45 Gy) in the intestine. Heron et al. [2] also believed that IMRT can reduce >30 Gy doses by 52% in the intestine, by 66% in the rectum, and by 36% in the bladder. D’Souza et al. [3] found as well that IMRT can reduce the intestinal dose by 33%. By a meta-analysis involving 4,580 cases and 19 trials of IMRT with concurrent chemotherapy, Green et al. [4] found improvements in the tumor control rate and overall survival. However, higher incidences of blood and gastrointestinal toxicity occur in patients with concurrent chemoradiotherapy. Beriwal et al. [5] reported early results of intensity-modulated and concurrent cisplatin therapy but made no comparison with conventional treatment. Folkert et al. [6] used intensity-modulated technology in postoperative patients, and found that the three- and five-year disease-free survival rates were 91.2% and 91.1%, respectively. IMRT has also been found to have certain dosimetry advantages in cervical cancer radiotherapy, with a number of good results observed in postoperative patients. However, based on the positive results of conformal pelvic radiotherapy in previously untreated cervical cancer patients, uncertainties remain on whether intensity-modulated technology can replace traditional treatment for these patients. Using IMRT and concurrent platinum-based chemotherapy may reduce acute radiation reaction. Thus, tolerance to treatment must be promoted, which requires further investigation.

Materials and Methods
In this study, 72 patients were randomly divided into two groups (36 patients each) by the envelope method.

General information
From September 2006 to September 2009, 72 patients with clear pathological diagnosis of Grades IIa–IIb cervical cancer that met the following criteria were included in the randomized control study: 1) the patient signed and agreed to participate in the study by signing an informed consent form; 2) the patient was 18 to 70 years old; 3) the pathological diagnosis was squamous cell carcinoma, and the clinical stage was within Grades IIa–IIIb; 4) the patient was to undergo treatment for the first time and had no history of cancer or chemoradiotherapy; and 5) the patient had ≥110 g/L hemoglobin, ≥3.5 × 10⁹/L WBC, ≥100 × 10⁹/L platelets, <1.25 times the normal upper limit of liver and kidney function, and normal blood sugar. Based on the FIGO staging criteria, the patients were randomly divided into two groups by the envelope method. All procedures were approved by the hospital ethics committee. This study was conducted in
accordance with the declaration of Helsinki and with approval from the Ethics Committee of Huai’an First People’s Hospital, Nanjing Medical University. Written informed consent was also obtained from all participants.

**Posture**
During therapy, the patient was supine with hands clasped and elbows and legs naturally closed.

**Computed tomography (CT) scan**
A SensationOpen was used for CT simulation. The bladder was emptied 90 minutes before CT scan, and then filled with meglumine diatrizoate injection in order to displace the intestine during scanning. Diatrizoate was taken before the scan. The scan was started after an injection of iohexol, and the range of sweeping surface was from L1 to five cm under the ischial tuberosity (five mm thickness).

**Treatment planning**
The clinical target volume (CTV) contained the primary tumors of the uterus, cervix, vagina, and other regions such as below the lymphatic drainage area (L4, iliac, internal iliac, external iliac, obturator, pararectal, paracervical, and presacral lymph nodes from L5 to L3). The CTV did not include pelvic tissue. Based on the actual situation, the planning target volume (PTV) was formed by the CTV boundary out-expansion of 1.0 cm. The sensitive tissues including the rectum, bladder, small intestine, and femoral head were sketched. After target delineation CMS radiation treatment planning system, the authors designed IMRT plans for the patients based on the reverse design principle. Seven field irradiations and six MV X-ray were used as follows: 1) 50 Gy for the pelvic wall lymph drainage (excluding the presacral lymphatic drainage area); 2) 45 Gy/22 for the uterus, cervix, vagina, and other primary tumor areas; 3) the dose gradient PTV was ≤ 10%; and 4) 40 Gy irradiated volume \( V_{40} \) of rectum and bladder were less than 40%, and \( V_{40} \) of intestine was < 30%. The priorities for PTV were the rectum, bladder, small intestine, femoral head, and spinal cord. To the patients, a conformal conformal pelvic radiotherapy of 45 Gy/22F was given, and then, another 6.0 Gy/3 F was subsequently supplied by the radiation field to the center while sheltering the pelvic wall. An oncor linear accelerator was used.

**Chemotherapy and detection**
Two groups were treated with nedaplatin 30 mg/m² weekly for six cycles. During treatment, routine blood analysis was conducted twice a week to monitor the liver and kidney function, once a week for blood biochemistry, and once a month for ECG (monthly review). If needed, the granulocyte colony-stimulating factor was used for symptomatic treatment.

**Toxicity evaluation**
Acute toxicity was evaluated by the Common Terminology Criteria for Adverse Events v3.0 [7].

### Table 1. — Clinical data.

<table>
<thead>
<tr>
<th>Group</th>
<th>Conventional (n=36)</th>
<th>Intensity-modulated (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>24-73</td>
<td>22-75</td>
</tr>
<tr>
<td>Median</td>
<td>56</td>
<td>57</td>
</tr>
<tr>
<td>Pathological type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>35</td>
<td>34</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
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<td>2</td>
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<tr>
<td>PS grade</td>
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<td></td>
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<tr>
<td>0-1</td>
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<td>36</td>
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<tr>
<td>FIGO staging</td>
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<td></td>
</tr>
<tr>
<td>IIA</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>IIb</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>IIIa</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>IIIb</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 2. — Adverse reactions.

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Control group (n=36)</th>
<th>Study group (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea, vomiting</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hemoglobin deduction</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Neutrophils deduction</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Platelet deduction</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

### Statistical analysis
The primary endpoint for the treatment was toxic reaction. The secondary endpoint was the overall survival rate and tumor-free survival rate. SPSS17.0 statistical software was used, and the Kaplan–Meier method and line Log-rank test were used for survival analysis. Count data were analyzed by the \( \chi^2 \) test, and \( p < 0.05 \) was considered significant.

### Results
Follow-up observations were conducted every three months in the first year, every six months in the second year, and then once a year starting from the third year. The follow-up rate was 100%. The patients’ characteristics were similar (Table 1).

### Toxicity
Nausea, vomiting, hemoglobin, and neutrophils decreased in the two groups, but were not significantly different. In the grades I and II IMRT group, the diarrhea rate was 55.6% (20/36); in the grade III and above group, the rate was 5.6% (2/36). In the grades I and II 3D CRT group, the diarrhea rate was 69.4% (25/36); in the grade III and above group, the rate was 30.6% (11/36). For grades III and above, significant differences were observed between the two groups (Table 2).

### Survival
In the IMRT group, the one-, two-, and three-year overall survival rates were 94.4%, 86.1%, and 77.8%, respectively.
The one-, two-, and three-year disease-free survival rates were 91.7%, 75.0%, and 72.2%, respectively. In the 3D CRT group, the one-, two-, and three-year overall survival rates were 91.7%, 86.1%, and 75.0%, respectively. The one-, two- and three-year disease-free survival rates were 91.7%, 72.2%, and 69.4%, respectively, and no significant difference was observed (Figures 1 and 2).

Discussion

Concurrent platinum-containing chemoradiotherapy is opted for patients with Grade IIb and above cervical cancer [8]. In this study, 70.8% of the patients met this standard. Traditional external radiation is a combination of conformal pelvic radiotherapy and intracavitary radiotherapy. However, traditional technology affects large amounts of normal tissue, especially sensitive ones such as intestinal, rectal, and bladder tissues. IMRT optimizes the 3-D coverage of the conformal area of the target, improves the target dose, and reduces the irradiation of normal tissue surrounding the target area [1-3]. Song et al. [9] compared IMRT with 3-DCRT and determined the optimal dose distribution for the treatment of cervical cancer. They found that the small bowel and bladder average $V_{10}$ and $V_{20}$ decreased by 10.8% and 7.4% ($p = 0.001$ and 0.04), respectively.

Chen et al. [10] conducted a study on early cervical cancer treated by IMRT with concurrent chemotherapy. They found that the local control and disease-free survival rates were 93% and 78%, respectively, in three years. However, no clear conclusion was drawn for patients with late-stage cancer. Kavanagh et al. [11] suggested that cervical cancer patients unsuitable for brachytherapy can be subjected to IMRT for primary tumor. Guerrero et al. [12] proposed intensity-modulated integrated dosage (SIB) technology to replace traditional total pelvic irradiation. In SIB, 25 splits are performed for each 3.1 Gy. Salama et al. [13] applied intensity-modulated treatment with concurrent chemotherapy to 12 cases of cervical cancer, endometrial cancer, and other gynecological tumors, and found high tolerability. These studies suggested that intensity-modulated treatment can partly exert a positive effect on cervical cancer, and can be regarded as an alternative to endovascular treatment in some patients. However, no conclusion was drawn regarding concurrent chemoradiotherapy under the conventional fractionation mode in the initial treatment.

To examine the positive results of the traditional radiation mode (conformal pelvic radiotherapy and intracavitary brachytherapy) in the treatment of cervical cancer, the dose-administration mode in this study was made to be consistent with that of the traditional cassette mode. Conventional technique and IMRT treatment gave 45 Gy at point A and 50 Gy at the pelvic wall point B. The start time and dose of endovascular treatment were the same, i.e., the physical and biological doses for patients were the same as the traditional method. This result differed from the improvement in tumor physical and biological doses in nasopharyngeal intensity-modulated therapy [14]. The problems that remain to be solved are as follows. First, can the physical advantage produce increased amounts of minor radiation reac-
tions? Second, is the survival rate the same as traditional treatment methods? Third, can the external dose be further improved to reduce adverse reactions and enhance the therapeutic effect?

Although improved effects of concurrent chemotherapy have been observed, the acute reaction remains noteworthy [15,16]. The improvement in acute adverse reactions by excellent intensity modulated plans is the focus of current clinical works. IMRT reduces radiation reaction and ensures the smooth progress of radiochemical synchronization. Chen et al. [17] studied cervical cancer patients after intensity-modulated therapy with synchronnal cisplatin, and found that intensity-modulated radiation reduces the acute response of the intestinal and urinary tract, indicating improved tolerability. This randomized control study for previously untreated patients with cervical cancer, radiotherapy, and concurrent chemoradiotherapy was completed on all patients. The present data showed that: IMRT significantly reduced acute intestinal reaction, which is its main advantage. No significant difference was observed in the overall survival and tumor-free survival of the first, second, and third years between the IMRT and 3D CRT groups. Furthermore, the intensity-modulated group did not reduce the effect of traditional conformal pelvic radiotherapy treatment. A significant difference was observed in diarrhea, suggesting that IMRT considerably protected the intestine and rectum, and reduce pain from radiation. Hematologic toxicity in the two groups was one of the major side effects of nedaplatin in clinical settings [18]. Moore et al. [19] noted that carboplatin can replace cisplatin to reduce the gastrointestinal response to chemotherapy. The present study also showed that nedaplatin was a better choice. In the IMRT group, nausea and vomiting, decreased hemoglobin, decreased neutrophils, and thrombocytopenia insignificantly differed from those in the 3D CRT group. Furthermore, more normal tissue was observed at low-dose levels in IMRT, and no serious effects on the hematopoietic function occurred.

This study confirmed that IMRT technology had obvious advantages in protecting sensitive tissue and reducing radiation reaction (especially the diarrhea incidence) over traditional conformal pelvic radiotherapy technology. In three years, the overall survival and recurrence-free survival indicated no disadvantages compared with traditional treatment, especially for large lumps or patients with Grade IIIb and above tumors. Thus, this treatment can replace traditional conformal pelvic radiotherapy technology for the treatment of cervical cancer.

Molla et al. [20] suggested that stereotactic radiotherapy can replace traditional brachytherapy and four Gy for nonsurgical treatment. Grigsby et al. [21] observed that tumors with different sizes have significantly different local control rates and survival rates. Therefore, the excellent dose distribution formed by IMRT can partly replace traditional close-intracavity after loading therapy for previously untreated patients or individualized doses for larger tumors, which are all worthy of further research.

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References


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e-mail: changhuyu@126.com
Identification of potential targets for ovarian cancer treatment by systematic bioinformatics analysis

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Summary
Purpose of investigation: To provide a systematic overview to understand the mechanism of ovarian cancer. Materials and Methods: Data of GSE14407 downloaded from Gene Expression Omnibus (GEO) database and differentially expressed genes (DEGs) were identified. Gene ontology and pathway enrichment analysis were performed by Database for Annotation, Visualization and Integrated Discovery (DAVID). Furthermore, the authors constructed the protein-protein interaction (PPI) network and co-expression networks by Cytoscape. Results: A total 1,442 genes were identified to be differentially expressed. Regulatory effects of DEGs mainly focused on cell cycle, transcription regulation, and cellular protein metabolic process. Significant pathways were determined to be p53 signaling pathway, amino sugar, and nucleotide sugar metabolism. The most significant transcription factor was aryl hydrocarbon receptor nuclear translocator (ARNT). Abnormal spindle-like microcephaly-associated protein (ASPM), Aurora kinase (AURKA), Cyclin-A2 (CCNA2), G2/mitotic-specific cyclin-B1, (CCNB1), and Cyclin-dependent kinase 1 (CDK1) were significant nodes in PPI network. Conclusion: The significant genes and pathways show potential targets for the treatment of ovarian cancer.

Key words: Ovarian cancer; Protein-protein interaction; Co-expression network; Gene ontology analysis; Pathway enrichment analysis.

Introduction
Ovarian cancer is a gynecologic malignancy arising from the ovary and is characterized by uncontrolled tumor growth [1, 2]. It is one of the leading causes of cancer death among women [3]. The symptoms of patients with ovarian cancer are subtle at early stage, including bloating, pelvic pain, and frequent urination [4]. The five-year survival rate of ovarian cancer patients with advanced stage is only 30% after initial diagnosis [5]. It is reported that more than 90% of ovarian cancers originate from surface epithelium of the ovary [6]. In 2012, there were around 22,280 new cases of ovarian cancer [5]. Ovarian cancer is a health concern highlighted all over the world.

Many studies have been conducted to explore the mechanism underlying ovarian cancer progression. One of the important mechanism of ovarian cancer has been determined to be the dysregulation of transcription factors in ovarian cancer [7, 8]. A series of transcription factors (TFs) formed complex regulatory network to regulate gene expressions in cancers [9]. TFs played critical roles in regulating transcription activation by suppressing or triggering target genes with binding sites in regulatory regions [10]. It is reported that TFs in the progression of ovarian cancer are mainly involved in the regulation of cell cycle and cell differentiation [4]. Furthermore, the development of ovarian cancer is revealed to be the accumulation of genetic changes [11]. The oncogenes or tumor suppressor genes have been found to be expressed abnormality in ovarian cancer such as human epidermal growth factor receptor 2 (HER-2/neu), c-myc (Myc), V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (K-ras), and p53 (tumor protein 53) [12]. However, the molecular mechanism underlying the progression of ovarian cancer is largely unknown.

Here, the authors applied bioinformatics technology to identify the differentially expressed genes between human ovarian cancer epithelia tissues and normal ovarian surface epithelial tissues. They constructed the Protein-Protein interaction network and co-expressed network of the differential expression genes. Through investigation of critical genes in function levels, they further explored systematically the mechanism underlying ovarian cancer.

Materials and Methods
Affymetrix microarray data and differential expression analysis
The gene expression profile (GSE14407) was downloaded from Gene Expression Omnibus (GEO) which is a public functional genomics data repository. The expression data was collected by Bowen N.J., et al [13]. A total of 24 samples were available for analysis, including 12 samples of human ovarian cancer epithelia tissues and 12 samples of normal ovarian surface epithelial tissues. The raw CEL data and the annotation files for probes were downloaded based on the platform of GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array) for further analysis.

The CEL source files were processed into expression estimates by Robust Multi-array Average (RMA) algorithm in R [14] and the probe numbers were converted into gene symbols...
Table 1. — GO analysis for DEGs in BP, CC and MF (Top10).

<table>
<thead>
<tr>
<th>Group</th>
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<th>Term</th>
<th>Count</th>
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</thead>
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### Table 2. — Significant pathways by KEGG pathway enrichment analysis.

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<th>Term</th>
<th>Count</th>
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<td>KEGG_PATHWAY</td>
<td>hsa03010: Ribosome</td>
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<td>KEGG_PATHWAY</td>
<td>hsa04610: Complement and coagulation cascades</td>
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<td>KEGG_PATHWAY</td>
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<td>KEGG_PATHWAY</td>
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<td>hsa00280: Valine, leucine and isoleucine degradation</td>
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<td>hsa04110: Cell cycle</td>
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<td>hsa04115: p53 signaling pathway</td>
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</table>

by Bioconductor algorithm in R combined with annotation files. The differentially expressed genes were identified by limma package in R [15]. The authors defined |logFC|>1.0 and p-value < 0.01 as the cut-off value.

**Gene Ontology (GO) and pathway enrichment analysis**

GGO database provides structured and controlled information for community use in annotations of genes [16]. It is commonly used to analyze the over-represented terms for a set of genes [17]. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database for systematic analysis of genes functions and involved pathways [18]. The Database for Annotation, Visualization and Integrated Discovery (DAVID) is a program for assessing the significance of clustering for GO and KEGG terms [19].

The authors performed GO analysis and KEGG pathway analysis for DEGs identified in this paper by DAVID online tool and set p-value < 0.05 as threshold value.

**The construction of protein-protein interaction (PPI) network**

Search Tool for the Retrieval of Interacting Genes (STRING) database consists of integrated information of known and predicted protein–protein associations [20]. The interactions of DEGs were mapped into protein associations based on STRING database. The protein interactions were displayed with confidence score. The authors established the PPI network of significant protein pairs with confidence score > 0.4 using Cytoscape [21] and the higher expressed network clusters were further analyzed with the application of plugin Molecular Complex Detection (MCODE) of Cytoscape [22].

**Co-expression network construction of differentially expressed genes**

The University of California, Santa Cruz (UCSC) Genome Browser is publicly available for a large collection of related annotations, which records the integrated information of transcription factor binding sites [23]. To evaluate the co-expression power in DEGs, the authors calculated the genes correlations by Pearson’s correlation coefficients (PCCs). The gene pairs which had transcription factors regulation were filtered based on UCSC database [24]. The threshold value with |PCC| > 0.90 and p-value < 0.01 was set to select the significant co-expressed gene pairs. Then the co-expressed networks were visualized by Cytoscape package and subnetworks were scored by plugin MCODE.

**Results**

**Differentially expressed genes (DEGs)**

In order to analyse the DEGs between ovarian cancer epithelia tissues and normal ovarian surface epithelial tissues, the authors download the gene expression profiles of GSE14407 from GEO. After analysis, they obtained total of 1,442 DEGs including 1,187 upregulated ones and 255 downregulated ones.

**GO analysis and pathway enrichment analysis**

To investigate the DEGs in function level, the authors carried out GO analysis and KEGG pathway analysis. In GO analysis, the DEGs were mainly classified into three categories including biological process (BP), cell component (CC), and molecular function (MF). As shown in Table 1, the over-represented GO terms of upregulated DEGs were mainly related with proteolysis, protein localization, regulation of cell death, and regulation of programmed cell death. The upregulated DEGs were mainly enriched in pathways of complement and coagulation cascades, amino sugar and nucleotide sugar metabolism, and fatty acid metabolism (Table 2). The downregulated DEGs enriched GO terms included DNA binding, purine nucleotide binding, and ATP binding (Table 1) and the mainly involved pathways were cell cycle, oocyte meiosis, pathways in cancer, and p53 signaling pathway (Table 2).

**PPI network analysis**

The PPI network was constructed using the protein pairs with confidence score > 0.4 (Figure 1). After MCODE analysis, the authors obtained three sub-networks: subnetwork 1, subnetwork 2, and subnetwork 3 (Figure 2). Thirty-seven nodes in PPI networks were found to be with the maximum connective degree (24) such as abnormal spindle-like microcephaly-associated protein (ASPM), Aurora kinase (AURKA), Cyclin-A2 (CCNA2), G2/mitotic-specific cyclin-B1 (CCNB1), and Cyclin-dependent kinase 1
Figure 1. — PPI network of DEGs (red: upregulated genes; green: downregulated genes).

Figure 2. — Three sub-networks in PPI network by MCODE.

Figure 3. — Five modules of co-expressed network Sub-network 1-3 for 357 TFs; Sub-network 4-5 with ARNT and RIM3 as core nodes.
(CDK1). The three subnetworks involving biological processes were cell cycle, cell division; translation, regulation of cellular protein metabolic process, and cell surface receptor linked signal transduction, and G-protein coupled receptor protein signaling pathway, respectively (Table 3).

Co-expression network analysis
A total of 357 transcription factors with |PCC| > 0.90 and \( p \)-value < 0.01 were used for co-expressed network construction. In the co-expressed network, three sub-network with highest scores and were filtered by MCODE. Two transport factors with the highest connectivity were selected and the sub-networks with the two transport factors as core nodes and their first adjacent nodes were investigated for function analysis (Figure 3). The five modules mainly involved biological process were listed in Table 4.

Discussion
Ovarian cancer is regarded as heterogeneous cancer, which remains the leading cause of death from gynecologic cancer [12]. With high mortality and low cure rates, highlighted attentions from all over the world have been focused on disclosing the potential mechanism of ovarian cancer progression. In order to better understand ovarian cancer, the present authors explored the molecular mechanism with the application of bioinformatics method.

The present results suggested that 1,442 genes were involved in the progression of ovarian cancer, including 1,187 upregulated genes and 255 downregulated genes. Coregulation mechanism is an important approach to understand the development of cancers. Based on the DEGs identified in the present study, the authors further investigated the gene expression correlations with TF related regulatory pairs. Results showed that the main co-expression sub-networks were involved in cell cycle, regulation of transcription, and cellular protein metabolic process. To obtain information of genes communications from mass genomic data, they also constructed the PPI networks. The PPI networks also showed that the main biological processes of DEGs between ovarian cancer epithelia tissues and normal tissues included cell cycle, signal transduction and cellular protein metabolic. The similar results can be found in the GO and pathway analysis. The DEGs enriched GO terms related with cell cycle, proteolysis, and molecular binding.

Significant pathways and genes have been determined to be associated with the primary function modules. Pathway enrichment analysis suggested that p53 signaling pathway was significant in ovarian cancer. Protein 53 (p53) as a tumor suppressor protein has been reported to be excessively expressed in ovarian cancers [25]. The expression of p53 is implicated in the modulation of apoptosis which may result in inhibition of drug induced apoptosis [26]. Previous study has reported that the action of p53 is associated with drug resistance of ovarian cancer cells [27]. The activation of p53 was triggered in the caspase-dependent mitochondrial death pathway induced by drug and the expression of p53 protected tumor cells from apoptosis and delayed S-phase arrest [26]. The critical role of p53 pathway contributing to drug resistance has been highlighted in the treatment of ovarian cancer. The p53 signaling pathway identified in the present work function in signal transduction and cell apoptosis process.

By co-expression network analysis, the transcription factors: aryl hydrocarbon receptor nuclear translocator (ARNT) was found to play key roles in regulation regions of ovarian cancer. ARNT gene encodes aryl hydrocarbon receptor nuclear translocator protein which is the composition of the aryl hydrocarbon receptor (AhR) complex. ARNT forms AhR complex in nucleus with activated AhR in response to aryl hydrocarbon receptor (AhR) ligands [28]. AhR-ARNT complex has a close association with the xenobiotic stress response. The heterodimer of AhR:ARNT functioned in regulating multiple gene expressions to respond to xenobiotic stress, including cytochrome P450 subfamily polypeptide 1 (CYP1A1) [29]. The overexpression of ARNT is positively related with CYP1A1 expression levels [30]. A series of evidences proved that CYP1A1 gene polymorphism played role in the development of epithelial ovarian cancer [31]. The accumulation of CYP1A1 elevated the risk of having ovarian cancers. Therefore, ARNT is a critical regulator in the development of ovarian cancer by inducing the associated genes.

### Table 3. — The main biological process of 3 sub-network of PPI network.

<table>
<thead>
<tr>
<th>Subnetwork number</th>
<th>Number of nodes</th>
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<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>cell cycle, cell division</td>
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<tr>
<td>2</td>
<td>37</td>
<td>translation, regulation of cellular protein metabolic process</td>
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<tr>
<td>3</td>
<td>12</td>
<td>cell surface receptor linked signal transduction, G-protein coupled receptor protein signaling pathway</td>
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### Table 4. — The main biological processes of different modules in co-expression network.

<table>
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<tr>
<td>1</td>
<td>139</td>
<td>protein localization, regulation of cellular protein metabolic process</td>
</tr>
<tr>
<td>2</td>
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<td>protein transport, establishment of protein localization</td>
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<tr>
<td>3</td>
<td>113</td>
<td>cell cycle, translation, cell cycle process</td>
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<tr>
<td>4</td>
<td>161</td>
<td>regulation of transcription, cell cycle</td>
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<tr>
<td>5</td>
<td>161</td>
<td>regulation of transcription, cell cycle</td>
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</table>
PPI networks also indicated some significant genes with higher degrees such as ASPM, AURKA, CCNA2, CCNB1, and CDK1. ASPM encoding for a mitotic spindle protein is localized in the spindle poles during mitosis. The expression of ASPM was found to be upregulated in proliferating tissues and malignant cells [32]. ASPM expression is determined to be correlated with the grade and survival of epithelial ovarian cancer [33]. Different levels of ASPM expression were observed in ovarian tumor cells correlating with the grade of tumors. ASPM has been considered to be a potential molecular target in glioblastoma and also has potential application in other cancers [34]. AURKA served as a member of serine/threonine kinases also played a key role mitosis process. The peak activity of AURKA is presented in the G2 phase to M phase transition of the cell cycle [35]. As outlined in previous reports, the overexpression of AURKA is a common and significant event in the progression of ovarian cancers [36]. The amplification of AURKA may be a premalignant marker for ovarian carcinogenesis. Other node proteins such as CCNA2, CCNB1, and CDK1 also showed significant effect on cell cycle process. Therefore, these genes played a primary role in regulating cell cycle.

In summary, this work provides a systematic overview to understand the mechanism underlying ovarian cancer progression. The differential expressions of genes result in changes of pathways and function modules. The critical nodes in networks and the significantly disturbed pathways showed potential targets for ovarian cancer treatment. Further studies should be conducted to explore the clinical application of the targets.

