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**Contents**

**ORIGINAL ARTICLES**

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab for the treatment of recurrent ovarian cancer: a retrospective cohort study</td>
<td>113</td>
</tr>
<tr>
<td>(S.N. Akers, G. Riebandt, A. Miller, A. Groman, K. Odunsi, S. Lele - Buffalo, NY (USA))</td>
<td></td>
</tr>
<tr>
<td>The role of bevacizumab in patients with recurrent ovarian cancer remains controversial and warrants further investigation.</td>
<td></td>
</tr>
<tr>
<td>UGT1A1 genotype-specific phase I and pharmacokinetic study for combination chemotherapy with irinotecan and cisplatin: a Saitama tumor board study</td>
<td>120</td>
</tr>
<tr>
<td>UGT1A1 genotype-specific dose-finding phase I study of combination therapy with irinotecan and cisplatin for gynecologic cancer patients.</td>
<td></td>
</tr>
<tr>
<td>Analysis of the risk factors for the recurrence of cervical cancer following ovarian transposition</td>
<td>124</td>
</tr>
<tr>
<td>(C. Zhao, J.L. Wang, S.J. Wang, L.J. Zhao, L.H. Wei - Beijing, R. CHINA)</td>
<td></td>
</tr>
<tr>
<td>The authors found pathological type, differentiated degree, and cancer embolus in vessels or lymphatic metastasis that may be the risk factors related to the recurrence of cervical cancer following ovarian transposition.</td>
<td></td>
</tr>
<tr>
<td>The correlation between expression of synuclein-γ, glucose transporter-1, and survival outcomes in endometrioid endometrial carcinoma</td>
<td>128</td>
</tr>
<tr>
<td>(D.G. Hong, N.Y. Park, G.O. Chong, Y.L. Cho, I.S. Park, M.J. Jeong, J.Y. Park, Y.S. Lee - Daegu, REPUBLIC OF KOREA)</td>
<td></td>
</tr>
<tr>
<td>The synuclein-γ and glucose transporter-1 were not considered to be a prognostic factor and were not related to survival.</td>
<td></td>
</tr>
<tr>
<td>Differential gene expression profile in cervical cancer and parenchyma infected with human papillomavirus 16 screened by cDNA microarray</td>
<td>132</td>
</tr>
<tr>
<td>(Y.L. Wang, X.P. Ding, Z.R. Xiong, H. Liao - Chengdu, CHINA)</td>
<td></td>
</tr>
<tr>
<td>The expression of many genes in cervical cancer and in HPV 16-infected tissues is statistically different in relation to normal cervical tissue.</td>
<td></td>
</tr>
<tr>
<td>Nodal involvement evaluation in advanced cervical cancer: a single institutional experience</td>
<td>138</td>
</tr>
<tr>
<td>(C. Gonzalez-Benitez, I. Zapardiel, P.I. Salas, M.D. Diestro, A. Hernandez, J. De Santiago - Madrid, SPAIN)</td>
<td></td>
</tr>
<tr>
<td>Para-aortic lymphadenectomy still seems to be the gold standard to assess nodal status in advanced cervical cancer.</td>
<td></td>
</tr>
<tr>
<td>Mifepristone sensitizing cisplatin for cervical adenocarcinoma HeLa cell sensitivity to chemotherapy and its mechanism</td>
<td>142</td>
</tr>
<tr>
<td>(Caihong Li, Hong Ye - Hubei, CHINA)</td>
<td></td>
</tr>
<tr>
<td>Preliminary study on mechanism of action of cisplatin combined with mifepristone for cervical adenocarcinoma HeLa cell.</td>
<td></td>
</tr>
<tr>
<td>Diagnostic value of CA125 as a predictor of recurrence in advanced ovarian cancer</td>
<td>148</td>
</tr>
<tr>
<td>Increment of eight IU/ml for the CA-125 level among the assessed CA-125 increments as the best predictor of recurrent ovarian cancer.</td>
<td></td>
</tr>
<tr>
<td>Livin and caspase-3 expression are negatively correlated in cervical squamous cell cancer</td>
<td>152</td>
</tr>
<tr>
<td>(M. Xu, L.P. Xia, L.J Fan, J.L. Xue, W.W. Shao, D. Xu - Yancheng, P.R. CHINA)</td>
<td></td>
</tr>
<tr>
<td>Livin may inhibit apoptosis in cervical squamous cell carcinoma by downregulating caspase-3, thereby promoting disease progression.</td>
<td></td>
</tr>
<tr>
<td>Laparoscopically-assisted radical vaginal hysterectomy with five years follow-up: a case control study</td>
<td>156</td>
</tr>
<tr>
<td>(J.J. Yu, W.X. Yang, X.M. Wang - Wuxi, CHINA)</td>
<td></td>
</tr>
<tr>
<td>Laparoscopically-assisted radical vaginal hysterectomy is a safe and alternative approach to open radical hysterectomy, reducing operative complications.</td>
<td></td>
</tr>
<tr>
<td>Analysis of one year follow-up of women with cervical cytology report of atypical squamous cells and the diagnostic role of high-risk HPV infection</td>
<td>159</td>
</tr>
<tr>
<td>(K. You, Y.L. Guo, L. Geng, J. Qiao - Beijing, CHINA)</td>
<td></td>
</tr>
<tr>
<td>Colposcopy and biopsy are mandatory in patients with abnormal cytology, especially in developing countries, due to the high rate of loss to follow-up.</td>
<td></td>
</tr>
</tbody>
</table>
The value of mesothelin in the diagnosis and follow-up of surgically treated ovarian cancer
Naian Qiao, Haiying Li - Jinan, CHINA
Serum mesothelin dosage has a significant value for differential diagnosis of benign and malign ovarian tumor and is useful for follow-up of treated epithelial ovarian cancer.