Acknowledgements

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References

Identification of potential targets for ovarian cancer treatment by systematic bioinformatics analysis


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Prognostic factors for survival in cervical cancer patients with bone metastasis


1Department of Obstetrics and Gynecology, 2Department of Radiation Oncology, 3Department of Biomedical Statistics
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Summary
Purpose: To investigate the prognostic factors for survival in uterine cervical cancer patients who developed bone metastasis. Materials and Methods: Cervical cancer patients with bone metastasis who were treated at the present institute from April 1996 to September 2010 were identified from the authors’ institutional tumor registries. Primary disease, follow-up, and recurrence data were collected and retrospectively reviewed. Univariate and multivariate analyses of prognostic factors for survival were performed. Results: A total of 37 patients that developed cervical cancer bone metastasis were included in the authors’ database. The median survival time after recurrence was 12 months. Univariate analysis revealed that patients with a disease-free interval (DFI) of ten months or less achieved significantly shorter survival after bone metastasis detection than those with a DFI of 11 months or more (median: 8.5 months versus 17 months, \( p < 0.0001 \)). Multivariate analysis also showed that DFI of ten months or less was a significant predictor of short survival (\( p = 0.0018 \)). Conclusions: The DFI was found to be independent prognostic factors for survival in cervical cancer patients who developed bone metastasis.

Key words: Bone metastasis; Cervical cancer; Prognostic factors; Survival.

Introduction
Cervical cancer is one of the most common malignancies in women worldwide. It has been estimated that 12,710 new cases and 4,290 deaths occurred in the US in 2011 [1]. In Japan, 6-7,000 new cases are reported annually [2]. Despite the recent advances in surgery [3] and the introduction of concurrent chemoradiotherapy [4], substantial treatment failure still occurs, especially in advanced-stage patients [5]. Patients that suffer recurrence or metastatic cervical cancer have a dismal prognosis with a one-year survival rate of 15-20\% [6].

Bone metastasis from uterine cervical cancer is generally uncommon, however, as bone metastases have substantial negative effects on patients’ quality of life and are associated with short survival [7-9], deeper understanding of this condition as well as the development of optimal treatment strategies that prolong survival and improve the patient’s quality of life are necessary.

The prognostic factors in patients with recurrent cervical cancer have been investigated in several studies, and recurrence within the previously irradiated area, young age, poor performance status, and a short disease-free interval (DFI) have been reported to be significant predictors of shorter survival [10-12]. However, to the best of the present authors’ knowledge, only limited survival information on bone metastasis in cervical cancer patients is available in the literature.

The authors conducted a retrospective analysis to investigate the prognostic factors for survival in cervical cancer patients with bone metastasis.

Materials and Methods
Patients
Permission to proceed with the data acquisition and analysis was obtained from Osaka University Hospital’s institutional review board. A list of patients who were treated for cervical cancer from April 1996 to September 2010 was generated from each institutional tumor registry. Then, patients who developed bone metastasis were identified through a chart review, and their clinical information was retrospectively reviewed. Clinical data on the following characteristics were collected for all patients: initial clinical stage, cell type, primary treatment, DFI, site and number of bone metastases, the presence or absence of symptoms, salvage treatments, and survival after the diagnosis of bone metastasis.

Salvage treatments were classified as follows: chemotherapy (platinum-based chemotherapy with or without palliative external beam radiotherapy delivered to the bone lesion), and palliative care (palliative care including palliative external beam radiotherapy). Patients with incomplete medical records were excluded. DFI was defined as the time from the initial diagnosis of cervical cancer to the detection of bone metastasis.

Statistical analysis
The authors performed univariate analyses by comparing the Kaplan-Meier curves of each subgroup using the log rank test. Cox proportional hazards regression analysis was performed to identify significant independent prognostic factors for survival. All \( p \) val-
ues of < 0.05 were considered statistically significant. All analyses were performed with SAS version 9.1 for Windows

Results

Patients

From April 1996 to September 2010, 713 Japanese patients with cancer of the uterine cervix were treated at the present institution. Among these patients, 37 (5.2%) developed bone metastasis during the follow-up period. The clinicopathologic characteristics of the patients are summarized in Table 1. The median age was 58 years (range 29-83). Of these 37 patients, 19 (51.4%) had Stage I-II and 18 (48.6%) had Stage III-IV disease. Histologically, 30 patients (81.1%) had squamous cell carcinoma (SCC), and seven (19.9%) had non-squamous histology. The primary treatments were definitive radiotherapy (or chemoradiotherapy) in 18 patients (48.7%), surgery with or without adjuvant radiotherapy (or chemoradiotherapy) in 16 (43.2%), and chemotherapy in three (8.1%). Twelve patients had multiple bone lesions (32.4%). Twenty-nine patients (78.3%) had concomitant metastasis affecting other organs, including the lungs (eight cases), supraventricular nodes (five cases), liver (four cases), para-aortic nodes (five cases), brain (one case), skin (two cases), and pelvic (ten cases).

The median DFI (the time from the initial diagnosis of cervical cancer to the detection of bone metastasis) was ten months. Twenty patients (54.1%) had developed bone metastasis within ten months of being diagnosed with uterine cervical cancer. Of these, seven patients were found to have bone metastasis at the time of their initial treatment for cervix cancer.

Fifteen patients (40.5%) who developed bone metastasis were treated with chemotherapy, and 22 patients (59.5%) were treated with palliative care alone. Among the patients in chemotherapy-group or in palliative care-group, two or 12 received palliative external beam radiotherapy, respectively.

As shown in Table 2, the most common site of involvement was the pelvic bones, and the vertebral column was the next most frequent site of involvement. Bone metastasis was diagnosed by X-rays in two patients (5.4%), computed tomographic scanning in six (16.2%), magnetic resonance imaging in six (16.2%), FDG-PET/CT in eight (21.6%), and skeletal scintigraphy in 15 (40.5%). Only one patient (2.7%) had a confirmed pathological diagnosis of bone metastasis. At the time of bone metastasis diagnosis, 22 patients (59.5%) had bone pain as their presenting symptom, and the remaining 15 (40.5%) were asymptomatic.

Survival analysis

The median survival period after bone metastasis diagnosis of all patients was 12 months (range, 2-188). To investigate the independent predictors of survival in cervical cancer patients with bone metastasis, the authors performed univariate and multivariate analyses. As shown in Table 3 and Figure 1, the univariate analysis showed a statistically significant difference in survival after bone metastasis between the patients with shorter and longer DFI (median: 8.5 versus 17 months, p < 0.0001). Consistent with the results of the present univariate analysis, Cox multivariate analysis also showed that DFI was a significant prognostic factor in terms of survival after bone metastasis (Table 4). The univariate and multivariate analysis also showed that age, clinical stage, histology, number of bone metastases, other organ involvement, and modality of salvage treatments was not a significant prognostic factor for survival (Tables 3-4).

Discussion

Bone is the third most common site of hematogenous spread from uterine cervical cancer after the lungs and liver [7]. According to recent reports, the incidence of clinical bone metastasis derived from uterine cervical cancer varies

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>≤ 57</td>
<td>17</td>
</tr>
<tr>
<td>≥ 58</td>
<td>20</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
</tr>
<tr>
<td>Ia-Ib</td>
<td>5</td>
</tr>
<tr>
<td>Ila-Ilb</td>
<td>14</td>
</tr>
<tr>
<td>Illa-IIlb</td>
<td>5</td>
</tr>
<tr>
<td>IVA-IVb</td>
<td>13</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>30</td>
</tr>
<tr>
<td>A or AS</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>5</td>
</tr>
<tr>
<td>Primary treatment</td>
<td></td>
</tr>
<tr>
<td>Definitive RT/CCRT</td>
<td>18</td>
</tr>
<tr>
<td>Surgery with or without adjuvant RT/CCRT</td>
<td>16</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>3</td>
</tr>
<tr>
<td>DFI</td>
<td></td>
</tr>
<tr>
<td>DFI &lt; 10 months</td>
<td>20</td>
</tr>
<tr>
<td>DFI &gt; 11 months</td>
<td>17</td>
</tr>
<tr>
<td>No. of bone metastasis</td>
<td></td>
</tr>
<tr>
<td>Single bone lesion</td>
<td>25</td>
</tr>
<tr>
<td>Multiple bone lesions</td>
<td>12</td>
</tr>
<tr>
<td>Other organ involvement</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>29</td>
</tr>
<tr>
<td>No (bone lesions alone)</td>
<td>8</td>
</tr>
<tr>
<td>Salvage treatments</td>
<td></td>
</tr>
<tr>
<td>Palliative care alone</td>
<td>22</td>
</tr>
<tr>
<td>Chemotherapy with or without palliative RT</td>
<td>15</td>
</tr>
</tbody>
</table>

SCC: squamous cell carcinoma; A: adenocarcinoma; AS: adenosquamous carcinoma; RT/CCRT: radiotherapy or chemoradiotherapy; DFI: time from the initial diagnosis of cervical cancer to the detection of bone metastasis.

<table>
<thead>
<tr>
<th>Site of bone metastasis</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skull</td>
<td>2</td>
</tr>
<tr>
<td>Rib</td>
<td>2</td>
</tr>
<tr>
<td>Vertebrae</td>
<td>10</td>
</tr>
<tr>
<td>Upper extremities</td>
<td>1</td>
</tr>
<tr>
<td>Pelvis</td>
<td>20</td>
</tr>
<tr>
<td>Lower extremities</td>
<td>3</td>
</tr>
</tbody>
</table>
Prognostic factors for survival in cervical cancer patients with bone metastasis

from 1.1% to 8.2% [7, 9, 13-15]. In addition, autopsy studies have reported a higher incidence of 8.6-17.9% [8, 16]. The incidence of bone metastasis in the present study was 5.2%, which was consistent with these previous reports.

In the current study, the vertebrae and pelvic bone were two most common sites of bone metastasis (Table 2). This finding is also consistent with previous reports showing that the vertebrae and pelvic bone are common sites of bone metastasis and that the distal appendicular skeleton is rarely involved [7, 9, 15, 17-19].

Table 3. — Univariate analysis for survival after the diagnosis of bone metastasis.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>No. of patients</th>
<th>Survival (months)</th>
<th>Univariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
<td>p value</td>
</tr>
<tr>
<td>Age (years) ≥ 57</td>
<td>17</td>
<td>11</td>
<td>2-188</td>
</tr>
<tr>
<td>Clinical stage I-II</td>
<td>19</td>
<td>15</td>
<td>6-188</td>
</tr>
<tr>
<td>Clinical stage III-IV</td>
<td>18</td>
<td>11</td>
<td>2-40</td>
</tr>
<tr>
<td>Histology SCC</td>
<td>30</td>
<td>14</td>
<td>2-188</td>
</tr>
<tr>
<td>Histology Others</td>
<td>7</td>
<td>9</td>
<td>6-17</td>
</tr>
<tr>
<td>DFI ≤ 10 months</td>
<td>20</td>
<td>8.5</td>
<td>2-27</td>
</tr>
<tr>
<td>DFI ≥ 11 months</td>
<td>17</td>
<td>17</td>
<td>4-188</td>
</tr>
<tr>
<td>No. of bone metastasis Single</td>
<td>25</td>
<td>14</td>
<td>2-188</td>
</tr>
<tr>
<td>No. of bone metastasis Multiple</td>
<td>12</td>
<td>11.5</td>
<td>4-50</td>
</tr>
<tr>
<td>Other organ involvement No</td>
<td>8</td>
<td>12.5</td>
<td>6-188</td>
</tr>
<tr>
<td>Other organ involvement Yes</td>
<td>29</td>
<td>12</td>
<td>2-40</td>
</tr>
<tr>
<td>Treatments Palliative care</td>
<td>22</td>
<td>11.5</td>
<td>3-37</td>
</tr>
<tr>
<td>Treatments Chemotherapy</td>
<td>15</td>
<td>12</td>
<td>2-188</td>
</tr>
</tbody>
</table>

SCC: squamous cell carcinoma; BSC: best supportive care; DFI: time from the initial diagnosis of cervical cancer to the detection of bone metastasis; 95%CI, 95% confidence interval.

Survival period after bone metastasis diagnosis.

Treatments for bone metastasis and concomitant metastasis affecting other organs.

Among the present patients, 29 out of 37 had concomitant metastatic lesions in other organs. This is consistent with previous reports suggesting that more than 50% of patients have multiple metastatic lesions at the time of bone metastasis diagnosis [9, 14, 15]. These results strongly indicate that a systemic metastatic work-up is necessary in cases of suspected bone metastasis.

Traditionally, skeletal scintigraphy has been the most common diagnostic procedure for bone metastasis. Recent reports have suggested that FDG PET/CT is also useful for the detection of bone metastases [7, 20]. However, the diagnostic abilities of skeletal scintigraphy and FDG PET/CT have never been directly compared in patients with cervical cancer. The present authors have recently experienced a case exhibiting multiple areas of focal bone FDG uptake mimicking bone metastasis from uterine cervical cancer [21]. Thus, to exclude the possibility of the false-positive accumulation of radioisotopes or FDG in osteoporotic, osteonecrotic, or inflammatory lesions, other useful diagnostic tools including X-rays, CT scans, or MRI should be used in combination with skeletal scintigraphy or FDG PET/CT [20-22].

There are no guidelines regarding the therapeutic options for bone metastasis from cervical cancer. As most patients die within 12 months of the discovery of bony metastatic lesions [7, 9, 14, 15], previous reports recommended that treatment should be directed towards improving the patient’s quality of life and palliating their symptoms. Palliative radiotherapy is reported to be beneficial for pain relief and to decrease the risk of fractures [3-5]. According to a report by Matsuyama et al., 67% of patients experienced pain relief after being treated with 30 Gy of external beam radiotherapy in ten fractions [9]. For systemic disease,
chemotherapy following palliative radiotherapy has also been reported to provide adequate symptom control [7, 9]. In the current study, of the 12 symptomatic patients who received palliative radiotherapy, 11 (91.7%) experienced pain relief (data not shown).

The prognostic factors for survival in this specific patient population are unknown. Previous reports have suggested that survival after bone metastasis diagnosis was dismal and was not associated with the number of bone metastases or the treatment modality [7]. In the current study, multivariate analysis showed that the DFI was an independent prognostic factor for survival after bone metastasis ($p = 0.0018$). The patients with DFI of ten months or less showed a significantly shorter survival than those with DFI of 11 months or more (median: 8.5 months versus 17 months, $p < 0.0001$). The present univariate and multivariate analyses did not identify the number of bone metastases or the modality of salvage treatments as independent prognostic factors of survival, which was consistent with the previous reports [7].

The limitations of the present study need to be addressed. One is the relatively small number of patients included in the current study. Moreover, due to its retrospective nature, potential confounding biases might have been missed in the analysis, such as the selection bias introduced by the physicians in determining the salvage treatments employed.

In conclusion, bone metastasis was observed in 5.1% of the patients in the present cohort. The DFI was found to be a significant prognostic factor for survival in cervical cancer patients who developed bone metastasis. Although retrospective, the result from the current study may enable us to identify a “long-term survivor” who could be offered the opportunity to receive salvage treatments or enter clinical trials with novel agents. The prognostic information obtained from the current study needs to be investigated further in prospective studies.

References


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The differences of phyllodes and acoustic attenuation in breast lesions diagnosed with Breast Imaging-Reporting and Data System for Ultrasonography (BI-RADS-US) category 4C

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Summary

Objective: This study aimed to discuss the differences of malignant findings in breast lesions diagnosed with Breast Imaging-Reporting and Data System for Ultrasonography (BI-RADS-US) category 4C to determine which malignant findings are more important. Materials and Methods: A total of 159 cases of breast lesions diagnosed with BI-RADS-US category 4C were analyzed retrospectively. All patients got pathological results (81 cases of benign; 78 cases of breast cancer). Two doctors scanned and diagnosed the patients, with another doctor recording ultrasonographic findings retrospectively. The differences were compared by means of the Chi-square (χ²) test. Results: Phyllodes and acoustic attenuation had statistical differences in the comparison of breast lesions (p < 0.05). Irregular shape, indistinct boundary, poorly-defined margin, penetrating or tortuous surrounding vessels, RI ≥ 0.7, and microcalcification had no statistical differences in the comparison of benign and malignant breast lesions. Conclusions: Phyllodes and acoustic attenuation are the more important malignant ultrasonographic findings of breast cancer. The malignant ultrasonographic findings are not unique for breast cancer.

Key words: Malignant ultrasonographic findings of breast; BI-RADS-US; Differential diagnosis of breast lesions.

Introduction

Breast cancer is one of the common malignant tumors in the female. The incidence of breast cancer is the third in the malignances [1]. At present, the diagnosis of breast cancer is mainly dependent on mammography in America and Asia [2, 3]. However, with the improvement of ultrasonic resolution and the development and application of new ultrasonic technology, breast ultrasonography has gained widespread acceptance as a diagnostic tool for the evaluation of human breast disorders [4].

As reported and found by the present authors’ daily work, some of the breast lesions diagnosed with Breast Imaging-Reporting and Data System for Ultrasonography (BI-RADS-US) category 3 were diagnosed as malignant by pathology after biopsy or surgery [5, 6] and some of the breast lesions diagnosed with BI-RADS-US category 4C were diagnosed as benign by pathology after surgery [7, 8]. Sonographers make a diagnosis promptly according to the ultrasonographic findings of breast, but it is not clear which one is more important when the decision is made.

The present authors will discuss the differences of ultrasonographic findings in breast lesions diagnosed with BI-RADS-US category 4C in this work to determine which malignant findings are more important.

Materials and Methods

Patients

All patients in inpatient departments underwent surgery (January 2009-December 2009), including 81 cases of benign breast lesions (age range 18-79 years, mean 47.23 ± 10.80; 47 cases of adenosis, 21 cases of intraductal papilloma; 13 cases of fibroadenoma), and 78 cases of malignant breast lesions randomly selected (age range 26-73, mean 47.5 ± 8.93 years). All female patients were examined by the department of ultrasonic diagnosis and diagnosed with BI-RADS-US category 4C. The American College of Radiology BI-RADS-US lexicon (ACR BI-RADS-US) was used. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of the First Affiliated Hospital of China Medical University. Written informed consent was obtained from all participants.

Sonographic equipment and the scanning method

A color ultrasound diagnostic system was used with the linear probe at the frequency of 7-14 MHz. All patients took supine position. Two arms were placed on both sides of the head; bilateral breast and axillaries were fully exposed. Two doctors (with more than ten years of experience) scanned and diagnosed the patients, with another doctor (more than ten years experience) recorded the ultrasonographic findings retrospectively.

Malignant findings in BI-RADS-US category 4C

The authors defined malignant findings in BI-RADS-US category 4C: A-Irregular shape, B-indistinct boundary, C-phyllodes,
D-poorly-defined margin, E-acoustic attenuation, F-penetrating or tortuous surrounding vessels, G-RI ≥ 0.7, H-microcalcification.

Statistical analysis
Statistical analysis was performed by using SPSS 19.0 software. The differences in the study group and the control group were compared by means of the Chi-square x (Chi-square) test. The results were considered statistically significant whenever $p$ was < 0.05.

Results
Ultrasonographic findings in breast lesions diagnosed with BI-RADS-US category 4C
The ultrasonographic manifestations of malignant ultrasonographic findings in benign and malignant breast lesions diagnosed with BI-RADS-US category 4C are shown in Figures 1 and 2.

The differences of malignant findings in breast lesions diagnosed with BI-RADS-US category 4C
Phyllodes and acoustic attenuation had statistical differences in the comparison of breast lesions ($p < 0.05$), irregular shape, indistinct boundary, poorly-defined margin, penetrating or tortuous surrounding vessels, RI ≥ 0.7, and microcalcification had no statistical differences in the comparison of benign and malignant breast lesions ($p > 0.05$, Table 1).

Discussion
The pathological morphologies are the basis of ultrasonographic manifestations. A total of 159 cases in BI-RADS-US category 4C were analyzed and the differences of ultrasonographic findings in breast lesions were discussed in this paper. Interestingly the present results found
that phyllodes and acoustic attenuation had statistical differences in the comparison of benign and malignant breast lesions, but the others had no statistical differences.

The ultrasonographic finding of phyllodes, which is the most important imaging finding, is due to the multi-center growth and uneven blood supply of the tumor. The imaging findings of phyllodes in invasive lobular carcinoma are consistent with their histopathological features. Phyllode is the basic and essential diagnostic tool and can distinguish between benign and malignant tumors according to Choi et al. [9]. Tan et al. [10] also reported that ultrasonographic findings can be used to help pre-operatively determine breast phyllodes tumors. The present authors found in this study that the phyllodes are more important malignant ultrasonographic findings and its accordance with above research. The authors found in this study that phyllodes and acoustic attenuation are the important malignant ultrasonographic findings in diagnosing breast cancer. The diagnostic value of poorly-defined margin needs further study.

Acknowledgements

Yinyan Li is greatly indebted to all my teachers who have helped me directly and indirectly in my studies. Any progress that I have made is the result of their profound concern and selfless devotion. Among them the following require mentioning: Professor Xuemei Wang.

References

Cancer testis antigen OY-TES-1: analysis of protein expression in ovarian cancer with tissue microarrays

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Summary

Objectives: The purpose of this study was to determine the potential of cancer testis antigen OY-TES-1 as a vaccine for ovarian cancer (OC). Materials and Methods: A tissue microarray (TMA) containing 107 samples from OC tissues and 48 samples from OC adjacent tissues was analyzed by immunohistochemistry with the OY-TES-1 polyclonal antibody. The correlation between OY-TES-1 and clinicopathological traits of OC was statistically analyzed. Results: The expression of OY-TES-1 protein was found in 81\% (87/107) of OC tissues and 56\% (27/48) of OC adjacent tissues. The immunostaining intensity of OY-TES-1 in OC tissues was significantly higher than that in OC adjacent tissues tested ($p = 0.040$). OC adjacent tissues only demonstrated lower immunostaining intensity, whereas some of OC tissues presented higher immunostaining intensity and majority showed the heterogeneity of protein distribution. There was no statistically significant correlation found between OY-TES-1 expression and any other clinicopathological traits such as age, FIGO stage, pathological grade, and histological type. Conclusions: OY-TES-1 was expressed in OC tissues with a high proportion, and some of OC tissues presented OY-TES-1 expression in high level vs OC adjacent tissues. OY-TES-1 could be an attractive target for immunotherapy for OC in the future.

Key words: OY-TES-1 protein; Ovarian cancer; Immunohistochemistry; Antigen; Immunotherapy.

Introduction

Ovarian cancer (OC) is the ninth most common cancer and the fifth leading cause of cancer death in women [1]. Survival rates approaching 90\% have been reached among OC patients who were diagnosed at an early stage. Nonetheless, a challenge for early detection of OC still remains because non-specific symptoms of early ovarian lesions go unnoticed until the patient presents with an abdominal distension due to late-stage tumor growth and accumulation of ascites. Despite great improvements in surgical resection, chemotherapy, and radiotherapy, the long-term survival rate is only at 20\% to 30\% for advanced OC [2]. These “high-risk” patients have a short remission duration of 10 to 12 months and a recurrence rate of >70\% [3]. Therefore, additional treatment such as immunotherapy for prolonging the life-span of patients with OC is needed [4].

An ideal candidate antigen for immunotherapy of any cancer type should show both inherent immunogenicity and differential expression in the cancer tissues. Cancer testis (CT) antigens represent a unique class of tumor antigens, which are expressed in a variety of cancerous tissues and are silent in normal tissues except for the testis. So far, there are many reports demonstrating that patients with tumors are able to elicit both specific cellular and humoral immune responses to these antigens. Therefore, CT antigens are considered to be ideal candidates for novel cancer immunotherapies with encouraging preliminary results [5-7].

OY-TES-1 has been firstly characterized as a CT antigen by Ono et al. [8]. It is the human homologue of acrosin binding protein (ACRBP), which was as the precursor of sp32 (sperm protein 32) originally identified in porcine, guinea pig, and mouse [9]. As of yet, it has been reported that OY-TES-1 can express in different cancerous tissues, but restrictively or does not express in normal adult tissues except for testis. Moreover, it can raise humoral response in patients with a variety of tumor types including in bladder, prostate, liver, colon, lung, and ovary [8, 10]. However, there is limited information regarding the OY-TES-1 expression in OC tissues, and expression status of OY-TES-1 protein in OC adjacent tissues is not yet available. Therefore, in this study, the authors determined the frequency and intensity of expression of OY-TES-1 protein in OC tissues as well as adjacent tissues of OC, and evaluated the relationship between OY-TES-1 expression and clinicopathological parameters.

\*Contributed equally to this work.

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Materials and Methods

Patients and specimens
A panel of formalin-fixed paraffin-embedded human tissue microarray (TMA) included 107 cases of OC and 48 cases of OC adjacent tissues (Table 1). All tumors were classified according to World Health Organization (WHO) criteria [11]. The OC adjacent tissues were taken from the periphery of the lesion. The absent of pathological cells was confirmed by microscope. The testis tissue was collected from an elderly patient with prostate cancer. The use of tissue was approved by Ethics Committee of the Hospital and informed consent was obtained from all patients.

siRNA transfection
The OC cell line SKOV3, known to express OY-TES-1, [10] was obtained from Shanghai Cell Collection of the Chinese Academy of Science and used for RNA interference (RNAi). The siRNA (sense: 5’- GACUAUAUCCAGUACCCAATT-3’, antisense: 5’-UUGGGUACUGGGAUAUAGUCTG-3’) targeting OY-TES-1 from 1486 to 1504 nucleotides (UniGene no. Hs.123239) and a scrambled siRNA were designed and synthesized. Briefly, transfection procedure was as following: 1×10^5 cells/well was incubated in 24-well board, when cell confluence reached around 50-60%, the culture medium was changed with serum-free L-DMEM 24 hours before the transfection, then the cells were transfected with OY-TES-1 siRNA by using X-tremeGENE siRNA transfection reagent. OY-TES-1 protein was detected in 24, 36, 48, and 72 hours after siRNA transfection with immunocytochemistry using OY-TES-1 antibody (Cat no. ab64809).

Immunocytochemistry and immunohistochemistry
TMA sections were deparaffinized in xylene, rehydrated by transfer through graded concentrations of ethanol to distilled water, and submitted to antigen retrieval in an 800W microwave containing 0.01 mol/L citrate buffer (pH = 6) for 15 minutes. Then, endogenous peroxidase activity was blocked by incubation with 0.03% H2O2 for five minutes at room temperature. The slides were then incubated with polyclonal OY-TES-1 antibody (5 μg/ml) at 4°C overnight followed by horseradish peroxidase (HRP)-conjugated second antibody at room temperature for one hour. For immunocytochemistry the cells treated with siRNA were cultured on the cover slide and fixed with methanol, following immunostaining as above mentioned, but without antigen retrieval. All slides were developed by diaminobenzidine (DAB) for ten minutes and lightly counterstained with hematoxylin. Stained slides were dehydrated consecutively in graded ethanol, and finally transferred into xylene and mounted [12].

The expression status of OY-TES-1 was scored based on the number of immunostaining cells in three different fields of each slide. The extent of expression was graded as follows: negative, staining of single cells or small clusters of cells (<5% of cells stained); +, 5%-25%; ++, >25%-50%; +++, >50%-75%; ++++, >75% of cell stained. For quantitative analysis of OY-TES-1 protein the integrated optical density (IOD) was used by special image analysis software.

Statistical analysis
The associations between OY-TES-1 expression and clinicopathological parameters were evaluated using the χ^2 test or Kruskal Wallis test as appropriate. Statistical program for social sciences (SPSS) software (version 15) was used in all statistical analysis. A p value less than 0.05 was statistically considered significant.

Results

Study population
The characteristics of the study population are presented in Table 1. The median age of patients was 48 years (range 12-75). The 107 cases tested were in Federation International of Gynecology and Obstetrics (FIGO) Stage I to IV and included nine cases with metastatic cancer from other sites, all specimens presented pathological grades 1 to 3.
Figure 1. — Validation of OY-TES-1 antibody by RNAi reduced OY-TES-1 expression in SKOV3. (a) cells without transfection (Mock 1); (b): cells transfected with scrambled siRNA (Ctrl siRNA); (c-f) cells transfected with OY siRNA for 24, 36, 48, and 72 hours (OY siRNA), respectively. All sections were counter-staining with Hematoxylin (Bar: 20 μm). Bar graph represents quantitative analysis of OY-TES-1 protein with integrated optical density (IOD). Error bars represent average error from the mean (asterisk: *p < 0.05).

Figure 2. — Immunohistochemical staining of OY-TES-1 protein with OY-TES-1 antibody. (a, d): OC adjacent tissues; b-c: OC tissues; All sections were counter-staining with Hematoxylin (Bar: 50 μm).
with different histological types. The majority of specimens was in FIGO Stage I (46%, 49/107), pathological grade 3 (42%, 45/107), and papillary serous (73%, 78/107). The OC adjacent tissues were taken from the periphery of the lesion. The absence of pathological cells was confirmed by microscope. The median age of patients was 40 years (range 14-69).

Validation of OY-TES-1 antibody

To validate the specificity of OY-TES-1 antibody, OY-TES-1 positive cell line of SKOV3 was used to RNAi. Down-regulation of OY-TES-1 was observed after transfection of OY-TES-1 siRNA in SKOV3 for 24 hours (Figure 1), which suggested that the OY-TES-1 antibody can specifically target OY-TES-1 protein.

Expression of OY-TES-1 in OC and OC adjacent tissues

The location of OY-TES-1 protein showed a predominantly, although not exclusively, cytoplasmic staining in OC tissues (Figure 2). The OY-TES-1 protein was detected in 81% (87/107) of OC tissues and 56% (27/48) of OC adjacent tissues, respectively. The expression frequency of OY-TES-1 protein in OC tissues was significantly higher than OC adjacent tissues ($p = 0.001$). Furthermore, IOD was used for quantitative analysis of OY-TES-1 protein. The results showed the difference of IOD median between the OC tissues and OC adjacent tissues ($p = 0.040$), and confirmed the higher expression of OY-TES-1 protein in OC tissues (IOD median = 542.30) as compared to OC adjacent tissues tested (IOD median = 243.42).

All specimens of OY-TES-1 protein positive from OC adjacent tissues only demonstrated lower immunostaining intensity (+ and ++). Whereas 87 specimens of OY-TES-1 protein positive from OC presented in different immunostaining intensity from + to ++++, among which 68% (59/87) of OC specimens with staining intensity of + and ++ demonstrated apparent heterogeneity of OY-TES-1 protein distribution (Table 2).

Correlation between OY-TES-1 expression and clinicopathological parameters

To evaluate whether the OY-TES-1 expression might be related to the clinicopathological parameters, 107 specimens from OC were analyzed. There was no statistically significant correlation between OY-TES-1 expression and any other clinicopathological traits (age, FIGO stage, pathological grade, and histology type) as shown in Table 1.

Discussion

OY-TES-1 has been annotated into a CT database by Ludwing Institute for Cancer Research as CT23 according to its list in the database (http://www.cta.lncc.br). In previous studies, the mRNA expression spectrum of OY-TES-1 in tumors included bladder cancer, breast cancer, colon cancer and liver cancer [8, 12, 13]. However, only a single study has been conducted with OC in which mRNA and protein of OY-TES-1 were detected in 23% (23/100) and 60% (60/100), respectively [10]. Here, the authors present the result with 81% of OC expressing OY-TES-1 protein. The discrepancy of OY-TES-1 protein expression rate in OC may relate to the different race and histological types. It may also result in use of different antibody. Tammela et al. [10] used monoclonal antibody against OY-TES-1, while the present authors applied the polyclonal antibody which should be lower specificity than monoclonal antibody, which was commonly considered to lower specificity than monoclonal antibody. However, this antibody the present authors used has been validated through combination of recombinant OY-TES-1 protein and RNAi, confirming to be specific for the target antigen. Although the present result demonstrated more than half of OC adjacent tissues with OY-TES-1 protein positive, its expressive level was lower comparing to the OC tissues. Therefore, application of OY-TES-1 immunotherapy for OC patients may not severely affect those tissues with low OY-TES-1 expression.

Nowadays the expression of several CT antigens, such as SPAG9 in 90% [14], SP17 in 68% [15], CT45 in 37% [16], NY-ESO-1 in 30%, MAGE-1 in 28% [17], SCP-1 in 15% [18], SSX4 in 12.5% [19], MAGE-3 in 7% [17], and SSX2 in 2.5% [19], have been reported in OC. Among these CT antigens, NY-ESO-1 has been successfully applied to immunotherapy for OC patients. After primary surgery and chemotherapy, high-risk OC patients in first clinical remission received NY-ESO-1b peptide without serious vaccine-related adverse events. Vaccine-induced CD8+ and CD4+ T cell clones were shown to recognize

Table 2. Immunostaining in OC tissue and OC adjacent tissue.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Staining intensity N (%)</th>
<th>χ² value</th>
<th>p value</th>
</tr>
</thead>
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<tr>
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<td>+ 21 (78)</td>
<td>6 (22)</td>
<td>0 (0)</td>
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<tr>
<td>OC tissues (n=87)</td>
<td>++ 35 (40)</td>
<td>24 (28)</td>
<td>17 (20)</td>
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<tr>
<td>Grade 1 (n=23)</td>
<td>+++ 10 (44)</td>
<td>4 (17)</td>
<td>6 (26)</td>
</tr>
<tr>
<td>Grade 2 (n=27)</td>
<td>++++ 7 (26)</td>
<td>11 (41)</td>
<td>5 (18)</td>
</tr>
<tr>
<td>Grade 3 (n=37)</td>
<td>+ 18 (48)</td>
<td>9 (24)</td>
<td>6 (16)</td>
</tr>
</tbody>
</table>

Legend: + (1), ++ (2), +++ (3), ++++ (4)

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301
NY-ESO-1-expressing tumor targets. Long-lived and functional vaccine-elicited CD8+ and CD4+ T cells were detectable in some patients up to 12 months after immunization [20, 21]. As OY-TES-1 has higher frequency in OC demonstrated by the present authors and others, it seems to be potential in the future to develop OY-TES-1 immunotherapy for OC. However, the present result also indicated the majority of cases with immunostaining intensity and heterogeneity of OY-TES-1 protein expression, which may affect OY-TES-1 as an immunotherapeutic target applied at least in OC. It is unknown whether the heterogeneity of OY-TES-1 protein expression may result from DNA methylation and/or histone acetylation. It has been demonstrated that the epigenetic events influence some of CT gene expression, such as MAGE family is responsible for the gene demethylation [5,22]. Therefore, the mechanism of OY-TES-1 expression should be further investigated. If the expression of OY-TES-1 is regulated by the methylation of CpG islets in its promoter region, utility of combining demethylating agent 5-aza-2’-deoxycytidine (DAC) therapy with OY-TES-1 vaccine therapy may help to improve the heterogenic expression of OY-TES-1 that will greatly increase the efficiency of treatment.

Recently, it was found that OY-TES-1 was both necessary and sufficient for paclitaxel resistance in ovarian cancer cell lines and ovarian tumor explants. Moreover, high expression of OY-TES-1 was correlated with reduced survival time and faster relapse among ovarian cancer patients [10, 23]. Although no correlation between OY-TES-1 expression and clinicopathological parameters in OC was found in the present study, one-third of OC presented moderate (+ + +) to strong (++++) expression, which may imply immunotherapy suitability for these patients. The high level of OY-TES-1 expression may also be predictive of humoral immune response and cellular immune response presented in OC patients. It has been observed that an OC patient with specific humoral response to OY-TES-1 initially had optimal surgical debulking of Stage IIIC, and was without evidence of disease 40 months after chemotherapy [10]. Moreover, a HLA-A24-binding peptide of OY-TES-1 can be recognized by CD 8+ T cells and induced cytotoxic reaction against tumor cell line expressing OY-TES-1 [24]. Clearly, further extensive analysis of the immune responses in patients including serum antibody as well as T-cell responses will be necessary for development of OC immunotherapy based on the OY-TES-1 as a target.

Acknowledgement

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References


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Comparison of nine morphological scoring systems to detect ovarian malignancy

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Department of Obstetrics and Gynecology, Gülhane Military Medical Faculty, Ankara (Turkey)

Summary
Purpose of investigation: The aim of this study was to prospectively compare the diagnostic performances of nine gray-scale sonographic prediction models to detect ovarian malignancy. Materials and Methods: Clinical data of 322 women presenting with an adnexal mass were obtained and used in nine scoring systems. For each model a ROC curve demonstrating the capacity of the model to diagnose malignancy was constructed for all cases and for the subgroups of premenopause and postmenopause. The performance of each model was expressed as area under the ROC curve, sensitivity, and specificity. Results: The area under the ROC curve, sensitivity, and specificity of these models in the present study varied between 0.737 and 0.929, 70.7% and 87.9%, 60.2% and 80.3%, respectively. Conclusions: This study has revealed the usefulness of morphological scoring systems to correctly discriminate between benign and malignant pelvic masses.

Key words: Ovarian; Ultrasonography; Mass; Model.

Introduction
Adequate preoperative assessment of an adnexal mass is needed since the accurate diagnosis directs management. The preoperative definitive diagnosis of a malignant mass cannot always be made with current diagnostic modalities. However prior to surgery, it is important to differentiate between malignant and benign pathology of the adnexal mass in order not to face a malignancy unexpectedly and to avoid an inappropriate surgery.

Today, several parameters are available to distinguish between benign and malignant masses. For this purpose, gray-scale sonographic parameters are frequently used to evaluate the risk of malignancy. Other parameters that are used in discrimination between benign and malignant masses are Doppler ultrasonography, patient characteristics (menopausal state or age), and biochemical markers (CA 125, human epididymis protein 4 and prealbumin). Some authors [1-9] used only gray-scale sonographic markers as a preoperative diagnostic tool to assess the risk of malignancy while the others [10-12] combined these parameters for the same purpose. In most cases subjective assessment with ultrasonographic views by experienced sonologists is a main method for detecting the malignant mass [13]. However less experienced sonologists should still use morphological scoring systems that can be helpful to classify adnexal masses as benign or malignant. The present authors hence decided to reveal which gray-scale scoring model or models should be used by less experienced sonographers.

Overall, the aim of this study was to prospectively compare the diagnostic performances of nine gray-scale sonographic prediction models to detect ovarian malignancy.

Materials and Methods
A Medline search was performed to detect diagnostic models that are principally based on sonographic characteristics and are used to distinguish benign masses from malignant ones. “Ovarian”, “ultrasonography”, “mass”, and “model” were the key words. The authors also checked the references of the detected articles. Overall, they detected nine scoring systems six of which were morphological indices [3-5, 7-9] and three of which were morphological classifications [1, 2, 6]. All parameters used in these models were only gray-scale ultrasonographic signs.

The clinical data of 322 women with pelvic masses appointed for laparotomy or laparoscopy between October 1, 2008, and October 7, 2012, to the present hospital were obtained prospectively and used in nine scoring systems. Preoperative examination included vaginal examination and transvaginal sonography. The ultrasound was performed transvaginally by a 7.5-MHz transducer. A transabdominal repeat examination with full bladder was obtained if a mass was found to be too large to be observed completely transvaginally. The histopathological diagnosis was considered the gold standard for definitive outcome. Malignancy was defined according to criteria of histologic typing of ovarian tumors, according to WHO criteria [14], and staged according to criteria recommended by the International Federation of Gynecology and Obstetrics (FIGO). The authors included low malignant potential tumors as malignant. Cut-off levels we performed were 9, 4, 9, 5, 7, 3, 2, 8, and 5 for the scoring systems of Sassone et al., Lerner et al., Ferrazzi et al., DePriest et al., Finkler et al., Maggino et al., Granberg et al., Szpurek et al., and Ueland et al., 324x370 Canada Inc.

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Figure 1. — A: Comparison of ROC curves for all patients B: Comparison of ROC curves for premenopausal subgroup. C: Comparison of ROC curves for postmenopausal subgroup.
respective[1-9]. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS), Version 15.0. For each model a ROC curve demonstrating the capacity of the model to diagnose malignancy was constructed for all cases and for the subgroups of premenopause and postmenopause. The performance of each model was expressed as an area under the ROC curve, sensitivity, and specificity. The McNemar’s test was used for testing differences in performances between nine scoring systems. When each score was compared with the other eight in the series, the authors obtained thirty-six comparisons of paired samples. Score 1 was compared with scores 2, 3, 4, 5, 6, 7, 8, 9 (eight comparisons); score 2 was compared with scores 3, 4, 5, 6, 7, 8, 9 (seven comparisons); score 3 was compared with scores 4, 5, 6, 7, 8, 9 (six comparisons); score 4 was compared with scores 5, 6, 7, 8, 9 (five comparisons); score 5 was compared with scores 6, 7, 8, 9 (four comparisons); score 6 was compared with scores 7, 8, 9 (three comparisons); score 7 was compared with scores 8, 9 (two comparisons), and score 8 was compared with scores 9 (one comparison). The specificity was defined as the percentage of patients with malignant disease having a positive test result. The specificity was defined as the percentage of patients with a positive test result having malignant disease and a negative test result. The positive predictive value was defined as the percentage of patients with a negative test result having benign disease. Finally, the diagnostic accuracy was expressed as a proportion of correctly classified subjects (true positive + true negative) among all subjects. In the present study the authors assumed a concluding error risk of 5% and (correlated with the risk) significance level of $p < 0.05$ indicating statistically significant differences.

Results

Between 2008 and 2012, 322 patients were operated for an adnexal mass. The median age of these patients was 41.64 years, with a range from 16 to 79 years (SD: 13.262). Eighty-eight (27.3%) of the 322 patients were postmenopausal. Overall, 58 patients (18%) turned out to have a malignancy. Among the malignant diagnosis, there were 26 serous carcinomas, 16 mucinous carcinomas, one ovarian lymphoma, three Krukenberg tumors, one dysgerminoma, one granulosa cell tumor, three borderline serous tumors, and seven borderline mucinous tumors. Thirty (51.7%) of the patients had FIGO Stage I disease, ten (17.2%) had FIGO Stage II disease, 11 (19%) had FIGO Stage III disease, and seven (12.1%) had FIGO Stage IV disease; 264 cases were found to be benign. Among the benign diagnosis there were 18 simple cysts, 91 endometriosis, 51 dermoid cysts, 28 serous cystadenomas, 16 mucinous cystadenomas, nine fibromas, five thecomas, 12 corpus luteum cysts, 17 paratubal cysts, seven leiomyomas, four struma ovarii tumors, and six tuboovarian abscesses. The area under the ROC curves of these models in the present study varied between 0.737 and 0.929 (Figure 1A). When the sensitivity was fixed at 0.90, specificity varied between 0.42 and 0.79. The highest area under ROC for all cases was 0.929 with Szpurek index. For premenopausal subgroup, the highest area under ROC was 0.913 for Szpurek index (Figure 1B). For postmenopausal group the highest area under ROC were 0.949 for Szpurek index (Figure 1C). Corresponding ROC curves and other values of area under ROC curves are highlighted in Figure 1A, B, and C. Accuracies reported in the present and the original studies are shown in Table 1.

In all patients, the highest sensitivity was 87.9% for De-Priest index with a relatively low specificity value of 67.8% and positive and negative predictive values of respectively 37.5% and 96.2%. Diagnostic accuracy of De-Priest index was 71%. Sensitivities, specificities, positive and negative predictive values, and diagnostic accuracies of all other scoring systems in all cases are presented in Table 2.

In the premenopausal subgroup, sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy for Szpurek index were 82.1%, 80.6%, 36.5%, 97.1%, and 80%, respectively. Sensitivities, specificities, positive and negative predictive values, and diagnostic accuracies of all other scoring systems in the premenopausal subgroup are presented in Table 3.

In the postmenopausal subgroup, sensitivity, specificity, positive and negative predictive values, and diagnostic ac-

### Table 1. — Accuracies reported in the original and present studies.

<table>
<thead>
<tr>
<th>Author</th>
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<th>Specificity (%)</th>
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<th>Specificity (%)</th>
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### Table 2. — The sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values, and diagnostic accuracy (DA) of nine scoring systems in all cases.

<table>
<thead>
<tr>
<th>Author</th>
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<th>PPV (%)</th>
<th>NPV (%)</th>
<th>DA (%)</th>
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The sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values, and diagnostic accuracy (DA) of nine scoring systems in the premenopausal subgroup.

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The sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values, and diagnostic accuracy (DA) of nine scoring systems in the postmenopausal subgroup.

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Discussion

Assessing an ovarian mass with an ultrasonography is generally subjective and depends on the performer’s experience and skill. However in the preoperative evaluation of an ovarian mass, the correct way that takes us to the diagnosis of malignancy is still made by clinical impression and ultrasound examination. For this reason explicit criteria should be established and defined rigidly for adnexal mass malignancy. Higher diagnostic performance of Morphological indices or classification systems and six morphologic indices are useful in clinical practice, are easy to perform, and should therefore be the test of choice in the preoperative evaluation of the adnexal mass. Morphological indices or classification systems studied here can be used for the discrimination between benign and malignant pelvic masses and for selection of cases for optimal therapy. Although some degree of simple mathematical calculations are mandatory for gray-scale morphological indices, they appear to be very accurate, are useful in clinical practice, are easy to perform, and should therefore be the test of choice in the preoperative evaluation of the adnexal mass. Higher diagnostic performance of Szpurek Index is making it attractive to be used for detecting malignancy.

Subtle differences of examination technique and definitions may partly illuminate the lower performances in the present study than those in the original ones. The most important contributing factor to the differences in results is likely to be true differences in the tumor populations studied. In the present study, certain tumor types tended to be over-represented among the true negative, and false positive diagnoses. Simple benign cysts and endometriomas were usually truly classified as benign and were over-represented among the true-negative diagnoses. In the false positive group, cystadenomas, tuboovarian abscesses, leiomyomas, and fibromas were often misclassified as malignant.

Although it is believed that tumor volume estimation lowers index specificity [3], in the present study, indices using tumor volume as a parameter [4, 8, 9], had higher performances due to large sample of benign cysts. The present authors obtained low positive predictive values due to the high number of false-positive cases and they met high negative predictive values because of the fact that the number of the false-negative cases was low. The reason of this fact is that, in the tumor populations studied, low malignant potential tumors were not much and mostly classified as malignant because of their large volumes.
Comparison of nine morphological scoring systems to detect ovarian malignancy

References


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Cervical squamous cancer mRNA profiles reveal the key genes of metastasis and invasion

Yuan Cheng¹, Ding Ma¹, Youyi Zhang², Zijian Li²*, Li Geng¹*

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Summary
Purpose of investigation: To investigate mRNA expression profiles associated with cervical squamous carcinoma progression and to identify key genes involved in invasion and metastasis of cervical squamous cancer. Materials and Methods: The authors extracted the mRNA expression profile of eight normal cervical tissues by human whole genome microarray. The main functions of differentially expressed genes were identified by gene ontology (GO) analysis. Gene-networks were established based on bioinformatic approaches. Microarray data of the expressions level of key genes verified by qRT-PCR. Results: The authors identified 2036 differentially expressed genes between two groups including 1,282 down-regulated genes and 754 up-regulated genes (p < 0.05, FDR < 0.05). Gene-network revealed that PDGFRA, CAV1, and GJA-1 were critical for cervical cancer invasion and metastasis. Conclusions: PDGFRA, CAV1, and GJA-1 were revealed as key node genes for cervical cancer invasion and metastasis. The results may provide new evidences and ideas for early diagnosis and prognosis assessment of cervical cancer.

Key words: Cervical cancer; Gene expression profile; Microarray; mRNA.

Introduction
Cervical cancer is the third most common malignant tumor in women worldwide, preceded only by breast cancer and colorectal cancer. There is an estimated 500,000 cases of cervical cancer and 260,000 patients that die from the disease each year globally. The highest incidence rate is observed in Eastern, Western and Middle Africa; Central America; South-Central Asia, and Melanesia [1]. Premature mortality caused by cervical cancer in 23 countries is higher than that caused by breast cancer, according to the survey [2]. About 130,000 new cervical cancer cases are diagnosed in China each year, accounting for 1/5 of all cases worldwide. Recent trends suggest increased incidence of cervical cancer among younger women [3,4]. High risk human papillomavirus (HR-HPV) infection is an important risk factor in cervical cancer development. However, only few women infected with HR-HPV eventually develop cervical cancer [5]. It is apparently that oncogene activation, tumor-suppressor gene inactivation, and disruption of gene clusters of cell cycle, apoptosis, adhesion, immunity, and signal transduction occur during the progression from precancerous lesions to infiltrating carcinoma [6]. Clinical data show that the five-year survival rate among patients with cervical intraepithelial neoplasia (CIN) is nearly 100%. The five-year survival rate for patients with Stage I cervical cancer is about 70%-90% and for patients with Stage IV is only around 20% [7,8]. These data indicated that cervical cancer invasion and metastasis to other sites such as lymph node, lung, and bone seriously affects the clinical outcome and prognosis [9]. At present, the carcinogenesis of cervical cancer invasion and metastasis is still unclear, warranting further studies.

Biological processes such as cell division, proliferation, differentiation, and apoptosis are characterized by changes in gene expression. Gene expression is activated or inhibited in response to external environmental or biochemical signaling, producing distinct gene expression profiles, which are termed as “molecule signatures” of specific physiological or pathological conditions. Such signatures elucidate the relationships between gene function and signal transduction pathways and provide insights into various biological activities. With gene microarray technology, expression of tens of thousands of genes is analyzed simultaneously in single experiment, and multiple gene clusters associated with specific diseases and the key players of disease pathogenesis are identified. Rapid automated high-throughput gene analysis is achieved [10]. Screening for differentially expressed genes between normal cervical tissues and cervical cancer tissues with gene expression microarray provides important data underlying pathogenesis and molecular mechanism in cervical cancer development.

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and progression. As early as 2004, Sopov et al. extracted RNA from 20 CIN3 samples and ten cervical squamous cancer samples, amplified with self-monitoring, analysis and reporting technology (SMART), hybridized with 1,176 tumor-related gene probes in microarray and finally identified 92 differentially expressed genes [11]. In 2006, Chao et al. performed gene expression profile analysis of 16 cases of cervical squamous cell carcinoma and nine cases of adenocarcinoma of the cervical cancer by gene microarray and found 653 differential expressed genes [12]. They found a higher expression of CEACAM5, TACSTD1, S100P, and MSLN in adenocarcinoma, while S100A9 and ANXA8, were expressed higher in cervical squamous cell carcinoma. Lyng et al. analyzed gene expression profiles in 19 cases of cervical cancer without lymph node metastasis and ten cases of lymph node metastasis [13]. The data showed that eight genes were associated closely with cervical cancer metastasis and clinical prognosis.

Despite the decline in cervical cancer incidence rates due to early screening for HPV and cervical smear test of exfoliated cells, the incidence of cervical cancer is still the highest among malignant tumors of women’s reproductive system [1]. Squamous cell carcinoma is the most common pathological type of cervical cancer, accounting for about 75% of all cervical tumors [14]. It mainly occurs in the junction between columnar epithelium of the endocervical canal and the stratified squamous epithelium of the ectocervix (squamocolumnar junction). Therefore, the present study mainly focused on the gene expression profiles of normal cervical tissues and cervical squamous carcinoma tissues to explore the differentially expressed genes in the tumorigenesis. Microarray analysis revealed 2,036 differentially expressed genes, of which 755 genes were upregulated and 1281 genes downregulated. Further exploration of differential mRNAs with gene ontology (GO) analysis and gene regulatory network construction revealed PDGFRA, CA V1, and GJA-1 as the key genes involved in cervical cancer metastasis and invasion, providing evidence and insights for further investigation of cervical cancer pathogenesis and targeted therapy.

Materials and Methods

Source of samples
The study was approved by the Peking University ethics committee (approval No. IRB 00001052-06058). Informed consent was signed by all patients who diagnosed with IB-IIIB cervical cancer or benign gynecological diseases before operation for this study. The cervical cancer samples/CCS (n=8) and normal cervical samples/ NCS (n=8) were collected from Peking University Third Hospital. The average age of patients with cervical cancer HPV+ and benign gynecological diseases HPV- was 41.75±3.250 and 45.13 ±3.425 respectively. The case information was obtained from medical records and pathology diagnosis.
Microarray analysis
The authors extracted the microarray expression profile and standardized by Agilent G4450AA feature extraction software 10.0. This study analyzed gene expression profiling on Agilent human whole genome microarray (Agilent G4112F Design ID 014850, 4×44 K format).

GO analysis
GO analysis, the key functional classification of NCBI, was employed to analyze the main function of the genes. Generally, Fisher’s exact test and 2 test were used to classify the GO category, and false discovery rate (FDR) was calculated to correct the p value. The smaller the FDR, the smaller the error in judging the p value. FDR was defined as FDR = 1–Nk/T, where Nk refers to cases wherein the p value of Fisher’s test is less than that of 2 test. Only GOs with p < 0.05 and FDR < 0.05 were selected [15, 16].

Pathway analysis
Similarly, Kyoto encyclopedia of genes and genomes (KEGG, Japan), Biocarta (Germany), and Reatome (USA) are open-source for analyzing microarray data on biological pathway [17, 18]. In the present study, these were used to identify significant pathway correlated with cervical cancer.

Gene-gene network
Gene-gene network was built based on KEGG database. Gene-gene network was evaluated by graph theory method based on their “degree” in the network. The number of genes regulated by mRNA was defined as the degree of the mRNA.

qRT-PCR
Total RNA was extracted from cervical cancer samples and control samples. cDNA was synthesized by Quant reverse transcriptase, dNTP and random primer, and one ug total RNA. The cycling condition of qRT-PCR reaction consisted in an initial two minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C, 20 seconds at 64°C, and 30 seconds at 72°C.