How to prevent the iatrogenic diffusion of gynecological malignant tumors?
X. Tianmin, C. Weiqin, C. Manhua, L. Yang, S. Lihui, J. Shan - Changchun City, CHINA
To reduce the chance of iatrogenic diffusion of tumors, enhance the survival rate of tumor patients, improve the prognosis, and reduce the death rate as much as possible.

The regulation network and network motif analysis in ovarian cancer
A-juan Liang, Yan Hong, Yun Sun, Minzhi Gao, X. Zhao - Shanghai, CHINA
To interpret the transcription regulation network of ovarian cancer. The results provide a molecular mechanism and potential therapeutic targets.

An analysis of Turkey’s scientific contribution in ovarian cancer research
T. Guler, E. Yayci, T. Atacag, A. Cetin - Lefkosa, TURKEY
Turkey's contribution in ovarian cancer research has an increasing trend in the last decade.

Endometrial adenocarcinoma in a young woman
D. Caserta, G. Bordi, S. Scarani, M. Moscarini - Rome, ITALY
A case of a young woman with polycystic ovarian syndrome and endometrial adenocarcinoma who underwent surgical staging and treatment is reported.

Sustainable complete remission in recurrence yolk sac tumor patient treated with tandem high-dose chemotherapy and autologous stem cell
N.A. Abdullah, P.N. Wang, K.G. Huang, A.S. Adlan, J. Casanova - Tao-Yuan, TAIWAN
Recurrent ovarian yolk sac tumor successfully treated with high-dose chemotherapy and autologous stem cell transplantation following failure of conventional chemotherapy.

Coexistence of three benign and a borderline tumor in the ovaries of a 52-year-old woman
I.C. Kotsopoulos, P.A. Xirou, D.A. Deligiannis, V.S. Tsapanos - Patras, GREECE
A rare case of simultaneous presence of four histologically different types of ovarian tumors is presented.

Borderline ovarian tumor - a case report with genetic testing
J. Varga, M. Bilecová-Rabajdová, A. Ostró - Košice, SLOVAK REPUBLIC
A case of serous borderline ovarian tumor also including tumor vascular markers genetic assessment is described.

An undescribed coexistence of benign metastasizing leiomyoma in the lung with serous borderline tumor of the ovary
M.F. Gan, H.S. Lu - Zhejiang, CHINA
A case of pulmonary benign metastasis leiomyoma co-existing with ovarian serous borderline tumor is referred.

Vulvar melanoma and endometrial polyp following breast carcinoma: a case report
L. Shen, F. Zeng, L. Hong, G. Zhang, R. Mai - Shantou, CHINA
A case of vulvar melanoma and endometrial polyp in patient treated with radiotherapy and tamoxifene for previous breast cancer is reported.
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Bevacizumab for the treatment of recurrent ovarian cancer: a retrospective cohort study

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¹Division of Gynecologic Oncology, ²Department of Pharmacy, ³Department of Biostatistics
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Summary

Objective: To determine response rates (RR), progression-free survival (PFS), overall survival (OS), and toxicity in patients treated with cytotoxic chemotherapy, in combination with bevacizumab compared to cytotoxic chemotherapy alone, in the setting of recurrent ovarian cancer. Materials and Methods: After obtaining Institutional Review Board approval, two cohorts of patients with recurrent ovarian cancer were identified: 1) patients that received cytotoxic chemotherapy with bevacizumab from January 2006 to June 2009; 2) patients that received cytotoxic chemotherapy alone. RR were measured using RECIST criteria or by CA-125 levels using modified Rustin criteria. RR, OS, and PFS were determined using Kaplan-Meier survival analysis. Results: Thirty-two patients that received bevacizumab in combination with cytotoxic chemotherapy and 32 patients that received cytotoxic chemotherapy alone were identified. The control patients were matched for age, platinum response, histology, surgical outcome, grade, and number of previous chemotherapy regimens. There were no differences between the two cohorts in the rates of venous thromboembolism (VTE) (p = 0.39), bleeding (p = 0.15) or bowel obstruction (p = 0.40). The rate of hypertension in the bevacizumab cohort was greater than in the comparison cohort (p < 0.005). There were no differences in response rates PR/CR vs SD/PD (p = 0.46), OS (p = 0.79) or PFS (p = 0.43). Conclusions: With increased toxicity, increased cost of therapy and no improvement in PFS or OS, the role of bevacizumab in patients with recurrent ovarian cancer warrants further investigation.

Key words: Squamous cell carcinoma; Endometrial carcinoma; Ichthyosis uteri; Ovarian cancer; Chemotherapy; Bevacizumab.

Introduction

Ovarian cancer is the fifth most common cause of death from malignancies in women in the United States and the leading cause of death of gynecologic malignancies. It is estimated that 14,560 women will die from ovarian cancer in 2011 [1]. Approximately seventy-five percent of patients with epithelial ovarian cancer (EOC) present with advanced Stage disease (III and IV). It is most commonly treated with tumor debulking followed by adjuvant taxane and platinum-based chemotherapy [2, 3]. Although 70%-80% of patients demonstrate a response to primary adjuvant therapy, most of these patients will have disease recurrence within 15 months and often die from tumor progression [3, 4]. In the treatment of recurrent disease, there is only a 15%-20% response rate (RR) with no possibility of a cure. With almost no change in disease-specific mortality in the last three decades with standard of care treatment, these patients present a challenge for treatment and thus the use of novel targeted therapy warrants further investigation [5].