Results
Differentially expressed mRNAs in normal and cervical cancer
Tumor formation is a complicated process in which multiple genes and pathways are involved. Gene microarray technology is used for screening of genes involved in tumorigenesis. In this study the authors used Agilent gene expression microarray to analyze the mRNA expression changes between eight normal cervical tissues (HPV-) and eight cervical squamous cancer tissues (HPV+). Microarray data revealed 2,036 differentially expressed genes between two groups (754 genes upregulated and 1,282 genes downregulated) (p < 0.05, FDR < 0.05) and cluster analysis of differentially expressed genes was performed (Figure 1A). Colors in gene clustering map represent the expression level of mRNAs in tissues. The authors noticed that gene expression profiles in normal cervical tissues and in cervical cancer tissues are quite distinct. The top 25 upregulated...
and downregulated mRNAs suggested that the expression of TCAM1, AIM2, SYCP2, MMP12, CXCL9/10/11, and STAT1 were elevated significantly, while the expression of SPINK5, PPP1R3C, ZNF521, S1PR3, and TGF-β2 declined notably.

According to the NCBI gene functional annotations, the authors performed enrichment analysis of the 2,036 different genes (Figure 1B). Results showed that about 14.79% of the genes contribute to signaling, 12.92% to system development, 11.47% to cell proliferation, 7.64% to immune response, 4.41% to cell adhesion and 1.93% to cell migration.

Significant functional analysis of differential genes GO analysis

GO analysis provides valuable insights into the relationship between gene functions. The authors annotated the functions of 2,036 genes according to GO database (classified with NCBI gene function annotations) to identify significant GO functions, with a $p<0.05$, and FDR$<0.05$. They found 135 upregulated GO functions and 193 downregulated GO functions ($p<0.05$ and FDR$<0.05$). Significant GO functions of downregulated genes are mainly associated with mitotic cell cycle, immune responses, cell proliferation, and inflammations.
Cervical squamous cancer mRNA profiles reveal the key genes of metastasis and invasion

(Figure 2A), while the significant GO functions of upregulated gene are mainly involved in epidermis development, cell adhesion, extracellular matrix secretion, and keratin differentiation (Figure 2B).

**Significant interaction of networks among pathways—Path-Net**

The authors further analyzed the 2,036 genes by pathway analysis and constructed Path-Net map depending on the statistical significance (p value) of differential gene-associated pathways (Figure 3) (Table 1). They noticed that MAPK signaling, pathways in cancer, cytokine-cytokine receptor interaction, and focal adhesion were critical to the development of cervical cancer. Path-Net map showed that interaction of signaling pathways closely related to cervical cancer migration, invasion, and metastasis such as focal adhesion, regulation of actin cytoskeleton, Wnt signaling, adherens junction, and ECM receptor affected the development and progression of cervical cancer.

**Gene-gene networks in cervical cancer metastasis and invasion**

Cell adhesion declined significantly in cervical tumorigenesis in the GO and pathway analysis. Such changes favored the invasion and metastasis of cervical cancer. GO-Map and pathway analysis revealed that the interaction of signaling pathways closely related to cervical cancer migration, invasion, and metastasis such as focal adhesion, regulation of actin cytoskeleton, Wnt signaling, adherens junction, and ECM receptor affected the development and progression of cervical cancer. The authors therefore, listed the different genes involved in cell adhesion, focal adhesion formation and cytoskeleton, which are related to cervical cancer invasion and metastasis. In combination with signal transduction pathways and gene-protein relationships in KEGG database, they constructed gene regulatory signal transduction network (Figure 4) (Table 2). Depending on the value of centrality (a larger score representing higher influence of a specific gene in signal trans-
Yuan Cheng, Ding Ma, Youyi Zhang, Zijian Li, Li Geng

production) and degree (representing the number of genes interacting with a specific gene in a network) of genes in networks, they noticed that platelet-derived growth factor receptor alpha (PDGFRA), caveolin-1 (CAV1), gap junction protein, alpha 1, 43kDa (GJA1), and fibroblast growth factor receptor 1 (FGFR1) play a central role. They also noticed that members of integrin family including integrin alpha-3/4 (ITGA3/4) and collagen, type V, alpha 1/2 (COL5A1/COL5A2) are important in the network.

Microarray data verification
To evaluate the microarray data, the authors used qRT-PCR to analyze gene expression profiles in normal cervical tissues and cervical cancer tissues. They checked the expressions level of PDGFRA, CAV1 and GJA1, the critical genes involved in cervical cancer metastasis and invasion, and confirmed the reliability of microarray data (Figure 5).

Discussion
Cervical cancer is one of the most common cancers of the female reproductive system. Despite the decreasing incidence due to improved screening approaches such as cervical smear test, the prognosis of cervical infiltrating and metastatic carcinoma remains poor with a high recurrence rate. The five-year survival rate after surgery is still very low [19, 20]. Currently, the development of effective tools to predict invasive cervical cancer and metastasis is still ongoing, requiring additional studies. Genetic screening contributes to the understanding of cervical cancer pathogenesis by identifying novel biomarkers and candidate genes essential for tumorigenesis. The present study has established gene expression profiles of eight normal cervical tissues and eight cervical squamous cancer tissues. The authors discovered several well-known tumor-associated genes such as MMP-12, STAT1, and CXCL9, which were upregulated significantly in tumor samples and promoted tumor proliferation, metastasis, and invasion [21-23]. Multiple known cervical cancer-related genes such as CKS2, TBX3, and LAM2 were also identified. Lyng et al. performed microarray analysis of cervical cancer with or without lymph node metastasis. The results suggested that CKS2 and TBX3 were closely related with survival rate of metastatic carcinoma [13]. Imura et al. identified Lam-5 as an efficient biomarker in cervical infiltrating carcinoma diagnosis [24]. In addition, multiple genes such as TCAM1, AIM2, SYCP2, SPINK5, and PPP1R3C changed significantly in this study, which were not identified in previous studies. Testicular cell adhesion molecule 1, known as TCAM1, is highly conserved in the evolution of mammals.

Table 2. — Cervical carcinoma metastasis and invasion of regulatory networks of mRNA related degree value.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Description</th>
<th>Centrality</th>
<th>Degree</th>
<th>Indegree</th>
<th>Outdegree</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAV1</td>
<td>Caveolin 1, caveolae protein, 22kDa</td>
<td>0.03516</td>
<td>9</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>Platelet-derived growth factor receptor, alpha polypeptide</td>
<td>0.02962</td>
<td>27</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>GJA1</td>
<td>Gap junction protein, alpha 1, 43kDa</td>
<td>0.01494</td>
<td>8</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>FGFR1</td>
<td>Fibroblast growth factor receptor 1</td>
<td>0.01448</td>
<td>13</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>LRP6</td>
<td>Low density lipoprotein receptor-related protein 6</td>
<td>0.01053</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>EDNRB</td>
<td>Endothelin receptor type B</td>
<td>0.01031</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>ITGA3</td>
<td>Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)</td>
<td>0.00979</td>
<td>14</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>IRS1</td>
<td>Insulin receptor substrate 1</td>
<td>0.00952</td>
<td>15</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>CCR7</td>
<td>Chemokine (C-C motif) receptor 7</td>
<td>0.00940</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>ITGA4</td>
<td>Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)</td>
<td>0.00814</td>
<td>14</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>MAP1B</td>
<td>Microtubule-associated protein 1B</td>
<td>0.00811</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>COL1A2</td>
<td>Collagen, type I, alpha 2</td>
<td>0.00754</td>
<td>9</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>CDH2</td>
<td>Cadherin 2, type 1, N-cadherin (neuronal)</td>
<td>0.00728</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>ADCY1</td>
<td>Adenylate cyclase 1 (brain)</td>
<td>0.00662</td>
<td>10</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>
Higher expression of TCAM1 in cell membrane has been shown to be related with female infertility [25], but no reports have shown the role of TCAM1 in cervical cancer. These novel genes provide new ideas and challenges in elucidating the pathogenesis of cervical cancer.

Tumor invasion and metastasis is a complicated process involving multiple genes. Abnormal changes include cytoskeletal remodeling, diminished cell adhesion, and extracellular matrix degradation [26, 27]. Normal epithelial cells stick together by cell adhesion molecules to form tight connections. Local infiltration of carcinoma in situ is associated with decreased adhesion forces between tumor cells mediated by cell adhesion molecules [28]. Cell migration and transformation during invasion and metastasis is promoted by elevated cellular deformability. Cellular cytoskeleton is mainly composed of microtubules, microfilaments, and intermediate filaments. Microtubule depolymerization is the major driving force of cell movement. Previous studies showed that family members of the Rho Family GTPase (Rac1, Cdc42, RhoA, and RhoC) participate in cellular signal transduction and regulate the remodeling of cytoskeleton [29-31]. The Wnt-β-catenin signal pathway has been shown to be closely related with tumor invasion and metastasis. In tumor cells, E-cadherin overexpression blocked the transcriptional regulation by β-catenin and switched off the expression of target genes to inhibit the proliferation and migration of tumor cells [32]. In this study, GO analysis and Path-Net analysis of differential genes in cervical cancer indicated that cell adhesion, cell movement, and cellular cytoskeleton regulation are important in the development of cervical cancer. A network of genes associated with cervical cancer metastasis and invasion was constructed with genes involved in focal adhesion, Wnt signaling pathway, regulation of actin cytoskeleton and Rho family. Network analysis revealed the central role of PDGFRA, CAV1, FGFR1, and GJA1 in the whole network. The authors also checked the expression level of PDGFRA, CAV1, and GJA1 in cervical tissues with qRT-PCR and identified that the expression of these three key genes in cervical cancer was consistent with microarray data.

PDGFRA belongs to receptor tyrosine kinase family. Ligand binding to PDGFRA is related to cell growth and division [33, 34]. Studies have shown that PDGF-PDGFR signaling is associated with vascular smooth muscle cell migration and remodeling though Akt, ERK1/2, and EGFR [35, 36]. Taja-Chayeb et al. analyzed the expression level of PDGFRA in 36 cervical cancer samples (32SCC, 4ASC) with immunohistochemistry and found downregulation of PDGFRA expression in 58.4% of all cervical cancer samples [37]. With microarray and qRT-PCR analysis, the present authors found that PDGFRA expression in cervical cancer samples decreased significantly compared with normal samples. Regulatory network analysis of tumor invasion and metastasis-associated genes revealed that the indegree value of PDGFRA-related signal transduction pathways [10] and the outdegree value [22], suggest the indispensable role of PDGFRA in the entire regulatory network. GO and pathway analysis showed that PDGFRA is mainly involved in extracellular matrix organization and positive regulation of cell migration, which is essential for tumor metastasis and invasion. The authors also found that PDGFRA participated in cervical cancer invasion and metastasis by regulating the downstream genes such as SRC, PIK3CA, KRAS, and GRB2.

In recent years, loss of tumor suppression gene CAV1 was associated with tumor proliferation, invasion, metastasis, disrupted signal transduction, and multiple drug resistance [38, 39]. Miotti et al. found that CAV1 deletion leads to E-cadherin redistribution inside cells, reduced cellular interaction. Studies by Williams et al. revealed a negative correlation between CAV1 and MMP expression [44]. The present authors found that CAV1 deletion may have direct or indirect interaction with β-catenin or TGF-β to promote the invasion and metastasis of cervical cancer cells.

Connexin 43 protein, encoded by GJA1 gene, is involved in cell-cell signaling and related signal transduction pathways. Previous studies revealed that reduced or deficient connexin 43 expression resulted in abnormal cell-cell communication, diminished surveillance, and regulation of cellular function, excessive cell proliferation and elevated cell migration [45-47]. Crespin et al. reported that in neuroblastoma, connexin 43 affected cell morphology through regulation of actin cytoskeleton [48]. The present results showed that GJA1 expression declined significantly in cervical cancer tissues compared with normal cervical tissues. Network analysis of tumor invasion and metastasis-associated genes indicated that GJA1 participated in the process of cervical cancer adhesion, migration and metastasis to promote malignant transformation of cervical cancer.

**Conclusion**

In conclusion, gene microarray and bioinformatics analysis showed that PDGFRA, CAV1, and GJA1 are critical for cervical cancer metastasis and invasion. The results elucidate the key role of genes involved in cervical cancer de-
velopment and progression, providing insights into the underly- ing mechanism, and offer evidence for further identi- fication of novel biomarkers and key genes in cervical cancer.

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Comparative study of phosphorylated histone H2AX expressions in the cervical cancer patients of pre- and post-neoadjuvant chemotherapy

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3 GanSu Cancer Hospital, Lanzhou (China)

Summary
Objective: This study aimed to determine whether phosphorylation of histone H2AX (γH2AX) is a predictive marker for neoadjuvant chemotherapy patients of cervical cancer. Materials and Methods: The sections were divided into three sets. Set 1 consisted of 40 pre-treatment biopsies. Post-treatment tissues includes 38 patients in set 2 and 34 patients in set 3 who received cisplatin concurrent docetaxel treatment for one or two cycles, respectively. Formalin-fixed paraffin-embedded sections were analyzed after antigen retrieval and fluorescence antibody labeling for γH2AX staining. Results: The expressions of γH2AX in cervical cancer tissues of post-neoadjuvant chemotherapy decreased to 22.94 ± 14.02% and 23.68 ± 13.55% (one and two cycles treatment, respectively) compared to pre-neoadjuvant chemotherapy (28.29 ± 15.67%), however there was no significant difference for γH2AX expression between pre- and post-neoadjuvant chemotherapy patients (F=1.425, p = 0.245). There was no significant correlation between γH2AX expression and clinicopathologic parameters in patients of pre- and post-neoadjuvant chemotherapy. Conclusions: As a predictive marker for neoadjuvant chemotherapy of cervical cancer, more extensive research regarding γH2AX expression should be explored.

Key words: γH2AX; Cervical cancer; Neoadjuvant chemotherapy.

Introduction
DNA double-strand breaks (DSB) can arise from mistakes during DNA replication, from external agents, such as ionizing radiation or during genomic rearrangements. DSB can induce chromosomal aberrations that cause cells to malfunction, resulting in cell death or tumorigenesis [1, 2]. One of the earliest steps in the cellular response to DSB is the phosphorylation of histone H2AX at serine 139 (γH2AX), the site of γ-phosphorylation [3]. The number of γH2AX foci is a significant marker for DSB.

Invasive squamous cell carcinoma (ISCC) of the uterine cervix is one of the most frequent malignancies in women worldwide. Concurrent use of cisplatin with radiotherapy has become the standard of care in the treatment of patients with advanced cancer of the cervix [4, 5]. Prediction of response to treatment therefore requires a method that is sensitive to tumor response to both agents. Several papers have discussed the potential for γH2AX to serve as a predictive marker for cancer treatment [6-10]. immunohistochemical analyses of γH2AX have been reported for human cancers of the urinary bladder, breast, lung, colon, and prostate [11-13]. It was also reported that γH2AX-positive cells are overexpressed in ISCC of uterine cervix and the cervical cancer of radiochemotherapy [14-16]. These results suggest that staining of γH2AX correlates with DNA damage checkpoint activation in malignant lesions and the process of tumor therapy. Therefore, the existence of γH2AX foci might be a useful and sensitive marker for cancer, especially for detecting the results of cancers therapy.

In order to test if the expression of γH2AX in ISCC of uterine cervix could be a more accurate indicator for neoadjuvant chemotherapy, the authors analyzed γH2AX expression in cervical cancer tissues of pre- and post-neoadjuvant chemotherapy followed by surgery. In addition to their primary goal: to assess for a relation between γH2AX expression and clinicopathologic characteristics, as another more important goal the expression of γH2AX in cervical cancer tissues of pre- and post-neoadjuvant chemotherapy was also analyzed for its impact on response to chemotherapy.

Materials and Methods
Patients and tumor specimens
Formalin-fixed, paraffin-embedded sections were stained for γH2AX foci and were analyzed using visual scoring methods. Tumor incisional biopsies and surgical specimens, four-μm thick, were prepared from three patients sets. The first set of slides was ...
prepared from pre-chemotherapy incisional biopsies from 40 patients with cervical cancer. The second and third sets of slides were taken from 38 and 34 surgical specimens of cervical cancer patients who received the neoadjuvant chemotherapy. These patients received treatment of cisplatin (40 mg/m²) concurrent docetaxel (75 mg/m²) for one (set 1) or two cycles (set 2). Tumors ranged from FIGO Stage IB-IIIA, and all the tumors were squamous cell carcinomas (ISC). The mean ages of the patients pre- and post-neoadjuvant chemotherapy were 48.87 and 48.27 years, respectively (range: from 21 to 78 and 24 to 66) for ISC. All the slides were collected and prepared by the Pathology Department at the GanSu Tumor Hospital between 2012-2013, and the sections were kept at 4°C before dewaxing and staining.

Immunohistochemistry analysis of γH2AX

From each patient, one representative tumor block, including the tumor center and invasive front was examined by immunohistochemistry. In cases of large, late-stage tumors, different sections were examined to include representative areas of the tumor center, as well as of the lateral and deep tumor invasive fronts.

The same paraffin-embedded tissues as those used for the original hematoxylin and eosin-stained sections were chosen for immunohistochemistry. Paraffin-embedded tumor tissue was prepared, and after dewaxing and rehydrating in graded alcohols, slides were immersed in high pH target retrieval solution in a 95°C water bath for 30 minutes. Slides were washed in TBS, blocked for ten minutes, and incubated with mouse anti-γH2AX antibody (1:500 dilution) for two hours. Slides were rinsed and incubated for one hour with Alexa Fluor 594-conjugated AffiniPure goat anti-mouse IgG antibody (1:200 dilution). To stain nuclei, slides were submersed in 0.05 μg/mL DAPI for five minutes, rinsed, and finally mounted with ten μL Fluorogard. Tumor sections were viewed using a fluorescent microscope, using ×10, ×40, and ×100 Plan Neofluor objectives. As this was considered a feasibility study, no specific procedures were adopted to ensure that images were obtained randomly throughout the section. Because sections rather than whole cells were scored, cells with one or more γH2AX foci were counted as positive. Efforts were made to score only tumor regions, and obvious regions of necrosis or areas of stroma were not included in the analysis. Foci results are presented as averages of scores for several high-power images. Digitized images (eight to 12 images containing 50-100 nuclei each) were scored by eye for the percentage of nuclei presenting γH2AX foci, and the results are presented as averages [14, 15, 17].

Statistical methods

Data are presented as means ± SD. The levels of γH2AX expression in cervical cancer tissues of pre- and post-neoadjuvant chemotherapy were analyzed by One-way ANOVA. Association between γH2AX expression levels and clinicopathologic parameter (ages, lymph node metastasis or TNM stage) were evaluated using t-test. Association between γH2AX expression level and tumor grade was evaluated by One-way ANOVA too. SPSS version 17.0 software was used for the statistical analysis. A p-value < 0.05 was considered statistically significant.

Results

High power images of the biopsies were analyzed visually for fraction of nuclei that were γH2AX positive, defined as one or more foci per nucleus. γH2AX yielded granular, nuclear staining. The nuclei were frequently immunoreactive without any pattern, even pyknotic nuclei of the cells associated with horn pearls displayed immunostaining. The reactivity was frequently detected in mitotic and apoptotic cells or karyorrhectic debris, respectively. Normal glandular epithelium remained unstained. In addition, nuclei of immature squamous metaplasia were decorated by the antibody used, as were lymphocytes. Normal glandular endocervical epithelium showed occasional nuclear staining. Cells with pan-nuclear γH2AX staining, consisting of hundreds of individual foci, were observed in these tumor sections. The classic imagination of γH2AX expression in biopsies of pre- and post- chemotherapy are shown in Figure 1.

For the tumor biopsies of pre-neoadjuvant chemotherapy, the γH2AX positive cells ranged from 5% to 64%, and on average, only 28.29% of cells scored as γH2AX positive. The tissues taken from the patients after first cycle neoadjuvant chemotherapy showed a significant decrease in the percentage of nuclei with γH2AX foci on average 22.94% nuclei (range: 5–71%). A similar decrease was shown in tissues taken from the patients after second cycle neoadjuvant chemotherapy. There was 23.68% of the nuclei that scored as γH2AX positive with a range of 5% to 52%. However, there were no remarkable differences in γH2AX.
Comparative study of phosphorylated histone H2AX expressions in the cervical cancer patients of pre- and post-neoadjuvant chemotherapy

The authors then analyzed the association between γH2AX staining and clinicopathologic parameters in cervical cancer tissues of pre- and post-neoadjuvant chemotherapy. γH2AX staining showed no significant association with ages, tumor grade, lymph node metastasis or TNM stage in the cervical cancer tissues of pre- and post-neoadjuvant chemotherapy groups (Tables 2, 3).

Discussion

Impaired radiochemotherapy responsiveness of tumor is a major clinical problem in several solid tumor types including cervical carcinoma. Activation of DNA damage repair networks is central in the molecular responses to radiochemotherapy, and within these networks the γH2AX is the significant marker of DNA damage and repair [18]. In order to detect the potential of γH2AX as a predictive marker for neoadjuvant chemotherapy patients, the expression of γH2AX in cervical cancer tissue of pre- and post-neoadjuvant chemotherapy were researched in the present study.

When the expression of γH2AX was investigated in cervical cancer tissues of pre- and post neoadjuvant chemotherapy, three different categories of immunexpression was confirmed by statistical evaluation. The results of γH2AX expression in uterine cervix of pre-treatment was 28.29%, similar to those previously using 47 pre-treatment cervical cancer biopsies [16]. What the present authors are more concerned with is the information provided by γH2AX expression on cervical cancer response to chemotherapy and the potential for γH2AX to serve as a predictive marker of cervical cancer treatment. The expression of γH2AX in cervical carcinoma pre- and post radiochemotherapy were analyzed [15,16]. In one research, eight patients received weekly cisplatin (40 mg/m2) to a maximum of five cycles and concurrent external beam pelvic radiation of 45 Gy in 25 fractions over five weeks. Biopsies were obtained before the day’s radiation treatment. On average, only 25% of tumor nuclei exhibited one or more γH2AX foci before treatment and 74% after the start of treatment. In another research, 26 tumor biopsies taken before and 24 hours after the first fraction provided the opportunity to examine the retention of γH2AX foci in relation to local control and fraction size. Before treatment, 24% of cells contained γH2AX foci, 24 hours after exposure to the first fraction of 1.8–2.5 Gy, 38% contained foci. These results are in marked contrast to the present, in which cervical cancer of post-neoadjuvant chemotherapy showed a downregulation of γH2AX expression and may be explained by several factors. Thus, in the study of Bañuelos et al. [16] the biopsies were taken before the day’s radiation, however the exact time point, such as the cycle number of chemotherapy before the biopsies were taken, was unclear. Moreover, Olive et al. [15] analyzed γH2AX expression at 24-hour post-radiation, that is, at ongoing radiation-induced cell cycle perturbation, while the present analysis was per-

### Table 1. — Immunohistochemical analysis of γH2AX expression in cervical cancer patients of pre- and post-neoadjuvant chemotherapy.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>γ-H2AX expression</th>
<th>t/F</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td>Pre-neoadjuvant chemotherapy</td>
<td>40</td>
<td>28.29±15.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-neoadjuvant chemotherapy (one cycle)</td>
<td>38</td>
<td>22.94±14.02</td>
<td>1.425</td>
<td>0.245</td>
</tr>
<tr>
<td>Post-neoadjuvant chemotherapy (two cycle)</td>
<td>34</td>
<td>23.68±13.55</td>
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</tr>
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</table>

### Table 2. — Relations between γH2AX expression and clinicopathologic characteristics in cervical cancer patients of pre-neoadjuvant chemotherapy.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>γ-H2AX expression</th>
<th>t/F</th>
<th>p</th>
</tr>
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<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>26.76±13.77</td>
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<tr>
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<td>18</td>
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<td></td>
</tr>
<tr>
<td>Stages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>8</td>
<td>32.35±21.88</td>
<td>-0.816</td>
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</tr>
<tr>
<td>II–III</td>
<td>32</td>
<td>27.27±13.98</td>
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<tr>
<td>Tumor Grade</td>
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<tr>
<td>Low</td>
<td>16</td>
<td>27.92±12.45</td>
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<tr>
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<td>14</td>
<td>28.69±16.62</td>
<td>0.009</td>
<td>0.991</td>
</tr>
<tr>
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<td>10</td>
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</tr>
<tr>
<td>Lymph node metastasis</td>
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</tr>
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<td>10</td>
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<td>30</td>
<td>28.34±15.56</td>
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### Table 3. — Relations between γH2AX expression and clinicopathologic characteristics in cervical cancer patients of post-neoadjuvant chemotherapy.

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<th>t/F</th>
<th>p</th>
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</thead>
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<td>≥50</td>
<td>35</td>
<td>24.11±15.66</td>
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<td>Stages</td>
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</tr>
<tr>
<td>I</td>
<td>8</td>
<td>20.36±12.69</td>
<td>-0.709</td>
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<td>II–III</td>
<td>64</td>
<td>24.02±13.88</td>
<td></td>
<td></td>
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<tr>
<td>Tumor Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>27</td>
<td>21.63±10.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>23</td>
<td>23.61±16.54</td>
<td>0.171</td>
<td>0.843</td>
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<tr>
<td>High</td>
<td>17</td>
<td>23.67±13.90</td>
<td></td>
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<td>unknown</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>17</td>
<td>23.54±13.21</td>
<td>-0.080</td>
<td>0.937</td>
</tr>
<tr>
<td>Yes</td>
<td>55</td>
<td>23.85±15.68</td>
<td></td>
<td></td>
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</table>
formed at 21-31 days after first or second cycles of neoadjuvant chemotherapy. One potential mechanism that could explain the observed decrease in γH2AX expression is that tumor cells with high γH2AX expression will progress through the cell cycle after treatment and will thereby constitute a larger proportion of the residual tumor. Another potential mechanism is that apoptosis occurred for many γH2AX staining cells because 56% of cells with pan-nuclear γH2AX staining were also positive for the apoptosis marker, activated caspase-3 [16]. In fact except γH2AX, the other downstream signaling components of DSB, such as p53, p21, and mdm-2 have been researched with the aim of predicting tumor necrosis for many γH2AX staining cells because residual tumor. Another potential mechanism is that apoptosis progression through the cell cycle after treatment will be arrested either at G1 or at G2/M, whereas tumor cells with low γH2AX expression will be arrested either that tumor cells with high γH2AX expression or with capacity to induce γH2AX expression will be arrested either at G1 or at G2/M, whereas tumor cells with low γH2AX expression will progress through the cell cycle after treatment and will hereby constitute a larger proportion of the residual tumor. Another potential mechanism is that apoptosis occurred for many γH2AX staining cells because 56% of cells with pan-nuclear γH2AX staining were also positive for the apoptosis marker, activated caspase-3 except γH2AX, the other downstream signaling components of DSB, such as p53, p21, and mdm-2 have been researched with the aim of predicting radiochemo-therapy response in cervical cancer [19, 20]. The results reported by Beskow et al. [19, 20] are partly in accordance with the present. In their research a decreased expression of p21 was shown in cervical cancer biopsies of radiotherapy, and suggesting downregulation of p21 associated with radioresistant. The expression of γH2AX is decreased in cervical cancer tissues of post neoadjuvant chemotherapy, however, there were no remarkable differences in γH2AX expression between pre- and post- neoadjuvant chemotherapy groups. Perhaps, much more samples or corresponding tumors of pre- and post- neoadjuvant chemotherapy should be used to analyzed γH2AX expression in the future research.