Angiogenesis, a recently studied target in ovarian cancer patients, is crucial in the development and progression of ovarian cancer [6-12]. Some studies have demonstrated that increased angiogenesis activity is associated with increased aggressiveness of the tumor and may be a useful prognostic factor [8-10, 12-16]. Vascular endothelial growth factor (VEGF) is known to stimulate endothelial cell growth and promote vascular permeability leading to the formation of new blood vessels. VEGF has been shown to be a potential target for novel therapy and has been used in the treatment of recurrent ovarian cancer [17-19]. Bevacizumab, a humanized monoclonal antibody against VEGF, is FDA-approved for the treatment of metastatic colorectal cancer, unresectable non-squamous, non-small cell lung cancer, glioblastoma multiforme, and metastatic renal cell carcinoma [20-25]. Modest RR (8%-36%) and disease stabilization (8%-36%) have been reported in ovarian cancer [18, 26-28]. Bevacizumab is currently being evaluated in three phase III studies, Gynecologic Oncology Group (GOG) 218, ICON-7, and the OCEANS trial. GOG 218 and ICON-7 evaluate the use of bevacizumab with first-line adjuvant taxane/platinum-based chemotherapy for the treatment of ovarian cancer [29, 30]. Preliminary data on progression-free survival (PFS) are available for these two trials; however data on overall survival (OS) and quality of life have not yet been reported. PFS data from ICON-7 support the use of bevacizumab in combination with carboplatin/paclitaxel for front-line adjuvant therapy for the treatment of ovarian cancer, with a significant improvement in PFS of 17.3 vs 19 months (p = 0.004). Also a significant improvement in PFS with patients with Stage III/suboptimal debulking and Stage IV disease of 10.5 vs 15.9 months (p < 0.001) [31]. However the preliminary analysis of OS does not show an improvement in OS (p = 0.098). Preliminary data from GOG-218 also report an improvement in median PFS of 3.8 months (10.3 vs 14.1 months) when bevacizumab is
given as maintenance therapy for an additional ten months [32]. The OCEANS trial tests the benefit of bevacizumab in addition to carboplatin and gemcitabine in patients with platinum-sensitive recurrent ovarian cancer. The patients included in the study could have only received one previous line of chemotherapy to qualify for enrollment. Preliminary results demonstrate an improvement in PFS of 12.4 vs 8.4 months for patients with bevacizumab in addition to carboplatin and gemcitabine compared to placebo with carboplatin and gemcitabine. The OS data are not mature and have not been published [33].

The most common toxicities (> 10%) associated with the use of bevacizumab include: hypertension, proteinuria, epistaxis, headache, rhiitis, dry skin, back pain, exfoliative dermatitis, and rectal hemorrhage. Documented events associated with its use include, stroke, transient ischemic attacks, myocardial infarctions and angina. Age > 65 years has also been associated with an increased risk of thromboembolic events [34]. Bevacizumab received a black box warning for gastrointestinal perforations (GIP), wound healing complications, fistula formation, and hemorrhage. National Comprehensive Cancer Network practice guidelines in oncology list the use bevacizumab as an acceptable single agent therapy or as a part of combination chemotherapy for the treatment of patients with recurrent ovarian cancer.

Materials and Methods

After obtaining Institutional Review Board approval, two cohorts of patients receiving chemotherapy for recurrent ovarian cancer were identified: 1) 32 patients that received cytotoxic chemotherapy with bevacizumab (January 2006 to June 2009) and 2) 32 patients that received cytotoxic chemotherapy alone. The control patients were matched for age, platinum response, histology, surgical outcome, grade, and number of previous chemotherapy regimens. Patients were eligible if they had documented recurrent ovarian cancer by CA-125 or radiographic studies. All patients received taxane and platinum as front-line adjuvant therapy prior to their first recurrence. No patients received bevacizumab as part of front line therapy or as single agent therapy.

Patient demographics, clinico-pathologic data, and toxicities were extracted from patient charts. Bevacizumab was continued until disease progression or severe cytotoxic events occurred. PFS and OS were obtained using Kaplan-Meier curves. RR were calculated using response to treatment in solid tumors (RECIST) criteria or CA-125 levels according to modified Rustin criteria [35, 36]. Complete response (CR) was defined as no gross evidence of disease, resolution of measurable disease on computed tomography (CT) scan or normalization of CA-125 levels from an elevated level. Partial response (PR) was defined as a 30% reduction in lesions on CT scan or 50% reduction in CA-125. Progressive disease (PD) was defined as a 20% or greater increase in the lesions based on CT scan or doubling of CA-125 within eight weeks of starting therapy. Stable disease (SD) was any of the conditions that did not meet the above criteria. The best response for each patient was reported. CA-125 levels were routinely drawn with the pre-chemotherapy labs and imaging was not required to document a response. PFS was defined as the time from the initiation of treatment with bevacizumab or last cytotoxic chemotherapy until PD or date of last contact.