According to Brustmann et al.’s research, no statistical significance could be established for FIGO Stages I vs II in ISSC, because their study was limited to the low FIGO stages only [14]. Although the present authors chose 40 pre- and totally 72 post-neoadjuvant chemotherapy specimens with FIGO stages ranging from IB-IIIA, γH2AX staining showed no significant association with ages, tumor grade, lymph node metastasis or TNM stage in the uterine cervix.

The present authors report that the expression of γH2AX showed no significant association with ages, tumor grade, lymph node metastasis or TNM stage in the uterine cervix. They also observed a decreased expression of γH2AX in cervical cancer tissues of post-neoadjuvant chemotherapy, although there were no remarkable differences. The present results suggest that one mechanism of chemotherapy resistance in cervical cancer perhaps can include downregulation of γH2AX expression. However, the findings reported in this study need to be confirmed in larger materials.

Conclusion

The expression of γH2AX is not different between pre- and post-neoadjuvant chemotherapy patients. As a predictive marker for neoadjuvant chemotherapy of cervical cancer, more extensive research regarding γH2AX expression should be explored.

Acknowledgements

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Reference


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30 years of colposcopic studies: validity of local destructive treatments

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Clinica Hospital of Medical Specialties Chemsa, Panama City (Republic of Panama)

Summary
The effectiveness of local destructive treatments (LDT) applied in patients due to cervical pathology oncogenic risk (OR), were followed and verified in 396 patients who came to our attention, focusing on the type of pathology, type of treatment received, diagnosis clinical evolutionary, and results of the new study applying polymerase chain reaction (PCR), and above all, the time between the diagnosis, treatment, and PCR findings. The clinical evolution of the same reports achieved a healing rate of 82% followed by persistence 9.3%, improvement 4.8%, recurrence 2.8%, and only one case of progression 0.2%. The elapsed time in initial care and treatment was almost immediate, as the pathology diagnosis was considered on an emergency basis. Successive controls of these indicated that 119 studies of routine colposcopy were carried out, on an average of the first three years and with a maximum follow up of 30 years, with over 30 routine colposcopies that achieved healing in most of these. In 2011 and 2012, we added to the usual diagnostic methodology, molecular biology, and 119 studies were performed in those patients, resulting in only five negative cases. Most studies were classified as high risk papillomavirus (HR-HPV), corresponding to subtypes 31, 35, 18, and 16.

Key words: Local destructive treatment; Oncogenic risk; Colposcopy; Cervical cancer.

Introduction
Cervical cancer is the second female tumor worldwide with the highest incidence in the developing countries [1]. In the developed countries, the Papanicolaou test has significantly reduced its frequency [2]. Due to the role of human papillomavirus (HPV) infection in the carcinogenesis [3], two new approaches were adopted to fight this neoplasm: vaccination in younger women [4] and HPV detection in the remaining population. The organized screening prevention has reduced the incidence of cervical cancer by about 75% in higher income countries during the last 50 years [5]. The challenge for the future is to extend the possibility of effective prevention also in developing areas of the world, with the aim to detect this neoplasm that is today a diffuse cause of death.

Materials and Methods
In the 30 years of study, 12,679 patients were treated and 6,411 of them were diagnosed as oncogenic risk (OR), from which 4,353 corresponded to non-clinical HPV or clinical HPV associated with dysplasia or cancer. Most of these underwent local destructive treatments (LDT), and a follow up was performed to evaluate the effectiveness of the treatment, either cryosurgery or diathermo-coagulation or cautery [6].

We realized that achieving a natural immune response against HPV infection is almost impossible, since the acquired immune response requires inflammation. It is with cryosurgery and/or cautery that we managed to activate the immune system, which undoubtedly has been reached. This was demonstrated by routine colposcopy, applied three months until a clinical cure was achieved, which signified the destruction of viral cytopathic effect, visible to colposcopy. Similar experiences have been described by various authors, according to the criteria reported by S. Dexeus [7-9].

When we talk about cryo, we refer to provoking a lesion in the affected area by freezing it at approximately -60°C, killing the cells through water intoxication, while cautery produces desiccation of cells and thus, the expected immunological, local, regional, and systemic response.

The selected LDT method was applied, either by itself or combined, achieving good results. The preferred method was cryosurgery and the one that been applied was the slow method, in which we first froze the lesion for two to three minutes, then it was slowly thawed. The lesion was then frozen again for two minutes maximum and was slowly thawed once again. We complemented the treatment by using tetracycline, vitamin, and albutyl. Within a month, we conducted clinical inspection and then, after three months a routine colposcopy was performed [5].

We selected 396 patients with OR diagnosis, who had their follow ups during this century, which corresponded to 265 HPV, 117 dysplastic cancers, seven cancers, and 11 pure ORs (Table 1). Among the group, there were 321 cases that were treated with cryosurgery; only 51 cases with a combined treatment of cryosurgery and cautérisation and 87 cases with cautérisation only. We repeated this treatment as often as necessary, if the clinical lesion could not be eradicated within one or three months. Treatment was performed as soon as we diagnosed the OR and a follow-up was performed after three months and a new treatment was applied if the lesion persisted. After the lesion was removed, successive controls were performed every six months and/or annually.
Patients were assisted with clinical controls every six months or every year, achieving a definitive evolutional diagnosis, in which healing prevailed. The PCR test was applied at one to five years in 199 cases, at ten years in 94 cases, at 15 years in 41 cases, at 20 years in 25 cases, and at 30 years in 12 cases; they all resulted positive, except for the five negative cases and all of them resulted with HR-HPV (Table 2).

### Results

From the 396 cases, in which a follow up was given until date, 326 of them were considered cured due to the elimination of the viral cytopathic effect, verified by routine colposcopy. Only 33 cases persisted, 19 improved, 11 recurred and only one cases progressed (among dysplastic cases) (Table 3). Among the dysplastic cases, there were two pending cases and among HPV, four pending cases.

From a pathological classification perspective, healing was achieved in 100% of cancer cases and pure OR, but dysplastic and pure HPV cases prevailed in 210 and 98 cured cases, respectively, followed by persistence in 31 cases and improvement in 12 cases (Table 4).

Healing was the prevailing clinical presentation and in order to apply a quality control, PCR test was incorporated and applied in 119 women, and it was found that only five cases were negative and the remaining were considered to be HR-HPV, with 31, 18, 35, and 16 as the serotypes most frequently found, in 40, 27, 19, and 17 cases, respectively (Table 5). These results with healing clinical findings, despite the virus remaining in the human body, but without producing any cytological or histologic changes that might lead to cancer (as a host) over the last 30 years is what allowed us to say that the LDT has achieved on one hand, the HPV lesion destruction, and its neutralization by specific immunology, but on the other hand with the risk that the HPV chronic infection can lead to cancer (Table 6).

### Discussion

Patients that have been managed under a follow-up protocol allowed us to evaluate the therapeutic method, individual response, PCR linkage, evolutional diagnosis, and overall, the ability to convey clear concepts to the patients in order achieve a better life quality [10-12].

### Conclusions

In these 30 years of studies, we have demonstrated that local conservative treatments, are valid for three simple reasons:
1) The economic costs of aggressive treatments, compared with surgical ones are much more expensive;
2) The risk, to which the patient is exposed with aggressive treatments, sometimes causes significant damage to the organs;
3) It makes no sense that a low-risk diagnosis must be reached by applying aggressive treatments.

Our experience with the patients managed in this study, indicate that their evolution is flattering, by showing no progression to invasive cancer.

References


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Introduction

Small cell neuroendocrine carcinoma of the uterine cervix (SCNEC) is a rare gynecologic malignancy that represents less than 3% of all cervical cancer [1-3]. The histology and biologic behaviors of the tumor are similar to that of small cell lung carcinoma (SCLC), which is highly aggressive. The tumor is characterized by a high incidence of early distant metastases, resulting in poorer prognosis than other subtypes of cervical cancer [3-5]. Due to its rarity, studies exploring therapeutic efficacy in this setting generally require long enrollment periods to obtain a sufficient number of cases. Therefore, to date, most studies of neuroendocrine cervical cancer are comprised of small series and case reports, making it difficult to draw conclusions on prognostic factors and optimal treatment modalities.

Given the aggressive nature of neuroendocrine small cell cervical cancer, it is imperative to identify potential treatments that can improve the outcomes of these patients. The present authors therefore adopted a protocol of neoadjuvant chemotherapy (NACT) most of patients using the etoposide plus cisplatin (EP) regimen in an effort to improve outcomes at the present center.

In this study, we evaluated the efficacy and safety of NACT with EP before primary radical surgery followed by adjuvant chemoradiation or chemotherapy for Stage I-IIIB SCNEC.

Materials and Methods

Eligibility

Patients from Zhejiang Cancer Hospital from January 1997 to December 2010 for clinical Stage I-IIIB SCNEC. Patients were eligible if they had histologically confirmed small cell carcinoma in the cervix. Of the 23 patients with available paraffin blocks who were diagnosed as having small cell carcinoma on the basis of hematoxylin and eosin (H&E) staining, all had positive staining for one or more neuroendocrine markers. All tumors were staged according to the International Federation of Gynecology and Obstetrics (FIGO) clinical staging system for cervical cancer based on physical examination, chest X-ray, in-
travenous paleography, cystoscopy, sigmoidoscopy, and abdomino-pelvic computed tomography (CT) or magnetic resonance imaging (MRI) scan. When there were suspicious findings on chest X-ray or the presence of signs and symptoms upon physical examination, a CT scan of the chest and/or brain was carried out.

Treatment

All patients received one to four cycles of NACT and two to eight cycles containing cisplatin (NACT and adjuvant chemotherapy), 17 (73.9%) patients received NACT using a regimen consisting of EP. Eighteen (85.7%) patients received adjuvant chemotherapy using a regimen consisting of EP. Other chemotherapy regimens including paclitaxel and cisplatin (TP), bleomycin vincristine and cisplatin (BVP), doxorubicin etoposide and cisplatin (EAP), and ifosfamide together with IEP as shown in Table 1. After NACT, patients underwent radical hysterectomy and lymphadenectomy. Subsequently, external beam pelvic radiotherapy (EBRT) was initiated within six weeks of surgery. EBRT was delivered to a total dose of 45-48 Gy in 27 daily fractions over five to six weeks. External-beam therapy was delivered using anterior-posterior fields, box fields, or conformal radiotherapy and ten MV photons. Intracavitary treatment was delivered using Fletcher-suit after loading high-dose-rate applicators. Patients underwent concurrent or sequential adjuvant chemoradiation. Dose adjustment was based on the greatest toxicity grade, using the National Cancer Institute Common Toxicity Criteria for Adverse Event. Chemotherapy was repeated every three weeks, providing the patient’s absolute neutrophil count recovered to > 1,500/mm3 and platelets were > 100,000/mm3. The does of each drug was reduced by 20% of previous does in the case of grades 3 and 4 toxicities. Chemotherapy was withheld until resolution of any grade 3 or 4 non-hematologic toxicity.

Follow-up

The primary end point was any cancer-related death. All end points were calculated from the date of diagnosis to death, or censored at last follow-up. The date of death was obtained from the medical records, personal contact, or the National Registry of Death statistics of the China National Statistical Office.

Statistical analysis

All statistical analyses were performed using SPSS v.19 software. Survival curves were estimated using the Kaplan-Meier method, and p values were generated using the log-rank test. All tests were two-tailed with p values < 0.05 considered significant. All end points were updated in August 2013.

Table 1. Patient characteristics.

<table>
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</tr>
<tr>
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<td>6</td>
</tr>
<tr>
<td></td>
<td>IB2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>II A</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>III B</td>
<td>3</td>
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<tr>
<td></td>
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<tr>
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<tr>
<td></td>
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<tr>
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</tr>
<tr>
<td></td>
<td>Yes</td>
<td>9</td>
</tr>
<tr>
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<td>DSI</td>
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<tr>
<td></td>
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<tr>
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<td></td>
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<td>4 (4/8)</td>
</tr>
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<td></td>
<td>IIIB</td>
<td>2 (2/3)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>2 (2/2)</td>
</tr>
</tbody>
</table>

NACT: neoadjuvant chemotherapy; RT: radiation; CT: chemotherapy; CCRT: concurrent chemoradiation. RH: radical hysterectomy; LNI: lymph node involvement; LSI: lymphovascular space invasion; DSI: depth of stromal invasion.

Table 2. Demographic and treatment factors with associated five-year OS.

<table>
<thead>
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<th>n</th>
<th>5-year OS</th>
<th>p value</th>
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<td>13</td>
<td>46.2%</td>
<td></td>
</tr>
<tr>
<td>&gt; 40</td>
<td>10</td>
<td>60.0%</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>0.174</td>
<td></td>
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</tr>
<tr>
<td>I-II A</td>
<td>18</td>
<td>66.7%</td>
<td></td>
</tr>
<tr>
<td>III B-IIIB</td>
<td>5</td>
<td>0.0%</td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>0.196</td>
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<tr>
<td>≤ 4</td>
<td>8</td>
<td>75.0%</td>
<td></td>
</tr>
<tr>
<td>&gt; 4</td>
<td>15</td>
<td>42.7%</td>
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<tr>
<td>LNI</td>
<td>0.169</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>15</td>
<td>61.1%</td>
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<td>Yes</td>
<td>8</td>
<td>37.5%</td>
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<tr>
<td>LSI</td>
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<tr>
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<td>14</td>
<td>78.6%</td>
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</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>0.0%</td>
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</tr>
<tr>
<td>DSI</td>
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<tr>
<td>≤ 2/3</td>
<td>14</td>
<td>85.7%</td>
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<tr>
<td>&gt; 2/3</td>
<td>9</td>
<td>0.0%</td>
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<td>Histological homology</td>
<td>0.502</td>
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<tr>
<td>Pure</td>
<td>16</td>
<td>48.2%</td>
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<td>Mixed</td>
<td>7</td>
<td>71.0%</td>
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</tr>
</tbody>
</table>

LNI: lymph node involvement; LSI: lymphovascular space invasion; DSI: depth of stromal invasion.
the time of analysis. The response rate of NACT as primary therapy was 95.7% (CR 3, PR19, SD 1, PD 0). The median overall survival (OS) periods for those who survived and died during the evaluation period were 51.7 and 21.2 months. The estimated five-year disease-free survival (DFS) and OS rates were 61.1% and 66.7%, respectively for Stage IB1–IIA patients. The estimated five-year DFS and OS rates were 46.2% and 56.5%, respectively, for all patients. Currently 11 patients are alive and disease free, one patient is alive with disease, and 11 have died of disease. With the exception of three patients, relapse sites were unknown and there were one or multiple relapse sites for other nine patients. Relapse sites included the lung (n=5), liver (n=5), bone (n=3), para-aortic nodes (n=2), and brain (n=2). Eight patients (72.7%) with relapse were dead within three years of the first treatment.

### Results

From 1998 to 2010, 23 patients were enrolled and received at least one cycle of NACT. The characteristics of the patients included in this study are described in Table 1. The median patient age at diagnosis was 39 years (range: 25–65). The FIGO stage distribution was follows: six were Stage IB1, four were Stage IB2, eight were Stage IIA, three were Stage IIB, one was Stage IIIA, and one was Stage IIIB. The therapeutic regimens and clinical outcomes for all 23 patients are shown in Table 1. Five patients with advanced stage disease receiving NACT gained the opportunity of surgery. Three patients of which two were Stage IB1, one was Stage IIA had postoperative pathologic complete responses through one to two cycles NACT of EP regimen with a median follow-up duration 69.5 months (range: 51.1–177.1), without recurrence. However, the other eighteen patients who had not postoperative pathologic complete responses through NACT with a median follow-up duration 38.8 months (range: 7.3–81.5).

For all the patients, age, stage, tumor size, LNI, LSI, DSI, and histological homology were assessed; LSI and DSI (stromal invasion depth of cervix > 2/3) were found to be significantly associated with a worse prognosis compared to those patients without LSI and DSI (p = 0.005, p = 0.001, respectively). Although not statistically significant, age (≤ 40 years), Stage (IIB–IIIB), tumor size (> four cm), LNI, and pure histological homology tended to adversely affect survival (Table 2).

### Toxicity

Among 23 patients assessable for toxicity evaluation, the most common toxicity was hematologic, and the levels were mostly acceptable. The incidence of grades 3 and 4 toxicity was follows: anemia, 60.9%; neutropenia, 52.2%; thrombocytopenia, 0%; liver insufficiency, 4.3%, renal insufficiency, 0%; no treatment-related death occurred during therapy. The toxicities are summarized in Table 3.

### Pattern of recurrence and survival

The median survival was 40.8 months (range: 7.3–177.1 months) for all patients. The median survival was 48.9 months (range: 7.3–177.1) for Stage IB1–IIA patients. Eleven out of 23 patients were alive without recurrence at the time of analysis. The response rate of NACT as primary therapy was 95.7% (CR 3, PR19, SD 1, PD 0). The median overall survival (OS) periods for those who survived and died during the evaluation period were 51.7 and 21.2 months. The estimated five-year disease-free survival (DFS) and OS rates were 61.1% and 66.7%, respectively for Stage IB1–IIA patients. The estimated five-year DFS and OS rates were 46.2% and 56.5%, respectively, for all patients. Currently 11 patients are alive and disease free, one patient is alive with disease, and 11 have died of disease. With the exception of three patients, relapse sites were unknown and there were one or multiple relapse sites for other nine patients. Relapse sites included the lung (n=5), liver (n=5), bone (n=3), para-aortic nodes (n=2), and brain (n=2). Eight patients (72.7%) with relapse were dead within three years of the first treatment.

### Discussion

Based on reports from different hospitals, SCNEC is a rare disease [6]. That is associated with a poor prognosis. Because SCNEC occurs infrequently, it is difficult to perform a randomized controlled clinical trial to determine optimal therapy. The current study analyzed a large series of patients diagnosed with SCNEC from a single institution experience, which included an update of a previous reported series [7].

NACT has been recommended for patients with tumor size > four cm [5, 8]. However, another previous study that found that patients who received NACT tended to have a worse median OS that those who did not receive NACT [9]. Whether NACT can improve the prognosis for cervical cancer patients remains a matter of debate. We therefore carried out a retrospective trial to identify the efficacy and toxicity of NACT for patients with early-stage SCNEC. Although radical surgery is not associated with prolonged survival relative to definitive radiation for patients with SCNEC [10, 11], most gynecologic oncologists and patients in China favor radical surgery. Most patients with FIGO Stage IB1–IIA tumors underwent radical surgery as the main mode of treatment. Although favorable results have been reported for patients with SCNEC who received concurrent chemoradiation followed by several additional cycles of chemotherapy [4, 11], other studies have reported that radical surgery is an important component in the multimodal treatment of SCNEC [5, 12, 13]. However, patients with large lesions (> four cm) did poorly despite radical surgical treatment in this current series. Bermudez et al. [14] recommended NACT containing vincristine, bleomycin, and platinum for patients with large lesions > four cm. Based on his series of 13 patients who received NACT, it seems that preoperative chemotherapy may be a useful treatment method to enhance the resectability of the large tumors to improve outcome. However, Lee et al. [9] found that two of five patients with Stage IB1 and all six patients with Stage IB2–IIA tumors
treated with NACT died of their disease. They thought that although NACT might be useful for enhancing the resectability of bulky tumors, it did not improve survival.

The present results indicate that through preoperative chemotherapy, 18 patients with early-stage SCNEC received NACT including nine cases with bulky tumors; only one case had pelvic recurrence, seven cases died of their disease, all due to disease metastases, with a median survival of 48.9 months (range: 7.3–177.1) and an OS rate at five years of 66.7%. Although limited by the small number of patients included in this analysis, we did show improvement in the OS rate over the previously reported five year survival of 31.6–46.6% for Stage (I–IIA) patients [5, 9, 15]. In addition the present data showed that for 18 early–stage patients who received NACT, three (16.7%) patients achieved a complete response (CR) after one to two cycles of NACT of EP regimen. These three patients achieved long-term survival without recurrence, with a median follow-up duration 69.5 months (range: 51.1–177.1). Therefore, NACT may be an approach for assessing response to treatment.

We applied NACT to five patients with advanced stage disease (IIB–IIIB), of which gained the opportunity of surgery. While only one patient with Stage IIB disease is alive at the end of follow up, the remaining four patients died of their disease, two of which with pelvic recurrence. These results suggest that hysterectomy after NACT may confer little benefit in the setting of advanced stage SCNEC.

We also observed that DSI and LSI were poor prognostic factors. The five-year survival rate for patients without DSI was 78.6% compared to 11.1% for patients with DSI ($p = 0.001$). The five-year survival rate for patients without LSI was 71.4% compared to 22.2% for patients with LSI ($p = 0.005$). These results were consistent with those of a previous study [16]. Although not statistically significant, LSI tended to adversely affect survival.

We recognize the limitations of this study. Firstly, this was a small, single institute study that had inherent limitations. There was no comparative group for use as a control. Therefore, the favorable survival obtained in this study can only be compared indirectly with historical controls. Secondly, due to the fact that this study was not a prospective study, the chemotherapy regimens were not unified. However, despite these limitation, to the best of the authors’ knowledge, this is the first retrospective study that has tested an EP regimen in preoperative NACT follow postoperative adjuvant therapy for SCNEC.

Conclusion

We demonstrated a favorable outcome in OS in early-stage patients treated with EP regimen preoperative NACT follow postoperative adjuvant therapy. Toxicities are manageable. Therefore, this study suggests that a prospective, randomized controlled study should be designed to evaluate efficacy of this approach compared with the current primary radical surgery, followed by adjuvant chemotherapy for patients with early-stage SCNEC.

References


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Rare case of coexistence of primary ovarian carcinoid in mature teratoma with primary serous carcinoma in second ovary – a case report

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Summary
Ovarian malignant tumours are mostly ovarian cancers. The most frequent ovarian benign lesions are mature teratomas. A very rare ovarian neoplasm is carcinoid. It mostly occurs as a component of mature teratoma, what causes rare diagnosis before surgery. Study presents the case of patient with primary ovarian carcinoid in mature teratoma of one ovary, co-existing with primary epithelial carcinoma of another ovary. Surgical treatment of carcinoid involves adnexectomy or hysterectomy with adnexa and removal of great omentum, followed by chemotherapy and radiotherapy. In young women with early-stage tumours, treatment can be limited to adnexectomy followed by close monitoring. In the presented case, management associated with the diagnosis of ovarian carcinoid, resulted in the detection of early-stage ovarian epithelial cancer. This case seems to confirm the recommendations to take tissue samples from the other ovary for histopathological evaluation in cases of ovarian unilateral benign tumours.

Key words: Carcinoid; Teratoma; Serous carcinoma.

Introduction
Ovarian malignant tumours, usually occurring in the peri- and postmenopausal periods, are mostly ovarian cancers. In Poland, ovarian cancer comes after breast, lungs, uterine corpus, and the large intestine cancers to become the fifth most common malignant tumour in women. It is believed to arise from the epithelial cells covering the ovary. The morbidity rate for ovarian cancer is 11.2/100 000 per year. In Poland, over 3,000 new cases are recorded each year. The risk of this tumour suddenly increases in the fifth decade of life, and successively raises up to the eighth decade. Typically, clinical symptoms occur late, when the disease is already advanced [1, 2]. Approximately 40-50% of ovarian cancers are serous tumours. Some of the most frequently diagnosed benign lesions of the ovary are mature teratomas. They constitute 45% of all ovarian neoplasms, including 58% of benign tumours. About 80% of mature teratomas develop in the reproductive age, especially in the second and third decades of life. Teratomas are rated among germ-cell neoplasms. Tissues that they consist of are mainly ectodermal derivatives, such as sebaceous and sweat glands, epidermidis, and hair follicles [3]. Malignant transformation takes place in one to three percent of cases.

A very rare ovarian neoplasm is a carcinoid, which constitutes about 0.3% of ovarian tumours. Primary ovarian carcinoid, which represents 0.5-1.7% of all types of carcinoid tumours, belongs to the group of neuroendocrine neoplasms. It mostly occurs as a component of mature teratoma, which is why it is rarely diagnosed before surgery. As a rule, the diagnosis is based on the histopathological evaluation of the surgically removed ovarian lesion. There are no randomised studies on this issue, and the knowledge of biology, prognosis, and treatment comes from retrospective and case studies. Consequently, there are no uniform guidelines for the management of the disease [4]. In the available literature, the authors have not found reports on the simultaneous co-existence of epithelial carcinoma of one ovary and carcinoid of another ovary.

This study presents the case of a 43-year-old patient with primary ovarian carcinoid arising in mature teratoma of one ovary, co-existing with primary epithelial carcinoma of another ovary.

Case Report
In March 2011 in the Department of Gynaecology and Urogynaecology, a 43-year-old patient was hospitalized for a focal lesion of the left ovary. The lesion was being monitored from 2007, when it was visualised in the ultrasound examination of the abdominal...
of another one.

Discussion

This study presents the case of the premenopausal patient with a diagnosis of primary carcinoid arising in mature teratoma of one ovary and primary serous papillary carcinoma of another one.

Ovarian carcinoids belong to neuroendocrine neoplasms. They can occur as primary tumours, components of teratomas or mucous tumours (carcinomas, borderline tumours, benign cystic lesions), or secondary (metastatic) tumours. There are cases of lesions occurring in another ovary many years after surgical carcinoid treatment based on unilateral removal of the adnexa [5]. Most carcinoids arise in a dermoid cyst. Primary ovarian carcinoid was described for the first time in 1939 by Steward et al. [6]. In 1975 Godwin after the analysis of 2,837 cases of carcinoid tumours, gave details of three cases of primary ovarian carcinoid [7]. In 30% of cases, carcinoid causes typical symptoms of the carcinoid syndrome [8-10]. The cells of these tumours can produce substances such as: serotonin, bradykinin, histamine, the peptide YY, and gastrin. Hence, patients may complain of hot flushes, redness of the skin, diarrhea, stomach ache, and tachycardia. The carcinoid syndrome usually affects patients with the islet cell tumour [4, 11-14]. In the presented case, the patient only had hot flushes without any other symptoms of the carcinoid syndrome, therefore a diagnosis of this tumour before surgery was in her case practically impossible, especially because the diameter of a lesion was merely four mm.

A carcinoid component in a dermoid cyst is usually diagnosed postoperatively after histopathological evaluation [11, 15]. Carcinoids are mostly unilateral tumours, but it occur in 15% of cases that mature teratoma, mucous tumour, or Brenner’s tumour occurs in the other ovary [16]. A vast number of carcinoids (almost 50%) are diagnosed in the early phase of development, which gives a good prognosis. In such cases, the five-year survival rate is 90%. [4, 12, 17]. This is true that they are rare neoplasms, and information about biology, prognosis, and treatment comes exclusively from retrospective studies. Consequently, it is difficult to establish uniform guidelines for the management of patients with such diagnoses [4].

Surgical treatment involves the resection of the uterine adnexa or the uterus with adnexa and great omentum, followed by chemotherapy and radiotherapy. Carcinoids are not particularly sensitive to chemo- and radiotherapy, but in cases of malignant tumours, surgical treatment should be combined with platinum and etoposide-based radiochemotherapy. In young women who want to preserve their fertility, and have early-stage tumours, treatment can be limited to adnexectomy followed by close monitoring. The co-existence of such tumours as mature teratoma and carcinoid is usually observed in one ovary [15]. Malignant transformation mainly occurs in patients above 40 years of age. Preoperative diagnosis of dermoid tumours is possible due to a quite characteristic ultrasound image. The treatment of mature teratomas involves enucleation of the whole tumour. It is acceptable to perform laparoscopic surgery with an endobag.

The most important prognostic factor in ovarian cancer is the stage of its development. Unfortunately, clinical symptoms of ovarian cancer manifest relatively late, there-
fore these tumours are usually diagnosed when they are already very advanced. Recommended methods of surgical management at the early stage of the disease are: abdominal hysterectomy with adnexectomy, the removal of the greater omentum, and appendectomy. Peritoneal washings and swabs should be collected. Next, patients are usually qualified for postoperative chemotherapy based on paclitaxel and the derivatives of platinum. In the presented case, further management associated with the diagnosis of ovarian carcinoid resulted in the detection of early-stage ovarian epithelial cancer.

The literature describes cases of the co-existence of histologically diverse tumours. This refers both to the presence of different tumours in the same ovary, and their presence in both ovaries. There is a number of reports on patients with carcinoids arising in teratoma tissue [15]. It occurs that ovarian cancers co-exist with the large cell neuroendocrine carcinoma (LCNEC), which belongs to primary ovarian neuroendocrine tumours. There is also the description of ovarian serous carcinoma cells and neuroendocrine carcinoma cells arising in one tumour [12, 13, 18-20]. The available literature does not provide data about the co-existence of primary ovarian cancer of one ovary, and carcinoid arising in teratoma of another ovary.

In the case presented above, it can be said that considering the small sizes of the tumours and the lack of clinical symptoms, the histopathological diagnosis, and the presence of neoplastic tissues in both ovaries came as a surprise. Fortunately, neoplastic disease was diagnosed early, which gave a good chance of complete recovery.

This case seems to confirm the correctness of the recommendations to obtain tissue samples from the other ovary for histopathological evaluation in cases of ovarian unilateral benign tumours.

References

An unusual case of mammary gland-like carcinoma of vulva: case report and review of literature

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Summary

Introduction: Accessory breast tissue is a very rare finding in the general population with an incidence of one to two percent. An even rarer occurrence is accessory mammary-like tissue which developed breast carcinoma. The authors present a case of aggressive and metastatic carcinoma of vulvar originating from mammary-like tissue. Case: A 73-year-old Caucasian female presented with a lesion in her left vulva. The lesion was ulcerated and fragile. A dermatologist had evaluated the lesion and took a punch biopsy. Result was vulvar carcinoma. She was admitted to the gynecologic oncology clinic then after and was operated. After a radical vulvectomy and bilateral inguinal lymphadenectomy she received adjuvant radiotherapy because of lymph node metastasis. One year after the finish of radiotherapy patient was found to have lung and femur metastasis. She began to receive systemic chemotheraphy for metastasis. Conclusion: Primary mammary-like adenocarcinoma of the vulva is exceedingly rare. There is no consensus about the diagnosis, treatment, and follow up of these patients in literature. However, given that histological data confirms these cancers are behaving like breast cancers instead of known patterns of vulva cancer, the best treatment practices for breast cancer may be applied to treat these vulvar carcinoma patients.

Key words: Vulvar carcinoma; Ectopic breast tissue; Prognosis; Treatment.

Introduction

Ectopic breast tissue may occur anywhere along the primitive milk line from the axilla to vulva [1, 2]. These type of accessory breast tissue is a very rare finding with an incidence of one to two percent [3]. Usually these glands are silent and may only be active during pregnancy and lactation. Ectopic breast tissue may include all the normal components of normal located breast tissue; areola, nipple, and parenchyma [3]. This ectopic tissue responds to all physiological and hormonal changes like the normal one. Malignant changes can be also seen in these abnormal located mammary tissue. Vulvar primary carcinoma of ectopic mammary tissue is an exceptionally rare disease and only 26 cases being reported in the English literature between 1872 and 2012 [4, 5]. Here the authors described vulvar carcinoma arising from mammary-like glands of vulva.

Case Report

A 73-year-old G4P2 Caucasian female presented with a bleeding mass to an urban hospital dermatology clinic. Her general health was good and had a history of hysterectomy together with bilateral oophorectomy for endometrial hyperplasia 17 years ago. A topical steroid treatment was given for three weeks and a biopsy was carried out after that treatment period. Pathologic evaluation of the biopsy revealed that lesion was adenocarcinoma of the vulva with squamous differentiation areas. Patient was referred to the present gynecologic oncology clinic then after. There was a nearly two-cm mass in the left labium minus including the posterior forchette. She had a normal mammography result six months before the presentation. An inguinal lymph node survey was negative for masses. Speculum evaluation of the vaginal cuff was normal.

Computed tomography (CT) scan of the pelvis and abdomen with IV contrast showed no intra-abdominal metastasis. All preoperative blood tests were in normal ranges. Radical vulvectomy and bilateral inguinal lymphadenectomy were carried out in the present clinic. Final pathology revealed that it was a vulvar mammary-like gland adenocarcinoma of 3 x 2 x 3 cm. Histologic grade was 2 and there were two metastatic lymph nodes in every side. Immunohistochemical analysis of tumoral tissue was found as: GCDFP-1 weak positive, P63 negative, ER positive, PR positive, CK8/18, CK7, CK14 ,and E-cadherin all positive.

Patient was discharged after four days of hospital stay after operation and oncology board decided to give her an adjuvant radiation therapy. Between October 19, 2011 and December 1, 2011 vulvar tumor bed, bilateral inguinal and pelvic lymph nodes were treated curatively using volumetric modulated arc radiotherapy. Total dose of 6,000 cGy and 5,000 cGy was given in 30 fractions to the vulvar tumor bed, bilateral inguinal lymph nodes, and pelvic lymph nodes using simultaneous integrated boost technique. Six MV photons and daily kV-kV image guidance was used during treatment. Grade III dermatitis and grade II diarrhea occurred during treatment.

Eighteen months later after the first surgery, pulmonary lesions were found in pulmonary CT scan and positron emission tomog-
computed tomography (PET-CT) confirmed that these lesions were metastasis. Two more metastatic lesions in left femur and L3 vertebra was also diagnosed by this PET-CT scan. Patient had no complaint from pulmonary system and no finding in the vulvar operation area during this time period. She had only moderate degree bone pain in left humerus.

An oncology board consultation was done and it was decided to begin an aromatase inhibitor and zoledronic acid. A second palliative radiotherapy application to bone metastasis was also planned. In February 2013, total palliative dose of 2,000 cGy was given in five fractions to the L3 vertebra and left 1/3 upper humerus using 18 - 6 MV x-rays. Three months after beginning of this therapy regimen all the pulmonary lesions were found to be decreased in size and number and bone metastasis totally cured. Patient is still under same chemotherapy regimen without any complaint.

**Conclusion**

The first reported case of ectopic mammary gland adenocarcinoma located in vulva was reported by Hartung in 1872 [6]. There are nearly 26 cases worldwide in English literature to date [5]. This rare tumor type seems to be growing slowly for years in the vulvar area without complaints. The only way to correctly diagnose and treat this tumor type requires a good and detailed pathological evaluation of both biopsy specimen and surgically resected tissue. Adjuvant treatment regimens may include breast carcinoma experiences of medical oncologists and hormonal therapies like tamoxifen usage or aromatase inhibitors must be kept in mind. Management of patients must be directed like breast carcinoma in every step, although lesion seems to be a gynecologic oncology issue.

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Endoscopic surgery combining chemotherapy for vaginal yolk-sac tumor: a case report

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Summary

Purpose of investigation: To report and explore the effectiveness of the endoscopic surgery combining cisplatin, etoposide, and bleomycin (PEB) chemotherapy in treating vaginal yolk-sac tumor. Case Report: The clinical case of vaginal yolk-sac tumor in a young girl was analyzed. Hysteroscopy equipment was used to inspect and remove the majority of the tumor tissue, followed by six cycles of PEB chemotherapy. Hysteroscopy equipment was used again to examine the vagina and remove the tumor pedicle for biopsy, which revealed no tumor cells after two cycles of chemotherapy. The patient remained disease-free on follow up for a year. Conclusion: The use of hysteroscopy equipment to examine the vagina can diagnose early vaginal yolk-sac tumor and cytoreductive surgery which can preserve fertility can then be performed. Cytoreductive surgery may also be able to reduce the cycles of the chemotherapy to reduce the side effects and the risks of chemotherapy.

Key words: Vaginal tumors; Yolk-sac tumor; Hysteroscopy; Chemotherapy.

Introduction

Vaginal yolk-sac tumor is a rare gynecological malignancy, usually occurring in infants and young children [1, 2]. Aggressive surgery combined with radiotherapy and chemotherapy are primary treatments for this type of disease; however, it can cause serious damage to the organs and, the prognosis is very poor [3, 4]. With the rapid development in recent decades, chemotherapy, represented by cisplatin, etoposide, and bleomycin (PEB) reached a great success. Both chemotherapy and conservative surgery combined with chemotherapy receive good clinical results. However, because of the low disease incidence, the current data are mostly single case reports. It is still unclear how to obtain the maximum therapeutic effect and to minimize the side effects of treatment using the various methods. Here, the authors report a case of vaginal yolk-sac tumor diagnosed in a 11-month-old girl, who had satisfied clinical results after minimally invasive cytoreductive surgery using hysteroscopy equipments combined with chemotherapy.

Case Report

An 11-month-old girl was transferred to the hospital on December 29, 2011, with small amount of bright red vaginal bleeding for more than one month. Ultrasound, which was done in another hospital, showed vaginal fornix approximately 1.3 × 0.7 cm with uneven echo texture. The patient was diagnosed of “vaginal cancer; yolk sac tumor”. The patient’s mother is mentally retarded, with normal menstruation, gravida 1, para 1, with spontaneous vaginal delivery at term, denied using oral contraceptives or diethylstilbestrol during pregnancy. The newborn weighted 2,500 grams with Appgar score of ten for both one minute and five minutes post-delivery.

Current physical examination: good general condition, body temperature of 37.3°C, pulse 120 beats / minute, respiratory rate 22 breaths / minute, blood pressure 85/50 mmHg, height 72 cm, weight 10 kg; no abnormal findings on breast and the whole body. Gynecological examination: normal vulva, no thickening and edema seen on the hymen ring; a small amount of dark red vaginal bleeding seen with small finger rectal examination; a 2 × 2 cm substantive nodule was palpated at the upper middle section of the vagina, with no damage to the surrounding area and with no rectal involvement. Laboratory tests: the hematological and biochemical parameters were all within the normal range, along with the normal reproductive hormone, and the majority of tumor markers, but serum alpha-fetoprotein (AFP) was increased with 43.4 μg / L ( normal range 0-10 ug / L )

Vaginal examination was performed under intravenous anesthesia on December 30, 2011, using hysteroscopy equipment. The exam through the vagina revealed approximately 2 × 2 cm dark red spherical vegetation on the upper vagina, with uneven surface, ulcerated with bleeding spots; the base was wide, located on the left side of the vaginal wall, as shown in Figure 1a. The neoplasm was clamped step by step successfully using the gynecological hysteroscopy extract forceps, with five ml blood loss. The neoplasm was crispy and fish-shaped. Thereafter, the lens was reinserted for re-examination, which showed the majority of the neoplasm had been removed; the base was one cm wide with no active bleeding, located on the left side of the vaginal wall, as shown in Figure 2a. The neoplasm was clamped step by step successfully using the hysteroscopy equipment extract forceps, with five ml blood loss. The neoplasm was crispy and fish-shaped. Thereafter, the lens was reinserted for re-examination, which showed the majority of the neoplasm had been removed; the base was one cm wide with no active bleeding, located on the left side of the vaginal wall from two o'clock to six o'clock positions, five cm from the vaginal opening, approximately one cm from orificium externum uteri, as shown in Figure 2b. The re-examination also revealed that vagina was intact and age appropriate; mucosa on entire vaginal wall was smooth, with no bleeding. Samples were sent for histology.
One dose of antibiotics was given postoperatively to prevent infection. The vaginal bleeding stopped three days after the surgery. Serum AFP dropped to 9.4 μg / L (normal range 0-10 ug / L) two weeks after the surgery, which then reached a normal level. The remaining tumor markers were all within normal range. The pathological diagnosis was vaginal yolk sac tumor, with the immunohistochemistry as AFP (+), CK (cytoplasm+), CK7 (-), alpha-anti-trypsin (+), and PLAP (+), shown in Figure 2. The tumor was at Stage I, according to the International Federation of Gynecology and Obstetrics (FIGO) standard. PEB was given after the surgery as cisplatin 20 mg / m² and etoposide 100 mg/ m² from postoperative day 1 to day 5; bleomycin 20 mg/ m² postoperative day 2 every three weeks for total six cycles. AFP was checked before each cycle of chemotherapy, which was within normal range, as shown in Figure 3. There was a mild hepatic dysfunction and leukopenia at the end of the first course of

Figure 1. — Images of vagina during the first hysteroscopy: (a) preoperation; (b) postoperation.

Figure 2. — The immunohistochemistry of vaginal yolk sac tumor (×400): (a) HE; (b) CK (cytoplasm); (c) AFP; (d) alpha-anti-trypsin.

Figure 3. — Dynamic curve of AFP.
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treatment. Symptoms improved with liver protection and white blood cell symptomatic treatment. However, there were severe side effects that occurred from the second cycle through the last cycle of the chemotherapy, such as severe bone marrow suppression, drop of the complete blood count (CBC), lung infection and hyperpyrexia, which required the colony stimulating factor, red blood cell and platelet transfusion, and ceftriaxone intravenously treatment. The chemotherapy had to be postponed. The first and second cycle of chemotherapy was given every three weeks; afterwards, the interval varied from 23 to 43 days, which the longest occurred between the second and third course. During the chemotherapy, hysteroscopy equipment was used again to perform vaginal examination at the end of the two courses (March 29, 2012) to further clarify whether there was residual tumor tissue. The examination revealed a diameter of two mm light yellow nodule on the left side of the vaginal wall where the original neoplasm base located; no evidence of the bleeding seen on the surface; no abnormal findings on the rest of the vaginal wall; and intact cervical. The nodule was removed, and the biopsy confirmed a small piece of fibrous connective tissue and a small piece of squamous epithelium associated with necrosis with no evidence of tumor.

The patients completed six cycles of chemotherapy by July 20, 2012 and underwent follow-ups for nearly a year. During the follow-ups, she received rectal examination, checking pelvic ultrasound and serum AFP every three months, which all revealed to be cancer free. No evidence of recurrence of the disease was found.

Discussion

Yolk sac tumor, also known as endodermal sinus tumor, is highly malignant tumor derived from germ cells. The lesions develop rapidly with poor prognosis. Patient usually dies within 2 to 4 months after diagnosis without treatment [5]. Primary vaginal yolk-sac tumor is rare. Vaginal discharge and bleeding are the only early clinical signs. Elevated serum AFP can be differentiated from other vaginal diseases in girls with young age [5]. The pathological features and the treatment of yolk-sac tumor are similar with ovarian yolk sac tumor.

The treatment of vaginal yolk-sac tumor was limited to the local radical surgery and radiotherapy before 1965. These treatments brought severe side effects and poor prognosis. The survival rate was only 56.3% [3]. The radical surgery from total vaginectomy to pelvic exenteration, will not only lead to the loss of reproductive function and sexual function in patients, but also damage bladder and rectum function, which have huge impact on the quality of patients’ life. And pelvic radiation has the big possibility of causing ovarian castration, femoral head aseptic necrosis and bone marrow suppression; sometimes even induce a new tumor. After 1970s, there were big changes of yolk sac tumor treatment and the improvement of its prognosis, due to the application of VAC (vincristine, dactinomycin, cyclophosphamide) chemotherapy, and PVB (cisplatin, vincristine, ableomycin), PEB chemotherapy in most recent years [6, 7]. Tao and others reported six cases of using chemotherapy alone to treat vaginal yolk-sac tumor, all the patients obtained complete remission and no evidence of recurrent disease during follow-up of mean 75.5 months [8]. However, the side effects of chemotherapy were remarkable. Many of patients had to stop or delay the chemotherapy due to several serious side effects caused by the treatment. In addition, chemotherapy drugs also should be concerned about side effects with gonadal in children. Thus it is important to consider if using cytoreductive surgery can reduce the using of chemotherapy, thereby reducing its side effects.

It is not too difficult to early diagnose vaginal tumors since they usually have early symptoms such as vaginal discharge; and when the tumor starts to ulcerate and necrosis, it appears as meat-like tissue discharge, or vaginal bleeding. The six cases of vaginal yolk-sac tumor Tao reported showed vaginal bleeding or meat like tissue discharge [8]. The case presenting in this paper also had the first symptom of vaginal bleeding. Tao pointed out that most of the vaginal yolk-sac tumor rarely involves distant metastasis, rather the local invasion [8], which provides strong theoretical basis and operability for the cytoreductive surgery. In this case, we used hysteroscopy equipment to confirm the diagnosis and performed cytoreductive surgery. Post-operative serum AFP decreased to normal levels, suggesting that the tumor burden has been greatly decreased. Hysteroscopy equipment then used again to re-exam after two cycles of chemotherapy, with the biopsy result of “no evidence of tumor”, which suggests that the tumor in this case has reached complete remission at pathology perspective. Due to lack of experience, another four cycles of chemotherapy were still applied even AFP has remained within the normal range. Several severe side effects related to the repeating use of chemotherapy occurred during the last four cycles of chemotherapy. These side effects include severe bone marrow suppression, liver damage and lung infections. Currently, another two to three cycles of consolidation chemotherapy were recommended after normal AFP results, no evidence of tumor under imagine study, and negative pathological examination [8].

Conclusion

The authors propose that during the diagnosis and treatment of vaginal yolk-sac tumor, it is worth consider using hysteroscopy equipment to confirm the diagnosis and to perform cytoreductive surgery, which can reduce the cycles of chemotherapy and consequently its side effects, while improving the patient’s curative compliance and rate.

References


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Introduction

Vaginal neoplasms are uncommon accounting for less than 1% of female genital malignancies [1]. The majority of these vaginal tumours are squamous cell cancers. Rarely, one may encounter a vaginal sarcoma in an adult. It is estimated that primary sarcoma represents about only 2% of all vaginal malignancies. In adults, the most likely form of sarcoma is leiomyosarcoma whereas about 90% of rhabdomyosarcoma (sarcoma botryoides) are seen in children less than five-years-old.

Most cases of leiomyosarcoma occur in the uterus either de novo or less commonly as a result of malignant transformation of a leiomyoma. In common with other sarcomas, leiomyosarcomas display variability in size and shape of the cells and varying degrees of anaplasia in the nuclei. The chromatin is coarse and clumped and mitoses are often numerous and distinctly atypical. The site of origin of vaginal leiomyosarcoma is usually the smooth muscle of the vaginal wall but it may arise in smooth muscle cells in adjacent structures. Unlike other gynaecological malignancies in which the prognosis depends on the staging, leiomyosarcoma, which is confined to the vaginal wall, still carries a poor outcome [2].

Vaginal malignancies are even less common in pregnancy. Leiomyosarcoma which is rarely found in pregnancy is usually detected in the uterus or the vulva [3]. From the present authors' literature search, they report the first case of a vaginal leiomyosarcoma in pregnancy in an Afro-Caribbean woman.

Case Report

A 31-year-old Afro-Caribbean woman of gravidity 6, parity 4+1 presented at her 30-week antenatal appointment with a one-month history of a growth in the vagina which was increasing in size. There was no associated pelvic and abdominal pain nor any abnormal vaginal bleeding, urinary or bowel complaints. She had clinical diabetes mellitus which was well-controlled with a diabetic diet and soluble insulin. There was no past history of any sexually transmitted diseases.

On pelvic examination, two one-two-cm, non-tender wart-like growths were identified on the right posterior aspect of the lower third of the vagina. There was a thick cottage-cheese vaginal discharge consistent with vaginal candidiasis. The patient subsequently had a spontaneous vaginal delivery. She was discharged one day later with a six-week appointment for the postnatal clinic for further evaluation. However, she returned one week later complaining of pelvic discomfort and a burning sensation in the vagina. The growth had increased in size to four to five cm in diameter. The consistency was firm and the base appeared necrotic.

Under general anaesthesia, the tumour which was pedunculated was excised at the base of the stalk. Haemostatic sutures were inserted. On gross examination, two irregular nodular pieces of tissue measuring 1.5 x 2.0 x 3.0 cm were submitted for histological analysis which revealed that the neoplasm contained fascicles of spindle-shaped cells with fusiform, hyperchromatic, and pleomorphic nuclei with frequent abnormal mitoses (greater than ten per ten high-power fields). The surgical margins were minimally breached at two sites. The diagnosis was poorly-differentiated leiomyosarcoma of the vagina. The patient refused any further surgical intervention.

Extensive evaluation with chest X-ray, ultrasound of the liver, and CT scan with intravenous contrast of the abdomen and pelvis were performed. The findings included a mildly enlarged liver without any focal parenchymal lesions, normal gallbladder, spleen, adrenals and kidneys, no evidence of ascites or para-aortic lymphadenopathy, a mildly bulky uterus, normal adnexae, and bilateral...
inguinal lymphadenopathy with cystic degeneration of a right groin node which measured 2.4 cm in diameter. No focal lesions were seen in the lumbar and pelvic spine and the lungs were normal. After review by the gynaecological oncologist, she was referred for further adjunctive treatment.

Initially, she received a combination of vincristine, adriamycin, and cyclophosphamide which was followed by gemcitabine and cisplatinum. When a firm immobile irregular mass involving the posterior and right lateral aspects of the vagina with parametrial involvement was noted, a total of 3,000 cGy of radiotherapy, given in increments of 300 cGy per session was administered to the pelvis. This was followed by a similar dose to the brain for metastastic disease. She succumbed to the illness 11 months after the initial diagnosis.

Discussion

The authors’ literature search has revealed that this is the first reported case of vaginal leiomyosarcoma in pregnancy in an Afro-Caribbean woman. Behzatoglu et al. reported on the first possible case of leiomyosarcoma in a 21-year-old primigravida at 39 weeks’ gestation [3]. Primary vaginal malignancies are rare accounting for only approximately 2% of all female genital neoplasms. The majority of these are squamous cell carcinoma [4]. Primary sarcoma represents only about 2% of all vaginal malignancies, and leiomyosarcoma is the most frequent histological type in adults while rhabdomyosarcoma is more common in children. Ahram et al. identified only 138 cases of vaginal leiomyosarcoma in a thorough literature search [2]. Even rarer is this type of sarcoma in pregnancy with only a handful of cases reported thus far. This is the first case identified at the Mt. Hope Maternity Hospital in over 150,000 deliveries.

Our patient presented at 30-weeks’ gestation with a small swelling in the vagina which was assumed to be a benign wart and as a result the treatment was scheduled for after the delivery. The aggressiveness of this tumour was depicted by the rapid increase in size, as well as the presence of pain most likely from deep invasion in the sub-vaginal tissue in less than two months. A predilection for this tumour to occupy the posterior and lateral aspects of the vagina was also evident in the present case. Until the aetiology and pathogenesis of this tumour are fully elucidated, it is not possible to give a clear explanation for this occurrence.

Although at the time of the local excision the authors were aware that the swelling was likely to be malignant, they did not suspect a leiomyosarcoma. As a result, their plan was to excise the mass and to await the histological evaluation before making a definitive plan. The diagnosis confirmed by histology was a poorly-differentiated leiomyosarcoma with involvement of the margins. The patient refused any further surgical options instead she opted for adjuvant therapy.

Early diagnosis of leiomyosarcoma with the disease at Stage 1 is critical if simple excision is likely to yield good results [2]. In the present case there was a breach of the margin which may be indicative of the aggressiveness of the tumour. Tavassoli and Norris recommended extensive surgical excision of the lesions which are likely to recur [5]. Some of the risk factors for early recurrence include a tumour size of three cm or more in diameter, irregular contour, cellular atypia, and five or more mitoses per ten high-power fields. In the present patient all of these features were evident.

The lack of responsiveness of poorly-differentiated advanced disease is demonstrated in the present case who failed to respond to vincristine, adriamycin and cyclophosphamide, and also, a combination of gemcitabine and cisplatinum. The authors added both pelvic irradiation targeting the tumour and the involved groin nodes as well as external irradiation to the brain for cerebral metastases. Hensley advocated the need for primary surgery plus adjuvant irradiation to decrease local recurrences, and chemotherapy for persistent and recurrent disease [6]. The present patient experienced a fairly rapid downhill course despite several chemotherapeutic agents and irradiation which concurs with the findings of Ngan et al. who found that neither chemotherapy nor radiotherapy was particularly useful in late or recurrent disease [7]. It is unknown whether leiomyosarcoma behaves in a more aggressive manner in pregnancy and if so, what factors may be responsible. The present case highlights the fact that leiomyosarcoma of the vagina remains a difficult diagnosis to be made clinically, and it continues to carry a poor prognosis despite the advent of many newer and more effective chemotherapy. It should be reported due to its rarity in order for us to compile sufficient evidence on the most appropriate treatment. Furthermore, a greater sense of awareness is required by healthcare workers if we are to diagnose this highly malignant tumour early especially in pregnancy.