Results

A total of 64 patients were identified, 32 received cytotoxic chemotherapy in combination with bevacizumab and an additional 32 received cytotoxic chemotherapy alone for the treatment of recurrent ovarian cancer. The most commonly prescribed dose of bevacizumab was 15 mg/kg every three weeks (84%). No patients in the study group had received prior bevacizumab. The median age of patients in the bevacizumab cohort was 56.5 years and 58 years for the cytotoxic chemotherapy alone cohort (p = 0.23). Patient demographics are depicted in Table 1. Fifty-nine percent (19/32) of patients received weekly paclitaxel in combination with bevacizumab which was the most common regimen. Of the other regimens given, nine patients (28%) received cyclophosphamide, three patients (9%) received doxorubicin, and one patient (3%) received carboplatin/gemcitabine along with bevacizumab. In the chemotherapy alone cohort, nine (28%) patients received doxorubicin, eight (25%) patients received carboplatin/gemcitabine, five (16%) patients received paclitaxel, two (6%) patients received cyclophosphamide, two (6%) patients received topotecan, two (6%) patients received etoposide, one (3%) patient received carboplatin, one (3%) patient received navelbine, one (3%) patient received pemetrexed, and one (3%) patient received carboplatin/paclitaxel (Table 2). At the conclusion of the study, all 32 patients that received bevacizumab had died.

The cytotoxic regimens used for the treatment of recurrent ovarian cancer prior to the initiation of bevacizumab included taxanes, platinum compounds, doxorubicin, gemcitabine, and topotecan. The mean number of previous chemotherapy regimens prior to starting bevacizumab was 3.4 and 3.3 in the comparison cohort (p = 0.48) (Table 3). The mean number of cycles of bevacizumab was seven (range 1-18) and the mean cumulative dose was 8,329 mg (range 952-33,704 mg). Mean time of treatment length was 126 days (range 21-378 days). To determine response rates, PFS and OS, CA-125 values and radiologic studies were used.

Toxicity

Table 4 depicts the adverse events that occurred in each cohort. In the bevacizumab cohort, two patients experienced complications from bleeding, one patient developed severe epistaxis (11 cycles of bevacizumab - 19,640 mg cumulative dose), and one developed an upper gastrointestinal bleed (one cycle of bevacizumab - 1,680 mg cumulative dose), both requiring transfusions. Bevacizumab was discontinued for both patients and although there were no hemorrhagic events in the cytotoxic chemotherapy alone cohort, this difference was not statistically significant (p = 0.15). Two (6%) patients in the bevacizumab cohort developed a venous thromboembolism (VTE) compared to four (13%) in the cytotoxic chemotherapy alone cohort (p = 0.39). Seven (22%) patients in the bevacizumab cohort and ten (31%) patients in the cytotoxic chemotherapy alone cohort developed a bowel obstruction (p = 0.40). Nine (28%) patients developed hypertension requiring medical
therapy during treatment with bevacizumab compared to none in the cytotoxic chemotherapy alone cohort (p < 0.005). One patient receiving bevacizumab developed an enterocutaneous fistula (after first cycle of bevacizumab, 705 mg) and one (3%) patient in the cytotoxic chemotherapy alone developed a fistula (p = 0.55).

Bevacizumab was discontinued for the following reasons: disease progression (66%), bowel obstruction (13%), change in care (3%), hemorrhage (6%), congestive heart failure/myocardial infarction (CHF/MI, 6%), and enterocutaneous fistula (3%).

**Response data**

The median OS in the bevacizumab cohort was 10.4 months (range 5.6-13.8), while the median OS in the cytotoxic chemotherapy alone cohort was 4.9 months (range 3.5-6.6) as demonstrated in the Kaplan-Meier curve (Figure 1). This difference was not statistically different (p = 0.79). The median PFS was four months (range 2.8-7.1) in the bevacizumab cohort vs three months (range 2-4.9) in the comparison cohort (Figure 2). As in the OS, there was no statistical difference in PFS between the two cohorts (p = 0.43). In a subset analysis of platinum resistant patients there was no difference in OS (p = 0.34) or PFS (p = 0.37) between the two cohorts (Figures 3 and 4). In the platinum sensitive group there was no difference in OS (p = 0.87) or PFS (p = 0.43) between the two cohorts (Figures 5 and 6).

The overall RR was 19% in the bevacizumab group as compared to 16% in the control group. In the bevacizumab group, twenty-three (72%) patients had PD, six (19%) had a PR, and two (6%) had SD (one (3%) was unable to be assessed). In the control group, four (13%) had a CR, 24 (75%) had PD, one (3%) had a PR, three (9%) had SD.

**Discussion**

Bevacizumab, a humanized monoclonal antibody against VEGF, has shown activity in several solid tumors, including ovarian carcinoma. Despite the fact that most patients with ovarian cancer have good RR to adjuvant cytotoxic chemotherapy, a majority of these patients will recur within five years. Recurrent and platinum-resistant ovarian cancer continue to present treatment dilemmas, since RR in platinum-resistant patients are reported to be 15%. Five phase II studies have been performed to examine the use of bevacizumab in the setting of recurrent ovarian cancer: two single agent trials, two in combination with cytotoxic therapy, and one in combination with biologic chemotherapy. RR of 8%-36% and disease stabilization in 55%-75% of patients receiving bevacizumab were reported in these studies [18, 26-28, 37]. These results are promising for the treatment of recurrent ovarian cancer.

GOG conducted the largest single agent study, GOG 170D, which reported a 21% overall response rate (ORR) and a 40% six-month PFS in 62 women when treated with bevacizumab. Of these 62 women, 66% had received two prior regimens and 42% were considered platinum-resistant [26]. AVF2797, another single-agent industry sponsored trial reported an ORR of 16% in an 84% platinum-resistant population and 48% had received three prior

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**Table 1. — Patient demographics.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bevacizumab n = 32</th>
<th>Chemotherapy n = 32</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>56.5 (27-84)</td>
<td>54 (36-89)</td>
<td>0.23</td>
</tr>
<tr>
<td>Stage I &amp; II</td>
<td>2</td>
<td>0</td>
<td>0.15</td>
</tr>
<tr>
<td>Stage III &amp; IV</td>
<td>30</td>
<td>32</td>
<td>1.0</td>
</tr>
<tr>
<td>Histology Serous</td>
<td>25</td>
<td>25</td>
<td>0.72</td>
</tr>
<tr>
<td>Non-serous</td>
<td>7</td>
<td>7</td>
<td>0.77</td>
</tr>
<tr>
<td>Debulking status Optimal</td>
<td>27</td>
<td>28</td>
<td>0.46</td>
</tr>
<tr>
<td>Suboptimal</td>
<td>5</td>
<td>4</td>
<td>0.46</td>
</tr>
<tr>
<td>Overall response CR/PR</td>
<td>6</td>
<td>4</td>
<td>0.77</td>
</tr>
<tr>
<td>PD/SD</td>
<td>26</td>
<td>28</td>
<td>0.77</td>
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<tr>
<td>UTA</td>
<td>1</td>
<td>0</td>
<td>0.77</td>
</tr>
<tr>
<td>Platinum sensitivity Resistant</td>
<td>25</td>
<td>28</td>
<td>0.77</td>
</tr>
<tr>
<td>Sensitive</td>
<td>7</td>
<td>4</td>
<td>0.77</td>
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</table>

**Table 2. — Chemotherapy regimen.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bevacizumab n = 32</th>
<th>Chemotherapy n = 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td>19 (59%)</td>
<td>5 (16%)</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>9 (28%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>3 (10%)</td>
<td>9 (28%)</td>
</tr>
<tr>
<td>Carboplatin/Gemcitabine</td>
<td>1 (3%)</td>
<td>8 (25%)</td>
</tr>
<tr>
<td>Pemetrexed</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Carboplatin/Paclitaxel</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Etoposide</td>
<td>2 (6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Navelbine</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Topotecan</td>
<td>2 (6%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

**Table 3. — Number of previous chemo regimens.**

<table>
<thead>
<tr>
<th>No. of previous chemo regimens</th>
<th>Bevacizumab n = 32</th>
<th>Chemotherapy n = 32</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>5</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>8</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>6</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>5</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>5</td>
<td>0.01</td>
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<td>6</td>
<td>2</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>2</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Table 4. — Adverse events documented during treatment.**

<table>
<thead>
<tr>
<th>Event</th>
<th>Bevacizumab n = 32</th>
<th>Chemotherapy n = 32</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding</td>
<td>2 (6.3%)</td>
<td>0</td>
<td>0.15</td>
</tr>
<tr>
<td>VTE (during treatment)</td>
<td>2 (6.3%)</td>
<td>4 (13%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Bowel obstruction</td>
<td>9 (28%)</td>
<td>10 (31.2%)</td>
<td>0.40</td>
</tr>
<tr>
<td>Hypertension</td>
<td>7 (22%)</td>
<td>0</td>
<td>0.005</td>
</tr>
<tr>
<td>Fistula</td>
<td>1 (3%)</td>
<td>2 (6.3%)</td>
<td>0.55</td>
</tr>
</tbody>
</table>
Figure 1. — Kaplan-Meier plot demonstrating overall survival in the two cohorts.

Figure 2. — Kaplan-Meier plot demonstrating progression-free survival in the two cohorts.

Figure 3. — Kaplan-Meier plot for overall survival for platinum-resistant patients.

Figure 4. — Kaplan-Meier plot for progression-free survival for platinum-resistant patients.

Figure 5. — Kaplan-Meier plot for overall survival for platinum-sensitive patients.

Figure 6. — Kaplan-Meier plot for progression-free survival for platinum-sensitive patients.
The bevacizumab cohort had a RR of 19% compared to a RR of 16% in the chemotherapy alone cohort. The RRs for the bevacizumab cohort is similar to what has been reported by the previous phase II studies (15.9-24%). The OS and PFS in the bevacizumab cohort, 10.4 and four months respectively, are consistent with what Loizzi et al. previously reported in patients with recurrent ovarian cancer (OS and PFS, 13 and five months, respectively) [39].

Bevacizumab is generally tolerated but is known to be associated with certain toxicities including: hypertension, proteinuria, thromboembolic events, hemorrhage, GIP, wound-healing complications, and fistula formation [34]. Hypertension, new onset or exacerbation of existing hypertension, is the most common adverse event associated with bevacizumab. In randomized trials, grade 3/4 hypertension incidence ranged from 3%-15% compared to 0%-2% for controls [23, 24, 34, 40-46]. The patients receiving bevacizumab had a higher incidence of new onset hypertension or exacerbation of existing hypertension (28%) compared what has previously been reported.

Hemorrhage is another known adverse event associated with the use of bevacizumab. The etiology of the hemorrhage is thought to be due to lack of endothelial repair in areas where subendothelial tissues are violated by processes that may or may not be associated with malignancy [34]. Two patients in the bevacizumab group experienced hemorrhage, one had severe epistaxis, and the other had an upper gastrointestinal bleed (p = 0.15). VTE events have been reported in many trials using bevacizumab, but it is not clear whether this is greater than the baseline increase from the malignancy. Randomized trials have reported VTE rates to be no different in patients receiving bevacizumab compared to controls [23, 24, 40-46]. The rates of VTE in the two cohorts (6% vs 13%) was not statistically different (p = 0.39), consistent with what has been reported.