References


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Addition of bevacizumab to neoadjuvant chemotherapy for Stage IV ovarian serous adenocarcinoma with multiple lymph node metastases: a case report


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Summary
A 50-year-old female patient was diagnosed with Stage IV ovarian serous adenocarcinoma with multiple lymph node metastases. The CA-125 level normalized after four cycles of neoadjuvant chemotherapy (NACT) using paclitaxel, nedaplatin, and bevacizumab (BEV) before surgery. A positron emission tomography-computed tomography (PET-CT) scan showed significantly reduced bilateral adnexal masses after NACT fluorodeoxyglucose (FDG) metabolism in multiple lymph nodes was inhibited significantly, and the number and sites of metastatic lesions were decreased. The patient underwent optimal cytoreductive surgery. Chemotherapy was continued after surgery and image-guided radiation therapy (IGRT) (40 Gy) was applied for the remaining lymph nodes in the pelvic cavity and cervicothoracic region. No sign of recurrence has been observed in this patient nine months after surgery. The patient achieved a satisfactory outcome and no serious side effects were observed. Therefore, addition of BEV to NACT is a new method for the pre-operative treatment of advanced ovarian cancer.

Key words: Advanced ovarian cancer; Neoadjuvant chemotherapy; Bevacizumab.

Introduction
Currently, surgery is the standard treatment for ovarian cancer, and is supplemented by post-operative chemotherapy. Because of widespread metastasis and local invasion, only 20%-30% of patients with advanced ovarian cancer undergo optimal primary cytoreductive surgery (OPCS). Therefore, how to perform satisfactory cytoreductive surgeries for these patients is a difficult problem encountered by gynecologic oncologists. Neoadjuvant chemotherapy (NACT) is a new treatment method for advanced ovarian cancer. As with ovarian cancer chemotherapy, paclitaxel plus cisplatin therapy is the standard treatment for epithelial ovarian cancer in the NACT program. To improve the prognosis of patients with ovarian cancer, the vascular endothelial growth factor (VEGF) inhibitor, bevacizumab (BEV), is a major agent in biological therapy for ovarian cancer. The NICE guidelines recommend BEV plus paclitaxel and carboplatin as first-line chemotherapy for advanced ovarian cancer [1]. However, there have been few studies regarding the addition of BEV to NACT. The authors report a case of a 50-year-old patient with Stage IV ovarian serous adenocarcinoma who had multiple lymph node metastases and underwent OPCS after four cycles of NACT with BEV plus paclitaxel and nedaplatin. There were no serious or fatal side effects and the treatment outcome was satisfactory.

Case Report
On May 4, 2012, a 50-year-old female patient was admitted to the present hospital with a progressive mass in the left neck for one month. The pathologic examination in the local hospital showed that the lesion in the left neck was a lymph node metastasis from adenocarcinoma. The physical examination revealed an Eastern Cooperative Oncology Group (ECOG) of 1 and the blood pressure was 110/70 mmHg. In the root of the left neck, multiple enlarged lymph nodes fused into a hard mass with tenderness. The boundaries of the mass were unclear and the size was approximately 4 x 3 cm². The mass was essentially immobile. No abnormalities were noted in the heart, lungs, and abdomen. A hard mass (3 x 4 cm² in size) was palpable in the right adnexa, mobile, and non-tender. A mixed solid and cystic mass (5 x 4 cm² in size) was palpable in the left adnexa, mobile, and non-tender. A positron emission tomography-computed tomography (PET-CT) scan (May 4, 2012) showed multiple enlarged and clustered lymph nodes in the root of the left neck and the left shoulder (Figure 1). The fluorodeoxyglucose (FDG) uptake was abnormally increased, the maximum standardized uptake (SUV) value was 15.5, and the diameter of the largest lymph node was 1.4 cm. The lymph nodes in the right shoulder were also visualized with a maximum SUV value of 3.1 and the diameter of the largest lymph node was 0.9 cm. There were multiple enlarged lymph nodes on the left side of the aortic arch, the FDG uptake was abnormally increased, the maximum SUV value was 62, and the diameter of the largest lymph node was 1.3 cm. A lesion with abnormally increased FDG metabolism was observed anterior to the rectum;
the maximum SUV value was 27.7 and the size was 2.3 x 1.4 x 2.5 cm. There were multiple nodular lesions with abnormal FDG uptake located along the right ureterine wall; the maximum SUV value was 12.6 and the diameter of the largest lesion was 1.4 cm. There were multiple enlarged, beaded lymph nodes located medial to the bilateral iliac blood vessels, which extended superiority to the peripheral area of the abdominal aorta and extended further to the posterior space of the head of the pancreas. The total length of the beaded lymph nodes was approximately 2.3 cm, the diameter of the largest lymph node was 2.2 cm, and the maximum SUV value was 11.9. No abnormal activity in FDG metabolism was observed in other anatomic sites. The CA-125 level (1: before chemotherapy, Figure 2) was 164.40 U/ml. The PET-CT scan showed that the PT, APTT, and TT were normal, and the D-dimer and FDP were slightly increased. The fifth cycle of chemotherapy using paclitaxel (175 mg/m² iv on d1), nedaplatin (80 mg/m² iv on d1), and BEV (7.5 mg/kg iv on d1). The interval between each cycle was 21 days. Stage II hypertension, according to the Common Toxicity Criteria (NCI-CTC) criteria (version 3.0), occurred during chemotherapy and the blood pressure was controlled using oral felodipine. Subsequently, grade I bone marrow suppression occurred, which improved after appropriate treatment. Thirteen days after the third cycle of chemotherapy (July 5, 2012), a repeat PET-CT was obtained (Figure 1). No abnormal FDG uptake was observed in the bilateral shoulders and the neck. Multiple lymph node images were observed on the left side of the aortic arch; the diameter of the largest lymph node was 0.8 cm, but no FDG sign was observed. The FDG uptake mildly increased in the left adnexa; the maximum SUV value was 5.4 and the diameter was 0.8 cm. No lesions with abnormal FDG uptake were observed in the right adnexa, uterus, or pelvis. In addition, no abnormal activity of FDG metabolism was observed in other anatomic sites. After NACT, the sizes of the lesions in the bilateral adnexa were reduced significantly; the FDG metabolism of the lymph nodes in multiple sites of the body was inhibited, and the number and sites of tumor metastases were significantly decreased.

An exploratory laparotomy was performed on August 1, 2012. No ascites was noted during surgery. Adhesions involving loops of bowel and intestinal adhesions to the left pelvic wall were present. The left ovary adhered to the left pelvic wall and the posterior lobe of the broad ligament. The left ovary was slightly enlarged, the surface was rough, and scant necrotic tumor tissue was observed. No abnormalities were observed in the right ovary and fallopian tube. The size of the uterus was normal and some small intramural myomas were observed. The inferior diaphragm, liver capsule, omentum, appendix, small intestine, and mesentery were smooth without obvious tumor lesions (Figure 3A). The pelvic and para-aortic lymph nodes were significantly enlarged bilaterally, the texture was hard, and the adhesions were abundant. A left salpingo-oophorectomy was performed first. The results of the frozen biopsy revealed poorly differentiated serous adenocarcinoma in the left ovary and fallopian tube. Then, ovarian cancer cytoreductive surgery, including a total hysterectomy, right salpingo-oophorectomy, omentum resection, appendectomy, pelvic lymph node dissection, aortic lymph node sampling, and enterolysis was performed. The standard of satisfactory cytoreductive surgery is defined as no gross residual tumor after surgery (Figure 3B). The post-operative pathologic examination showed a small area with cancer tissue in the right ovary, but no cancer in the endometrium or right fallopian tube. Intramural leiomyomas and retention cysts in the cervix were observed. No cancer lesions were present in the appendix and omentum. Cancer metastasis was identified in the right common iliac lymph nodes (1 of 3), left internal iliac and obturator lymph nodes (1 of 2), and right ventral aortic lymph nodes (1 of 3). No cancer metastasis existed in the remaining lymph nodes.

A partial ileus occurred six days after surgery, which improved after continuous gastrointestinal decompression, acid suppression, and nutritional support. Vaginal bleeding was noted 14 days after surgery (August 15, 2012). The estimated blood loss was 60 ml. Hemostasis was achieved by packing gauze into the vagina. The second and third episode of vaginal bleeding occurred on August 18 and 22, 2012. The estimated blood loss was 300 ml during each episode. Active bleeding in the left apex of the remnant vagina was observed during a gynecologic examination, which stopped after packing gauze into the vagina, hemostatic medications, and rehydration. The coagulation profile showed that the PT, APTT, and TT were normal, and the D-dimer and FDP were slightly increased. The fifth cycle of chemotherapy using paclitaxel (175 mg/m² iv on d1) and lobaplatin (40 mg/m² iv on d1) was administered on August 27, 2012 without BEV. The sixth cycle of chemotherapy was administered on October 9, 2012 using paclitaxel (175 mg/m² iv on d1), lobaplatin (40 mg/m² iv on d1), and BEV (7.5 mg/kg iv on d1). Before the sixth cycle of chemotherapy, the CA-125 level was 10.76 U/ml. A PET-CT scan was obtained on October 15, 2012 (six days after the sixth cycle of chemotherapy). The result showed that there were multiple lymph nodes (0.2 - 0.3 cm in diameter) located in the roots of the neck bilaterally near the thoracic aortic arch, the peripheral area around the major retroperitoneal blood vessels, and the inguinal regions bilaterally. No increased FDG metabolism was observed. No sign of abnormal activity of FDG metabolism was observed in other anatomic sites. Between November 6, 2012 and December 5, 2012, the authors performed IGRT (40 Gy) in the remaining lymph nodes in the abdominal and pelvic cavities and the thoracic and cervical areas. No tumor recurrence was observed during nine months of follow-up visits after chemotherapy and radiotherapy (until September 2013). The patient has survived for 16 months.

Discussion
In the past few decades, a number of approaches have been attempted to deliver systemic treatment to improve the prognosis of patients with ovarian cancer. However, addition of a third drug to the paclitaxel and cisplatin chemotherapy regimen did not improve the prognosis, rather increased the side effects. Currently, many scholars have added molecular targeted therapy to paclitaxel and cisplatin-based chemotherapy in the treatment of ovarian cancer. Angiogenesis is an important factor of tumor invasion and metastasis and a necessary condition for tumor development. It has been reported that VEGF is highly expressed in ovarian cancer, and is related to ascites formation and a poor prognosis [2-4]. Previous studies have shown that anti-VEGF therapy can reduce the tumor load, inhibit the formation of malignant ascites, and there is a synergistic effect between anti-VEGF therapy and cytotoxic drugs [5]. Two large-scale randomized controlled clinical trials (GOG218 and ICON7) have captured the authors’ attention. It has been reported that the addition of BEV to standard chemotherapy and use of BEV as
Addition of bevacizumab to neoadjuvant chemotherapy for Stage IV ovarian serous adenocarcinoma with multiple lymph node metastases etc.

Maintenance therapy can significantly improve the progression-free survival (PFS) of patients with advanced ovarian cancers [6-7]. BEV has been widely used in pre-operative NACT for breast, colon, and prostate cancers. Van et al. [8] used BEV in pre-operative NACT for 50 patients with colon cancer and related liver and lung metastases. Radical surgery was performed on 36 patients (72%); the two-year overall survival was 80%, and the two-year recurrence rate was 64%. Clavarezza et al. [9, 10] used BEV in pre-operative NACT for patients with breast cancer and achieved a satisfactory disease response rate and pathologic complete response rate whether or not the receptors were positive or negative. Ross et al. [11] used BEV in NACT for patients with high-risk localized prostate cancer and observed that the tumor diameter and plasma PSA level decreased significantly. Nevertheless, there have been few studies involving the use of BEV in pre-operative NACT for advanced ovarian cancer. The
The authors administered four cycles of NACT using BEV, paclitaxel, and nedaplatin. After three cycles of chemotherapy, a PET-CT scan showed that a decrease in the tumor diameter >50%. Furthermore, the FDG value was significantly inhibited, and the maximum SUV value was reduced from 27.7 to 5.4. All enlarged lymph nodes shrank obviously after treatment and the diameter of the largest lymph node was reduced from 2.2 to 0.8 cm. There were no obvious abnormal signs of FDG and the CA-125 level normalized after two cycles of NACT. According to the NCI-CTC criteria (version 3.0), only a grade I gastric response, grade I bone marrow suppression, and Stage II hypertension occurred, but no serious side effects, including proteinuria, gastrointestinal perforation, and venous thrombosis, were noted during NACT. Anti-VEGF therapy induces VEGF-mediated damage during the repair of the endothelial cell surface, which results in bleeding. Based on a meta-analysis, the administration of BEV significantly increases the incidence of high-level bleeding, the risk is dose-dependent, and differs in various tumor types [12]. The risk of delayed wound healing decreased when BEV was withdrawn six to eight weeks before surgery and administered again >28 days after surgery [13-14]. In a recent study regarding the safety of surgery after NACT using BEV, paclitaxel, and carboplatin, four of five patients were FIGO Stage IV. All patients received six cycles of NACT; the mean number of cycles with BEV was three and the mean interval between the last pre-operative dose of BEV and surgery was 54 days (range, 34-110). Grade 3 complications occurred in one patient, and CT-guided lymphatic cyst aspiration was performed on this patient [15]. In the current study, vaginal bleeding after NACT using BEV might be related to...
the short interval between the last BEV dose and surgery (only 20 days). The half-life of BEV is relatively long (approximately 20 days). It has been reported that BEV is still active after patients in patients with colon cancer and related liver metastases, even if NACT using BEV is withdrawn six weeks before surgery. BEV inhibits VEGF in the circulation and local tissues, but does not increase the peri-operative mortality, which suggests that VEGF is not the most important factor during the acute recovery stage after surgery [8]. Thus, there should be a proper interval between the last administration of BEV and surgery because post-operative complications may occur within a long period after surgery. Stage II hypertension occurred after NACT using BEV, which may be related to the fact that BEV inhibits vascular endothelial cells from synthesizing nitric oxide, reduces the number of arterioles and capillaries, and thereby increases vascular resistance. Thus, blood pressure should be monitored routinely in patients treated with BEV.

Conclusion

NACT using paclitaxel, nedaplatin, and BEV achieved an excellent outcome in the treatment of a patient with Stage IV ovarian serous adenocarcinoma and multiple lymph node metastases. The patient underwent OPCS. Despite side effects, including hypertension, vaginal bleeding, gastric response, and bone marrow suppression, all these symptoms improved after symptomatic treatment. The addition of BEV to NACT did not increase the toxicity of chemotherapy drugs nor did BEV increase the incidence of surgery-related fatal complications after NACT.

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Carcinosarcoma in endometrial polyp. Diagnosis and treatment – case report

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Summary
This paper presents a case of carcinosarcoma localized in the endometrial polyp inside the uterus of a 74-year-old patient. This carcinosarcoma was diagnosed in the first clinical disease stage. Postoperative treatment was conducted but was not followed by additional chemo or radiotherapy due to the fact that the illness was in the first clinical stage. Secondary deposits in the abdomen and in the pelvis appeared exactly six months after surgery. Illness progression was sudden causing death three months after the appearance of secondary deposits or nine months after the illness was detected, despite attempts with chemotherapeutic treatment. Although uterine carcinosarcomas account for three to seven percent of all uterine malignities while malignant polyp degeneration occurs in only 0.36% cases, treatment of this malignant disease is a special challenge to all those involved in oncological gynecological practice with the aim of prolonging the progression-free interval and the overall survival of patients suffering from this rare malignity.

Key words: Uterine carcinosarcoma; Survival; Therapeutic approach.

Introduction
Uterine carcinosarcoma is rare, metaplastic subtype of endometrial cancer comprised of two distinct malignant components – epithelial and mesenchymal, with phenotypic features. Tumor behavior is very aggressive. Local recurrence is frequent as well as distant metastases [1]. Uterine carcinosarcomas are also known as malignant mixed Müllerian tumors. These tumors are generally thought to account for three to seven percent of uterine cancers [2]. Malignant degeneration of an endometrial polyp occurs rarely, i.e. in approximately 0.36% of all cases. Nevertheless, those rare cases of carcinomatous degeneration of an endometrial polyp described in literature have a good prognosis. Data from the literature describes individual cases of carcinosarcoma in the endometrial polyp [3].

Endometrial carcinosarcomas which show polypoidal arising in uterine cavity have aggressive clinical features [4]. According to references, such features cause dilemmas of whether or not systemic chemotherapy should be administered after surgical treatment in patients with first-stage disease, with histopathologically verified endometrial carcinosarcoma, and with polypoid growth inside the uterine cavity. Due to the aggressive nature of these tumors some authors recommend chemotherapy or even combined chemo-radiotherapy after surgical treatment even in early clinical stages of the disease in order to postpone or prevent relapse [5].

Case Report
This paper presents the case of a 74-year-old patient treated from endometrial carcinosarcoma localized in an endometrial polyp. One year prior, this patient underwent a complete gynecological examination, with colposcopy, cytology, explorative curettage, and color Doppler ultrasonography due to one day long postmenopausal bleeding of medium intensity, without results of malignancy. Furthermore a control with ultrasonographic exam performed after exploratory curettage resulted in completely normal findings. One year later, exploratory curettage was performed for new vaginal bleeding; this time it was scarce. It was then visualized by ultrasonographic exam that the endometrium was 32 mm thick honeycomb structure but with no pathological vascularization. Clinical findings were indicative either of endometrial carcinoma or endometrial polyp although scarce vascularization presented a differential diagnosis problem because it corresponded neither to endometrial carcinoma nor to endometrial polyp. Exploratory curettage was performed again. Histopathological finding was sarcoma stromae endometrii. After preoperative examinations with magnetic resonance imaging (MRI) of the abdomen and pelvis, a classic hysterectomy with bilateral adnexectomy, selective lymphadenectomy, and cytological analysis of the peritoneal lavage were performed. Definite histopathological findings verified polypoid carcinosarcoma in the uterine cavity known as mixed Müllerian tumor, comprised of 95% non-differentiated chondrosarcoma tissue and 5% endometrial adenocarcinoma, staged as FIGO Stage Ia. Postoperative period was normal. Patient recovered quickly. Follow-up consisted in regular check-ups, including MRI examination every three months. After six months the patient experienced dull low back pain which lasted for two days. Pain stopped spontaneously but was followed by bowel emptying problem, requiring detailed clinical examinations. The existence of a large abdominal tumor was verified by clinical
exam. Computed tomography (CT) scan of the abdomen and pelvis was performed, allowing visualization of expansive changes in the entire pelvis and abdomen, spreading from rectum to head of the pancreas, compressing the portal vein, right ureter, and large blood vessels in the pelvis. Patient, however, complained only of bloated abdomen. All laboratory results as well as patient’s general state were very good and did not correspond to clinical and CT findings. Systemic chemotherapy by mono-adriablastin in cycles was indicated. Patient completed the first chemotherapy cycle without side effects, but after a few days, clinical symptoms developed due to the expansion of the tumor lesions in the abdomen. The second chemotherapy cycle was prescribed. Patient resisted this treatment with difficulty because of previously described present clinical symptoms. Aside from nausea, complete appetite loss, heartburn, difficult bowel movement, pains, and exhaustion appeared. Although blood analysis showed only slight anemia, lymphostasis appeared especially in lower limbs. Swelling in legs progressively increased day after day despite the symptomatic therapy which was administered daily. Patient died before the third chemotherapy cycle i.e., three months after relapse was diagnosed and six months after radical surgical treatment.

It is interesting to mention that this patient underwent a gastric wall surgery at the age of 55 because of a tumor histopathologically diagnosed as Swannoma benignum which is a rare finding. Besides this, the patient had two sisters diagnosed and treated from malignant breast tumor.

Discussion

Uterine sarcomas are relatively rare mesenchymal malignant neoplasms with poor prognosis, accounting for 8% of all uterine malignant neoplasms. Reference data show that there are only a few moderately active cytotoxic agents for this entity, and therefore, chemotherapy for uterine sarcomas is palliative in most cases [6].

Monoadriablastin was prescribed to the patient without any success. References describe administration of several chemotherapy protocols: paclitaxel and ifosfamide which were the chemotherapy regimen which slightly improved both progression-free and overall survival; combination of ifosfamide and cisplatin which appears to improve progression-free survival but whose therapeutical application is limited by their cytotoxicity, while for leiomyosarcoma and undifferentiated endometrial sarcoma (formerly named high-grade ESS) doxorubicin, ifosfamide, and gemcitabine are used [6].

With regards to the application of radiotherapy to secondary deposits in abdomen, data references show that radiotherapy to the abdomen is not associated with improved survival [7].

The distinct biological behavior and poor overall survival of uterine sarcoma create challenges in the management of these tumors and urge us to perform even more thorough immunohistochemical, biological, genetic, and more extensive multidisciplinary research of this issue in order to prolong the progression-free interval and the overall survival.

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Steroid cell tumor of the ovary associated with endometrial adenocarcinoma – a rare case report

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Summary
Endometrial carcinoma is the most common invasive neoplasm of the female genital tract and is associated to the elevated levels of unopposed estrogen, especially in postmenopause. Ovarian Steroid cell tumors are rare tumors and they are named according to the origin of cell. The most common cancer of the female genital tract is the endometrial adenocarcinoma and it accounts for 7% of all invasive cancers in women. In the present case report, the authors present a 59-year-old multiparous woman with a postmenopausal bleeding complaint and they discuss the case of ovarian steroid cell tumor associated with endometrioid adenocarcinoma of endometrium. Endometrial adenocarcinoma is the most common cancer of female genital tract and its contemporarity with an ovarian steroid cell tumor is extremely rare.

Key words: Steroid cell tumor; Ovary; Endometrium; Adenocarcinoma.

Introduction
Ovarian steroid cell tumors, also known as “lipid cell tumors”, are rare neoplasms and account for less than 0.1% of all ovarian tumors [1]. It is a heterogeneous group of tumors arising from gonadal stromal origin and includes: stromal luteoma, Leydig cell tumor, and steroid cell tumor, not otherwise specified (NOS) [2]. Ovarian steroid cell tumor, NOS, is the most common subtype and mostly diagnosed by exclusion [3].

Endometrial carcinoma is the most common neoplasm of the female genital tract and it is strongly associated to the elevated levels of unopposed estrogen, especially in postmenopause [4].

In English-written literature, the present authors have found only a few cases of ovarian steroid cell tumor reported to be associated with endometrial carcinoma [5-7].

Herein the authors present a case of ovarian steroid cell tumor, NOS associated with endometrioid adenocarcinoma of endometrium.

Case Report
A 59-year-old multiparous woman suffering from postmenopausal bleeding was referred to the Department of Obstetrics and Gynecology of Goztepe Training and Research Hospital. Physical examination was normal; there was no virilisation and cliteromegaly. Estradiol level was found as 41.36 pg/ml, slightly higher than normal for postmenopausal status (normal < 32 pg/ml), and follicle stimulating hormone (FSH) level was 32.25 IU/L (normal 23.9 - 119.1). Blood analysis showed normal hemogram, creatinine and liver enzyme levels. Beta human chorionic gonadotropin (hCG) level was normal (0.97 mIU/ml) and the tumor markers levels were within the normal range; CA-125: 13 IU/ml (normal 0-35 IU/ml), CA15-3: 7 IU/ml (normal 0 - 31), CA 19-9: 0.8 IU/ml (normal 0 - 35), carcinoembryonic antigen (CEA): 1.9 ng/ ml (normal 0 - 3), and alpha-fetoprotein (AFP): 2.96 ng/ml (normal 0 - 9).

Although ultrasonographic examination was suboptimal due to obesity (body mass index; (BMI) = 48), the sonography showed a highly echogenic ovary, 5.25 x 4.56 cm. and there were no ascites (Figure 1A). Clinical history of patient revealed that she was operated because of right ovarian mass 33 years prior. The pathology report cites (Figure 1A). Clinical history of patient revealed that she was operated because of right ovarian mass 33 years prior. The pathology report.

Although ultrasonographic examination was suboptimal due to obesity (body mass index; (BMI) = 48), the sonography showed a highly echogenic ovary, 5.25 x 4.56 cm. and there were no ascites (Figure 1A). Clinical history of patient revealed that she was operated because of right ovarian mass 33 years prior. The pathological examination of that mass was done at another institution, but the authors could not found the pathology report.

Endometrial curettage was performed and the histopathological examination revealed endometrioid adenocarcinoma, FIGO Grade II. Therefore, the patient underwent an exploratory laparotomy; with total abdominal hysterectomy, and left unilateral salpingo-oophorectomy.

In gross examination, a fragile tumor, 1.5 x 1.5 x 1 cm. was seen in the uterine cavity. The tumor was limited to the upper half of the myometrium. A subserosal myoma, 10 x 7 x 6 cm, was located on the left side of the uterus. The gross examination of left ovary, 5 x 3 x 1.9 cm, showed a yellow colored, ill-defined solid lesion, 2 cm in diameter, with focal hemorrhage and cystic spaces (Figure 1B). Left fallopian tube was normal. Lymph nodes and omental sampling were not performed.

Multiple samples of the ovary were examined and it was seen that the lesion consisted of two different groups of cells; the first group resembled adrenal cortical cells while the second group was composed of cells resembling Leydig cells. The adrenal cortical-like cells were round and had large, pale, and vacuolated cytoplasm. Their nuclei were vesicular with marked nucleoli. The Leydig-like cells were also round to polygonal and had abundant eosinophilic cytoplasm with centrally located nuclei (Figure 2A). Reinke crystals were not seen. Mitotic figures were too rare and the Ki67 proliferation index was determined as < 1%. No significant atypia and no necrosis were observed. The microscopic examination of the endometrial samples revealed endometrioid adenocarcinoma,
Figure 1. — A: Ultrasonography: highly echogenic ovary, 5.25 x 4.56 cm in diameter. B: The tumoral mass has a yellow-grey cut surface.

Figure 2. — A: Endometrioid adenocarcinoma in endometrium (H&E x20). B: Steroid cell tumor in ovarian stroma (H&E x20).

Figure 3. — A: Steroid cell tumor consisted of two different groups of cells: Leydig-like cells and adrenal cortical-like cells (H&E x10). B: Diffuse membranous positivity with inhibin immunostaining (x10).
FIGO Stage II, consisting of focal solid areas and glands with confluent pattern (Figure 2B). Immunohistochemically, the tumor cells reacted positive for inhibin (Figure 3), CD56, and calretinin, while they were negative for WT1 and estrogen. This lesion was diagnosed as a steroid cell tumor, NOS, because of the absence of stromal hyperthecosis in surrounding stroma and Reinke crystalloids in tumor cells. The microscopic examination of endometrial samples revealed endometrioid adenocarcinoma, FIGO II, consisting focal solid areas and glands with confluent pattern (Figure 2). The tumor was limited to the upper half of the myometrium. Tubal metaplasia was observed in non-tumoral endometrium.