While the use of bevacizumab has also been associated with fistula formation, this adverse event tends to be rare. Garcia et al. reported an enterovaginal fistula in a recent phase II study [27]. The present authors report one (3%) case of enterocutaneous fistula after two cycles of bevacizumab (every three weeks, 15 mg/kg) in a platinum-resistant patient that had been treated with three prior lines of chemotherapy. However one patient in the comparison cohort also developed a fistula (p = 0.55). Bevacizumab has received a black box warning for its association with GIP. Cannistra et al. reported five (11%) GIP in forty-four patients and the study was terminated early due GIP [18]. GIP rates of 2%-4% have been reported. However in our group of patients receiving bevacizumab, no GIP occurred.

Current phase III studies (GOG-218 and ICON-7) have initially shown an improvement in PFS. GOG 218 showed an improvement in PFS of only four months for patients on standard therapy plus bevacizumab during standard therapy and up to ten months after completion of standard therapy compared to standard therapy alone [47]. However, these data cannot be extrapolated to the recurrent setting. The present RR, survival, and toxicity data are consistent with what has recently been reported. The reported toxicities were greater in the bevacizumab cohort compared to patients receiving cytotoxic chemotherapy for recurrent ovarian cancer, however only significant for hypertension (p < 0.005). There was no difference in RR (p = 0.46) between the two cohorts. Similar to what Garcia et al. reported, the two patients with clear cell histology that received bevacizumab had PR and SD [27]. Of the patients that received bevacizumab, it is important to note that eight patients had improvement in symptomatic ascites and two patients had improvement in symptomatic pleural effusions. However these patients also had PD based on CT scan and/or rising CA-125. Clinically these patients had improvement in their symptoms, yet all ten of these patients had PD. It is difficult to justify the use of bevacizumab in the setting of recurrent ovarian cancer with no change in RR, PFS or OS with an increase in cost and toxicity.

Currently there are no reliable markers that determine patients’ response to VEGF-targeted therapy. The results of this retrospective study show that in recurrent ovarian cancer, bevacizumab is tolerated with increased toxicity without an increase in RR, OS or PFS. However with inferior RR, increased toxicity and increased cost, the use of bevacizumab for the treatment of recurrent ovarian cancer, especially in heavily pre-treated patients and clear cell histology, warrants further investigation.

Some limitations of the study include: study design, limited number of patients, variability in treatment regimens, non-consistent radiologic follow-up, and lack of documentation of possible toxicities including proteinuria. Baseline radiologic studies were not available for all patients at the initiation of treatment with bevacizumab, thus limiting the ability to use radiologic studies to assess response to bevacizumab. It is difficult to assess hematologic toxicity of bevacizumab since many of the patients have been treated with multiple cytotoxic agents prior to the initiation of bevacizumab and the patients were receiving additional cytotoxic therapies during treatment with bevacizumab.

References


Bevacizumab for the treatment of recurrent ovarian cancer: a retrospective cohort study


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UGT1A1 genotype-specific phase I and pharmacokinetic study for combination chemotherapy with irinotecan and cisplatin: a Saitama tumor board study

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Summary

Introduction: Genotyping of UGT1A1 could be useful for prediction of severe toxicities for patients treated with irinotecan; however, genotype-based recommended dose (RD) has not been established. The aim of the present study was to determine the RD of irinotecan in combination with cisplatin (CPT-P) for individuals with or without UGT1A1 polymorphisms. Materials and Methods: According to polymorphisms of UGT1A1*28, *6, and *27, RDs were determined by three-case cohort methods for patients with wild-type and heterotype, and by inter-patient dose escalation for homotype patients. Pharmacokinetic studies were also evaluated. During May 2009 and July 2011, 18 Japanese patients were enrolled; 16 patients with ovarian carcinoma, and two cases with cervical cancer. The RD of irinotecan was determined as 50 mg/m² for the patients with wild-type, 40 mg/m² for those with heterotype, and 30 mg/m² for homo-type UGT1A1 genotype. Results: Patients with homotype UGT1A1 alleles had a significantly lower glucuronidation ratio in comparison with UGT1A1 wild-type and heterotype cases. Conclusion: UGT1A1 genotype-based RDs of irinotecan in CPT-P therapy were determined. Further studies to investigate efficacy of the RD including response evaluation are needed to confirm the present results.

Key words: UDP-glucuronosyltransferase 1A1 (UGT1A1); UGT1A1*6; UGT1A1*28; Irinotecan; Cisplatin; Phase I.

Introduction

Irinotecan hydrochloride is widely-used for a multiplicity of carcinomas, including colorectal and lung cancers. Combination therapy with irinotecan and platinum is often used for not only relapsed gynecologic cancers [1, 2], but also for untreated clear cell carcinoma of the ovary [3-5]. The efficacy of this therapy is currently being explored in the worldwide prospective randomized trial GCIG/JGOG 3017 to compare the survival of patients with ovarian clear cell carcinoma treated with either combination with paclitaxel and carboplatin or therapy with irinotecan and cisplatin [6].

Irinotecan-induced severe toxicities in patients with UGT1A1*28A1 allele have been reported in several studies [7-11]. A meta-analysis including ten sets of patients demonstrated that the risks of grades 3 and 4 hematologic toxicities were higher among UGT1A1*28/*28 patients than those with UGT1A1*1/*1 and UGT1A1*1/*28 genotype, when treated at medium- or high-dose of irinotecan (≥ 150 mg/m²) [12]. The significance was not observed in low-dose of irinotecan (100-125 mg/m²), which was commonly used at a therapeutic range for weekly and bi-weekly regimens. However, the effects might be potentially influenced by heterogeneity of patients and treatment schedules such as additive use of platinum agents. Another meta-analysis revealed that risk ratio (RR) of severe neutropenia was significantly increased in not only homozygous UGT1A1*28 cases (RR = 3.51), but also in heterozygous cases (RR = 1.82) [13]. The data suggested that the dose of irinotecan should be optimized according to three UGT1A1*28 genotypes; wild-type, heterozygous type, and homozygous type.

In the Asian population, allele frequency of UGT1A1*28 is quite low and another polymorphism of UGT1A1, UGT1A1*6 is more frequently observed compared with Caucasians or Afro-Americans [14, 15]. Recent studies have shown the significant relevance of UGT1A1*6 to severe toxicities of irinotecan-based chemotherapy [16-18].

In the present study, the authors performed UGT1A1 genotype-based phase I study for gynecologic cancer patients treated with weekly irinotecan combined with cisplatin to determine the maximal tolerated dose (MTD) and the recommend dose (RD), and analyzed the pharmacokinetics of irinotecan and its metabolites.

Materials and Methods

Eligibility criteria

The patients were considered to be eligible if they satisfied the following criteria: histologically confirmed diagnosis of ovarian or uterine cervical carcinoma; age range between 20 and 75 years; a performance status between 0 and 2 on the Eastern Cooperative Oncology Group (ECOG) scale; a life-expectancy of at least three months; treatment-free period of at least four weeks from previous chemotherapy or irradiation; adequate hematological (total white blood cell count ≥ 3,000/μl; absolute neutrophil count [ANC] ≥ 1,500/μl; platelet count ≥ 100,000/μl; and hemoglobin level ≥ 9 g/dl), hepatic (total bilirubin level ≤ 1.5 mg/dl; aspartate aminotransferase [AST] and alanine aminotransferase [ALT] levels ≤ three times the upper limit of normal), and renal (creatinine level ≤ 1.5 mg/dl and/or creatinine clearance ≥ 60 ml/minute)
function. The protocol included the following exclusion criteria: massive ascites and/or massive pleural effusion; serious infectious diseases or other complications, such as uncontrollable diabetes, intestinal pneumonitis, bowel obstruction, active bowel bleeding or colitis, active concurrent malignancies, symptomatic brain metastasis, nursing or pregnant women, medical record of hyperreaction to irinotecan or platinum agents, or other medical problems severe enough to prevent compliance with the present protocol. The study protocol was approved by each institutional review board. All study participants approved informed consent prior to the enrollment of the study.

**UGT1A1 genotyping**

Serum samples of the patients enrolled in the study were analyzed for polymorphisms of UGT1A1 by using the Invader UGT1A1 Molecular Assay which enabled genotyping of UGT1A1*28, *6, and *27 [19]. UGT1A1 polymorphisms were categorized into three groups: wild-type, heterotype, and homotype. Patients with heterotype UGT1A1 included UGT1A1*28 (+1/*28), UGT1A1*6 (+1/*6), and UGT1A1*27 (+1/*27), and those with no polymorphisms of UGT1A1*28, *6, *27 were categorized as wild-type (+1/*1) group. The cases with homozygous genotype of either UGT1A1*28 (+28/*28) or UGT1A1*6 (+6/*6), and those with double heterozygous polymorphisms of both UGT1A1*28 and UGT1A1*6 (+28/*6) were regarded as homotype patients.

**Drug administration**

The enrolled patients received chemotherapy consisting of 90-min intravenous infusion of 30-70 mg/m² of irinotecan on days 1, 8, and 15 and subsequently 120-min intravenous infusion of 60-70 mg/m² of cisplatin on day 1, q4 weeks. Treatment with irinotecan was withheld on days 8 or 15 if the patient experienced hematologic toxicities more than grade 3 or non-hematologic toxicities more than grade 2. Subsequent cycle of the therapy was initiated if the patients showed adequate hematological, hepatic, and renal function as was described in the criteria of patient enrollment, and also no sign of exclusion criteria. The prophylactic use of granulocyte colony-stimulating factor was not allowed.

Starting dose of irinotecan was 50 mg/m² for wild-type group patients, 40 mg/m² for heterotype patients, and 30 mg/m² for homotype patients, respectively. Dose of irinotecan was increased in 10 mg/m² increments, unless MTD was achieved. At least three patients were treated at each dose level as shown in Table 1. The patients with wild-type and heterotype were evaluated with three-case cohort methods. If no dose-limiting toxicity (DLT) was observed in the first three patients with wild-type/heterotype, the dose was escalated at next dose level when more than two patients experienced any DLT, the dose was defined as MTD. When one of three patients developed any DLT, additional three patients were added to the same dose level. If none or only one of additional three cases developed DLT, the dose level was escalated; however, if more than two patients experienced any DLT, the dose was determined as MTD. The RD was defined as one dose level under the MTD. Homotype patients were analyzed by interpatient dose escalation. Three homotype patients were treated with step 1 dose: 30 mg/m² of irinotecan and 60 mg/m² of cisplatin. If a patient developed no DLT, the patient would subsequently receive step 2 doses at the second cycle of the therapy. When two or more patients experienced any DLT, the dose was determined as MTD. The RD was defined as one dose level under the MTD.