Discussion

Ovarian steroid cell tumors are rare tumors and classified as stromal luteoma, Leydig cell tumor, and steroid cell tumor, NOS [2, 8] and they are named according to the origin of cell: stromal luteoma, when originating from ovarian stroma, Leydig cell tumor, when originating from Leydig cell, steroid cell tumor, NOS, when the original lineage is unknown and when the tumor cannot be categorized as either Leydig cell tumor or stromal luteoma [3, 9]. Steroid cell tumor, NOS may cause virilization in one half of the cases, while estrogenic manifestation were seen in approximately 10% [8] The tumor cells are round to polygonal with eosinophilic or vacuolated cytoplasm and have a centrally located nucleus that may contain a prominent nucleolus. Lipofuscin pigment is present in 40% of cases. Crystals of Reinke are not identified. The stroma is typically sparse and consisted of delicate connective tissue containing a rich vascular network. [8]. Indicators of malignancy are size (> seven cm in diameter), hemorrhagic areas or necrosis, moderate-to-marked nuclear atypia, and at least two MFs /10 HPFs [10, 11].

The most common cancer of the female genital tract is the endometrial adenocarcinoma and it accounts for 7% of all invasive cancers in women, excluding skin cancer. The endometrioid carcinoma is basically divided in two subtypes in terms of potential pathogenesis, endometrioid type, and non-endometrioid type. The endometrioid type typically occurs because of excess estrogenic stimulation, the process synthesizing estrogens from adrenal and ovarian precursors in body fats for postmenopausal women, ovarian dysfunction, and estrogen secreting tumors, and develops against a background of endometrial hyperplasia. Postmenopausal bleeding is the most common presentation of this disease. Obesity, diabetes, and hypertension are commonly seen in patients with endometrioid adenocarcinoma. The non-endometrioid type can occur as de novo [12]. The steroid cell tumors may exhibit occasional estrogenic effects and Huang and Holaday have described one case of steroid cell tumor associated with endometrial adenocarcinoma in 1970 [5].

In the present case, estradiol level was measured as 41.36 pg/ml, slightly higher than upper limit for postmenopause (normal < 32 pg/ml). She had no signs and symptoms except uterine bleeding and was diagnosed as endometrioid adenocarcinoma. It is known that the postmenopausal obesity is a risk of the endometrioid carcinoma in itself. Even though it is difficult to conclude a relationship between steroid cell tumor of ovary and endometrioid adenocarcinoma of endometrium, in the present case, the authors suggest that the increased production of hormones (androgen or estrogen) from steroid cell tumor, NOS, and from the adrenal and ovarian precursors may induce the uncontrolled endometrial cell proliferation and may result in endometrioid adenocarcinoma of endometrium.

Acknowledgements

Abdullah Aydin and Ebru Zemheri performed the histopathological diagnosis. Seyma Ozkanli and Burcinc Subasi wrote the case report. Kadir Guzin and Ahmet Gocmen operated the patient.

References


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Introduction

Ovarian cancer is the world’s fifth leading cause of cancer related death among women. This is mainly due to the biology of this cancer: a tendency toward asymptomatic spread in the peritoneum and lymphatic system. As a result, in nearly 75% of patients, the cancer is detected in the third and fourth stage of progression. Although complete remission after optimal cytoreduction and adjuvant chemotherapy is feasible in 80-85% of patients, the five-year survival rate in advanced stage disease does not exceed 30-50%, and in over 50-75% of patients, there is a recurrence of the neoplastic process [1-5].

Recurrence is observed in 80% of cases within the first two years of diagnosis, with the most common site being the peritoneal cavity. Less frequently, recurrence in the lymph nodes, liver, lungs or vagina may be observed [6-9].

Case Report

Case 1

A 54-year-old woman was diagnosed with ovarian cancer in March 2004. A total hysterectomy was performed in which the histopathologic examination confirmed solid adenocarcinoma of the ovary G3 according to FIGO IC. The patient received six courses of treatment with the first-line chemotherapy regimen: intravenously paclitaxel 135 mg/m2 and cisplatin 75 mg/m2 every 21 days. The patient completed treatment with full remission of the neoplastic process (confirmed in abdominal and pelvic computed tomography (CT), abdominal ultrasound, and chest X-ray). Due to the patient being treated in another cancer unit, there was a lack of some clinical data, including the level of the CA 125 marker.

Nine years post-treatment, cancer recurrence was diagnosed. On abdominal ultrasound a mass of 50 mm diameter in the right iliac fossa and lymph nodes in the hepatic hilus with a diameter of 15 mm were discovered. The CA 125 level was 418.6 U/ml. On April 25, 2013 an omentectomy was performed, which confirmed the metastasis of a poorly differentiated tumor G3.

Histological evaluation comparing material collected in 2004 and 2013 confirmed that the changes in the greater omentum are in fact metastasis from the ovary. From May 2013, the patient received second-line chemotherapy: intravenous paclitaxel 175 mg/m2 and carboplatin (AUC 5.0) every 21 days. Due to poor tolerability (hematologic complications: anemia, neutropenia, thrombocytopenia), the planned six cycles of treatment were not administered (patient completed four courses).

Case 2

A 63-year-old woman was diagnosed with a malignant tumor of the ovary. A transvaginal ultrasound examination diagnosed a tumor with both cystic and solid components in the pouch of Douglas, with a diameter of 120 mm (CA 125 marker: 266.5 U/ml). During the surgical procedure performed on March 4, 2004, the uterus, both adnexa, and omentum were excised and approximately 3,000 ml of fluid was evacuated from the abdominal cavity. Papillary serous adenocarcinoma G3 of ovarian or tubal origin, FIGO III C was diagnosed histologically. From March 2004 to June 2004, the patient received treatment with first-line cytostatic agents: six courses intravenously of paclitaxel 135 mg/m2 and cisplatin 75 mg/m2 every 21 days. The chemotherapy was well tolerated. The patient completed the treatment with total remission, including clinical, marker (CA125: 4.1 U/ml) and imaging (CT, ultrasound, chest X-ray), which was confirmed during a second-look surgery performed on July 27, 2004.

Nine years after the completion of treatment, a recurrence of malignant disease was diagnosed: abdominal and pelvic CT (July 23, 2013) displayed a cluster of lymph nodes with a cross section of 25 x 22 mm near the left renal vessels running the length of the aorta to the left common iliac artery (CA 125 marker: 22.2 U/ml). The patient was monitored until April 2013 in outpatient care. In March 2013, a control CT found that the described enlarged lymph
groups: of ovarian cancer, patients are divided into the following with platinum derivatives, and the appearance of recurrence elapsed between the completion of the first-line treatment. Such as: histological type, the stage of progression at the time of diagnosis, the degree of differentiation, the scope of surgery, the use of adjuvant therapy, and the sensitivity to platinum derivatives [9-11]. Depending on the time that has elapsed between the completion of the first-line treatment with platinum derivatives, and the appearance of recurrence of ovarian cancer, patients are divided into the following groups:

- Platinum resistant - recurrence occurs up to six months after completion of treatment with platinum.
- Partially platinum sensitive - recurrence occurs within six to 12 months after completion of treatment.
- Platinum sensitive - recurrence occurs more than 12 months after completion of therapy [6, 12, 13].

Among the studies of Robinson et al. [14] and Rauh-Hain et al. [15], it was concluded that patients who were additionally treated with bevacizumab within the standard treatment regimen of paclitaxel and platinum derivatives, cancer recurrence was in different anatomical sites: more often to the retroperitoneum, including the lungs and pleura, the central nervous system, the skin, and less commonly to the liver. This would explain the authors’ concept pertaining to altered immunoregulation in the peritoneal cavity.

In many studies, it is emphasized that the recurrence of ovarian tumors appearing after more than two years after achieving complete clinical remission, have a different biology than those recurrences occurring up to two years from the completion of the first-line therapy. It is undisputable that the condition for diagnosing a case as a recurrence, and not as a second, independent tumor, is the confirmation of identical histological structures of the primary and the recurrent tumor [16, 17]. Nevertheless, it is observed that in some cases of ovarian cancer, clinical recurrences behave like new tumors and respond well to treatment with platinum derivatives. This observation also supports the hypothesis that some cases of late recurrent ovarian carcinoma are in fact subsequent primary peritoneal tumors, and not a consecutive pathological proliferation of dormant ovarian cancer cells. Another theory on late recurrence states that, the same carcinogen acting newly on different groups of cells may cause a tumor identical to the primary tumor [16].

The results presented by Buller et al. [17] on the study of late recurrence of ovarian cancer indicate that 77% of tumors cells treated as late recurrences, had a different genotype than the cells of the original tumor. Thus, the concept of “field cancerization” of the carcinogen, which originally affected the ovarian cells, was developed. In the same study, it was hypothesized that the incidence of late recurrence ovarian carcinoma, whose cells differ in clonality from the primary lesion, may be characteristic for families predisposed to malignant tumors. The case raises the suspicion that ovarian cancer of epithelial origin may change its histological picture with the progression of the cancer process. The likely hypothesis of multifocal epithelial neoplasia of the primary site, supports the development of tumors of epithelial origin in patients after prophylactic oophorectomy due to a positive family history and as a result of late recurrences in women treated for ovarian cancer [9, 18].

Frequently, the increasing concentration of CA 125 is the first sign of the recurrence of ovarian cancer. If there are no clinical signs of the disease, treatment of the recurrence based solely on the increasing marker does not prolong survival, while causing cytotoxic effects. The average time between the CA 125 concentration increase and the clinical or radiological recurrence is two to four months. The three parameters, (clinical and radiological recurrence, and the CA 125 marker) are good indicators to initiate treatment [1, 13, 19, 20]. There are many deciding factors to the treatment strategy, most importantly, tumor size and the breaks in platinum therapy [21, 22].

Due to the described diverse biology of tumors occurring as late recurrence ovarian cancer, and more frequently appearing in the form of singular changes rather than metastatic disease, the patient with late recurrence, is a good candidate to attempt complete cytoreduction of the changes during a secondary surgery. Surgical treatment as second-line therapy in recurrent ovarian cancer has good clinical results also in the case of recurrence of tumors with borderline malignancy [11].

It has been proven that in the case of late recurrence ovarian carcinoma, using a treatment regimen analogous to first-line therapy, the surgical treatment allows for radical removal of tumor foci. With aggressive chemotherapy, in the majority of patients, it is possible to achieve a favorable clinical outcome [17, 23]. Only in the case of borderline ovarian tumor recurrence, the response rate to chemotherapy is low [14]. If we assume that late recurrences of ovarian carcinoma are in actuality another primary tumor, it would explain the favorable response to treatment with platinum derivatives. By comparison, in
cases when tumor recurrence presented shortly after first-line chemical treatment was concluded, the tumors were resistant to platinum derivatives, because there was formation of cell clones resistant to the chemotherapeutic agent [19].

In the two presented cases on late recurrence, the histopathologic picture between the primary tumor and the recurrence did not differ. One of the patients completed chemotherapy with total clinical remission, depressed markers, and in the upcoming months requires only regular oncologic follow up care. Therefore, the presence of late recurrence ovarian carcinoma and the high probability of effective second-line treatment, justifies the practice of a longer and more intense monitoring of patients after the culmination of the first-line therapy [16]. Similarly, in borderline ovarian tumors, where the recurrence tends to appear at different intervals from the completion of the first-line therapy (even years late), there is a necessity, in the case of the diseased, for regular long-term monitoring of patients, including the performance of full pelvic examinations, ultrasound evaluation, and measurement of the concentration of the CA 125 marker in the serum [24].

References


Granulosa cell tumor presenting with ovarian torsion and de novo borderline mucinous ovarian tumor in the contralateral ovary

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Summary
The authors report a case of 25-year-old women with a rare acute presentation of granulosa cell tumor (GCT) as an ovarian torsion. Right salpingoo-oophorectomy was performed. The pathological diagnosis was GCT. One month after the surgery there was a three-cm ovarian cyst in the contralateral ovary and the tumor size increased to six cm in diameter in the following month. Serum inhibin-B levels progressively increased. Cystectomy was performed to contralateral ovary as frozen-section examination indicated mucinous tumor. Final histopathological examination revealed borderline mucinous tumor. Regarding her request, the patient was reoperated again and unilateral oophorectomy and hysterectomy were performed. Clinicians must be aware of the possibility of an underlying malignancy associated with adnexal torsion even in young patients. Frozen section will be helpful in order to avoid incomplete surgeries. Cyst rapidly growing in the ovary in young women should raise the suspicion of a de novo malignancy.

Key words: Granulosa cell tumor; Ovarian torsion; Mucinous tumor; Frozen section.

Introduction
Granulosa cell tumors (GCT) are rare ovarian malignancies that arise from sex-cord stromal cells and account for approximately 1.5 to three percent of all ovarian malignancies [1]. Borderline ovarian tumors (BOTs) appear in young women accounting for 10–15% of epithelial ovarian tumors. They have a low invasive potential and most are cured with surgery [2, 3]. The authors came across an interesting case in which the underlying lesion behind torsion in a young woman was GCT, which is scarcely seen, and a de novo borderline mucinous tumor emerged in the contralateral ovary only two months after the index tumor.

Case Report
A 35-year-old women gravida 1, para 1 was admitted complaining of acute sharp abdominal pain increasing in intensity and nausea. Physical examination revealed a marked right lower quadrant abdominal tenderness with guarding and a large, palpable mass in the right lower abdomen above the umbilicus. Ultrasonography and colored Doppler showed a predominantly cystic mass approximately 15x12 cm with few septations in the right adnexial region with no arterial flow and free fluid in the Douglas space. The hemoglobin, hematocrit, leukocyte count, and tumor markers CA-125, CA 19-9, AFP, and CEA were all within the normal limits.

She was suspected of having ovarian torsion on the right ovary and underwent laparotomy. During operation, moderate amount of ascites was observed and the enlarged right ovary was found to be twisted twice around its pedicle. The opposite ovary and uterus were normal. Ascitic fluid was recovered from the abdomen for cytology and right salpingo-oophorectomy was performed. The final pathological diagnosis was adult-type GCT originating from the right ovary, and cytology of ascites fluid was suspicious of malignancy. Serum inhibin-B levels were normal. Serum inhibin-B levels progressively increased from 29 to 99 ng/l.

Because of the histology of the preceding tumor, rapidly increasing inhibin levels, and rapidly growing cyst on the contralateral ovary, the authors decided to perform a restaging surgery. The patient underwent laparotomy, a six-cm ovarian cyst was seen in the left ovary, cystectomy was performed, and the intraoperative frozen-section examination indicated mucinous neoplasm. Comprehensive surgical staging and appendectomy was performed. Final histopathological examination revealed GCT and BOT Stage 1a for both tumors. According to final pathologic diagnosis, the patient was fully informed about conservative surgery. The patient refused strongly to undergo conservative procedure. Therefore, patient was reoperated again and unilateral oophorectomy and hysterectomy were performed.

In young patients who desire to preserve fertility and have disease that is confined to one ovary according to staging surgery, a conservative unilateral oophorectomy would be preferred in GCT [4]. In the present case, an ovarian cyst in contralateral ovary doubling in diameter within one month occurred. In general, tumors demonstrating rapid growth should prompt suspicion of malignancy [5].

In the present case, the accurate diagnosis was not established on intraoperative frozen section due to mucinous histology. Inaccurate diagnosis was encountered more frequently with mucinous tumors than other tumor histologies [6].
It is acceptable to perform cystectomy or unilateral salpingo-oophorectomy to preserve fertility in Stage I BOT. However, simple ovarian cystectomy for BOT is associated with a higher risk of recurrence than unilateral salpingo-oophorectomy or Bilateral salpingo-oophorectomy [7]. Therefore patients should be very carefully informed about the risks of recurrence [8].

Discussion

Although ovarian masses large enough for torsion are accepted as malignant in postmenopausal women, malignancy risk should not be omitted in young women presenting with torsion. To avoid the second laparotomy, frozen section examination may be required even if there is no suspicion for a malignant lesion.

References


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Rare case of concurrent severe chylous ascites after radical surgery for cervical cancer

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Summary

Background: Cervical cancer case supervened with chylous ascites after extensive hysterectomy is rarely reported, and is very difficult to deal with. Case: A 40-year-old female patient complained of a small amount of vaginal bleeding after intercourse over the past seven years, and then was diagnosed as cervical squamous cell carcinoma IIa, with moderate anemia and leucopenia. The patient underwent radiotherapy and was given leucogen and iron dextran to elevate blood leukocyte at the same time. Considering blood routine examination was normal, the patient underwent extensive hysterectomy, bilateral adenectomy, and pelvic lymphadenectomy. By day 30 postoperatively, chyluria test showed positive which indicated chylous ascites in the patient. Since then, the patient successively had hypoproteinemia, electrolyte imbalance, high fever, fungal infection, etc. Very fortunately, the patient made a remarkable recovery from the serious condition after a series of flexible anti-infection and effective supportive treatments. Conclusion: Chylous ascites leads to the loss of lipid, plasma protein, fat-soluble vitamins, and subsequently malnutrition. Firstly, the primary disease should be dealt with through chemotherapy or radiotherapy for malignant tumors. Anti-infective therapy can prevent intra-abdominal infections and the occurrence of bacteremia. Considering postoperative treatments last longer in this case, the authors changed antibiotics several times to avoid drug resistance. However, the patient unfortunately had complication of fungal septicemia due to the serious condition, which should to be avoided next time. In addition, the balance of water, electrolyte, and acid-base is particularly important in the overall treatment.

Key words: Gynecological oncology; Chylous ascites; Cervical cancer; Peritoneal drainage.

Introduction

Chylous ascites are a clinical symptom that is rare and difficult to deal with, and mainly caused by surgery, trauma, inflammation, cirrhosis, tuberculosis, lymphatic malformations, lymphatic cancer, and other causes of reflux disorder or lymphatic rupture [1]. Tulunay et al. reported chylous ascites with an incidence of 2% after staging surgery for gynecological malignancies [2]. Chylous ascites are not a common complication after radical resection of cervical cancer, because there are only a few reports of such cases. The present cervical cancer case the authors report supervened with chylous ascites after extensive hysterectomy, bilateral adenectomy, and pelvic lymphadenectomy. They observed the evolution and early prognosis of lesions.

Case Report

A 40-year-old female patient complained of a small amount of vaginal bleeding after intercourse over the past seven years. The patient visited the present hospital due to large amounts of vaginal bleeding after intercourse on July 29th, 2012, and underwent cervical biopsy pathology in the out-patient department, which revealed: moderately differentiated squamous cell carcinoma in cervical tissue. She was admitted to the present hospital on August 13th, 2012. The patient had no history of tuberculosis, hepatitis, diabetes, and hypertension. Gynecological examination: a small amount of vaginal blood, cervical hypertrophy, moderate erosion, cauliflower-like tissues about four cm in diameter with contact bleeding on lateral lip. Infectious immune parameters: negative. Blood routine examination: WBC 3.10×10⁹/L, N 62.20%, RBC 3.43×10¹²/L, Hb 74 g/L; this patient was diagnosed with anemia, leucopenia. During August 14th to 25th, 2012, the patient underwent radiotherapy using 192Ir with the radiation dose: 20GY for four times, and was given leucogen and iron dextran to elevate blood leukocyte at the same time. Reviewed the hemogram after radiotherapy: WBC 3.50×10⁹/L, N 49.00%, RBC 3.15×10¹²/L, Hb 74 g/L; this patient was diagnosed as follows: cervical squamous cell carcinoma II a, moderate anemia, leucopenia. During August 14th to 25th, 2012, the patient underwent radiotherapy using 192Ir with the radiation dose: 20GY for four times, and was given leucogen and iron dextran to elevate blood leukocyte at the same time. Reviewed the hemogram after radiotherapy: WBC 3.50×10⁹/L, N 62.20%, RBC 3.43×10¹²/L, Hb 82 g/L. On September 11th, 2012 the patient underwent extensive hysterectomy, bilateral adenectomy, and pelvic lymphadenectomy. The authors found pelvic lymph nodes and abdominal para-aortic lymph nodes intumescent during operations, and placed a drainage tube in abdominal cavity, gave ceftizoxime, and ornidazole to prevent infection after operations. By day 6 postoperatively, peritoneal drainage fluid turned from light yellow to milky white, and gradually increased to 1,300 ml, a maximum capacity of 3,450 ml, followed by hypoproteinemia and electrolyte imbalance. Immediately, the patient underwent adequate abdominal drainage, given transfusion of fresh plasma, and albumin to cor-
rect hypoalbuminemia, was given potassium chloride and sodium chloride to correct electrolyte balance, attached to an intravenous drip with fat emulsion, amino acids, and vitamins to support compound treatment, and given low-fat, high protein diet for adjuvant therapy. For the next eight days, the amount of chylous fluid drained was maintained between 700-1,500 ml per day. Intra-abdominal fluid examination showed higher protein content suggesting that was exudate. By day 27 after operations, implementation of intravenous infusion of plasma and albumin was continued to correct hypoalbuminemia, with total parenteral nutrition therapy, subcutaneous octreotide 0.1 mg, q.d. to reduce lymph production. Considering the intra-abdominal drainage lasted too long, the authors changed the antibiotics to prevent infection. By day 29 after operations, peritoneal drainage fluid amount increased to 4,000 ml, the chyle test showed negative and adipocyte was not found. The chyluria test showed positive next day. Pleural effusion was TG 10.54 mmol/L and the above treatment was continued. By day 31 after operations, the patient’s temperature was 39.8°C. Chyle test for review showed positive. Total parenteral nutrition was suspended. Imipenem-cilastatin one g, tid, combined with ornidazole was given to strengthen anti-inflammatory effect. Recombinant human granulocyte-stimulating factor was injected via subcutaneous to promote leukocyte generation. Albumin and human serum gamma globulin were infused to improve immunity. Testosterone propionate was used to promote the synthesis of protein and water-soluble and fat-soluble vitamins were added to support the treatment. The 29th day after operations, fungal septicemia occurred, temperature continued at 39.3°C. Left lower lung had slight rales and a small amount of pleural effusion were showed in Chest X-ray examination. Type-B ultrasonic showed: cholecystolithiasis, the spleen was slightly larger, with abdominal and pelvic effusion. Fungal infection was revealed in blood culture. Candida parapsilosis was found in urine culture. Reviewed hemogram: WBC 3.7×10^9/L, CRP 75 g/L, ESR 25 mm/L, TP , AL 19.9 g/L, Na⁺ 128.6 mmol/L, K⁺ 3.3 mmol/L. Ornidazole was stopped and intravenous catheter was replaced, then continued to perform the above treatments. Fluconazole 0.2 g, q.d. was infused as an anti-fungal treatment. Fresh plasma was infused to correct hypoproteinemia. Sodium and potassium were supplied to maintain electrolyte balance. Thus the patient’s condition gradually improved. By day 54 after operations the patient complained of abdominal distension. Shifting dullness was positive and intravenous fluids were stopped. Furosemide was given by intravenous injection for diuresis. The next day, the patient underwent radiation therapy five times with 5 GY, and discharged. The patient was re-hospitalized on November 22th 2012, and underwent abdominal paracentesis and drained milky white liquid about 1,000ml by day 4. Temperature gradually became normal, peritoneal fluid significantly reduced, the patient was discharged and at six months follow-up, no obvious abnormalities were seen.

**Discussion**

The majority of reports on chylous ascites as a postoperative complication involve patients who underwent abdominal aortic surgery, lymphadenectomy for testicular and renal cancers, or pelvic surgery for advanced gynecologic malignancies [3]. Any surgery involving the retroperitoneal area or mesenteric root, or the anatomical variation of cisterna chyli or lymphatic plexus may initiate chylous ascites that caused by the lesion of intestinal lymphatic vessels, lymphatic trunk or chylocyst [4]. The present authors analyzed the reason for concurrent severe chylous ascites after operations may be related to the following factors: four to six days after radical resection of cervical cancer, chylous ascites could be extracted from peritoneal drainage fluid; due to lymphatic vessels rupture caused by lesions during the operation which may locate in lymphatic trunks, chylocyst or the lymph capillaries with no ligation; postoperative blocking of chylocyst or thoracic duct leading to lymphatic circumfluence suffocation, result in expansion and rupture of retroperitoneal lymphatic vessels; inflammation could cause lymph nodes hyperemia and lymphatic vessel wall edema, which induce lymphatic vessel stenosis or blocking, chyle permeate into abdominal cavity; others reasons may be related to the hypoproteinemia or poor physique.

Chylous ascites lead to the loss of lipid, plasma protein, fat-soluble vitamins and subsequently lead to malnutrition. Therefore, measures should be adopted as soon as possible to treat chylous ascites effectively: (I) the primary disease should be dealt with and perform chemotherapy or radiotherapy for malignant tumors. In this case, radiotherapy using 192Ir as the radiation source was performed in time when the patient could tolerate it; (II) change the dietary structure to high-calorie, high protein, low fat, low sodium type. The diet should contain only medium-chain triglycerides, which can be directly absorbed by the intestinal cells and pass through the portal vein in the form of free fatty acids and glycerol, reducing the amount of thracic duct chylous fluid; (III) parenteral nutrition can improve the nutritional status so as to provide necessary basic conditions for tissue repair and wound healing. At the same time, total parenteral nutrition can inhibit the secretion of gastrointestinal fluid and reduce the formation of lymph to ensure gastrointestinal tract to get sufficient rest, and promote the healing [5]; (IV) it is reported that somatostatin can be applied when chylous ascites occur, which can significantly reduce the drain, reduce production of intestinal lymph to speed up healing. Somatostatin or its analog (octreotide) showed high effectiveness in patients with protracted symptom of chylous ascites [6-7]. In this case, chylous ascites was so serious that the authors used somatostatin and got obvious effect; (V) anti-infective therapy can prevent intra-abdominal infections and the occurrence of bacteremia. Considering postoperative treatments last longer in these cases, antibiotics should be changed several times to avoid drug resistance and to prevent fungal septicemia as in the present case; (VI) the balance of water, electrolyte, and acid-base is particularly important to the overall treatment. We should be familiar with the distribution of retroperitoneal lymph nodes and its drainage regularity, and pay attention to distinguish
Rare case of concurrent severe chylous ascites after radical surgery for cervical cancer

lymphatic vessels, observe carefully whether there is milky white liquid flowing out, ligate the cutt off and isolated tissues one by one in operation. Only by dong so, will the occurrence of chylous ascites be avoid.

References


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