**Toxicity profiles and statistical analysis**

Physical examination and serum blood test were carried out at least days 1, 3, 8, 15, and 21 for toxicity evaluation. The toxicity profiles were determined by National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. DLTs were defined as follows; (a) grade 4 neutropenia or leukopenia, (b) grade 3 febrile neutropenia for more than three days, (c) grade 3 non-hematologic toxicities including diarrhea, (d) discontinuation of irinotecan on days 8 and 15 due to toxicities at the first cycle, and (e) delay of the second cycle beyond two weeks due to toxicities.

**Pharmacokinetic evaluation**

A pharmacokinetics study of irinotecan, SN-38, SN-38 glucuronide (SN-38G), and platinum was performed on day 1 of combination with irinotecan and cisplatin. Whole blood samples were collected at 0, 2, 4, 8, and 24 hours after completion of irinotecan infusion. Irinotecan and its metabolites concentration was measured by high-performance liquid chromatography as described previously [20]. The area under the plasma time-concentration curve (AUC) was calculated using a trapezoidal method. The degree of glucuronidation of SN-38 to SN-38G

---

### Table 2. — Dose-escalation scheme and incidence of dose-limiting toxicity (DLT)

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Irinotecan (mg/m²)</th>
<th>Cisplatin (mg/m²)</th>
<th>Number of patients</th>
<th>Number of DLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>60</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>60</td>
<td>3</td>
<td>2 (ANC, FN)</td>
</tr>
<tr>
<td>Heterotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>60</td>
<td>6†</td>
<td>1† (ANC)</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>60</td>
<td>3</td>
<td>2 (diarrhea, diarrhea + FN)</td>
</tr>
<tr>
<td>Homotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>60</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>60</td>
<td>3</td>
<td>2 (ANC + FN, ANC + ALT)</td>
</tr>
</tbody>
</table>

† Three additional patients were treated to confirm the feasibility of the dose level, ANC = neutrophil count decreased; FN = febrile neutropenia; ALT = alanine aminotransferase increased.
Table 3. — Pharmacokinetic parameter at the first cycle.

<table>
<thead>
<tr>
<th>UGT1A1 genotype</th>
<th>Inotocan AUC (ng*h/ml)</th>
<th>SN-38 AUC (ng*h/ml)</th>
<th>SN-38G AUC (ng*h/ml)</th>
<th>Platinum GR AUC (μg*h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wild-type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 1 (n = 5)</td>
<td>2,329 ± 121 46.5 ± 0.5</td>
<td>524 ± 214 11.2 ± 3.3</td>
<td>89.5 ± 15.5</td>
<td></td>
</tr>
<tr>
<td>Step 2 (n = 2)</td>
<td>4,574 ± 721 120 ± 28</td>
<td>779 ± 144 6.5 ± 1.0</td>
<td>92.2 ± 21.1</td>
<td></td>
</tr>
<tr>
<td><strong>Heterotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 1 (n = 6)</td>
<td>2,881 ± 307 50.5 ± 10.8</td>
<td>516 ± 197 9.8 ± 2.9</td>
<td>80.6 ± 16.7</td>
<td></td>
</tr>
<tr>
<td>Step 2 (n = 2)</td>
<td>3,107 ± 654 107 ± 21</td>
<td>873 ± 46 8.1 ± 1.4</td>
<td>75.5 ± 20.4</td>
<td></td>
</tr>
<tr>
<td><strong>Homotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 1 (n = 6)</td>
<td>1,730 ± 665 74 ± 37</td>
<td>336 ± 78 3.4 ± 0.6</td>
<td>68.9 ± 19.3</td>
<td></td>
</tr>
<tr>
<td>Step 2 (n = 2)</td>
<td>1,650 ± 528 136 ± 25</td>
<td>226 ± 36 2.4 ± 0.8</td>
<td>70.5 ± 16.9</td>
<td></td>
</tr>
</tbody>
</table>

†GR of homotype patients was significantly lower than that of wild-type/heterotype patients: 3.1 vs 8.7, p = 0.001 (Mann-Whitney U-test).

Estimated values of the pharmacokinetic parameters were reported as mean ± SE. Student’s t-test or Mann-Whitney U-test was used for statistical analysis by the Stat View software ver. 5.0 (SAS Institution Inc., Cary, NC, USA). A p value of < 0.05 was considered statistically significant.

Response evaluation

Response was evaluated with computed tomography (CT) or magnetic resonance imaging (MRI) after two cycles of chemotherapy in patients with measurable disease. Tumor response was assessed using Response Evaluation Criteria in Solid Tumors [21]. Responses were confirmed by CT at least 4 weeks later. Response evaluation of chemotherapy was not done by serum levels of CA125 in patients with ovarian carcinoma in the present study.

Results

Patients' characteristics

During May 2009 and July 2011, 18 Japanese patients (patients) were enrolled; 16 ovarian carcinoma patients, and two cervical cancers. The patients' characteristics according to each dose level are listed in Table 1. Genotype of UGT1A1 was wild-type in six patients (33%), heterotype in nine patients (50%), and homotype in three patients (17%). Heterotype included four patients of *28, five patients of *6, and no cases of *27. Genotypes of three patients with homotype were *6/*6, *28/*28, and *6/*28. Median age was 55 years, ranging from 35 to 66 years. Among 18 cases enrolled, 15 cases (83%) had received previous chemotherapy, and two cervical cases (11%) had undergone pelvic irradiation. There were no significant differences of total bilirubin levels among UGT1A1 genotypes.

Dose-escalation results

A summary of dose-escalation schema are shown in Table 2. At dose level 1 for wild-type cases, there were no DLTs in all three cases. At dose level 2 of wild-type cases, two of three cases developed DLTs: a case with grade 4 neutropenia, and another with grade 3 febrile neutropenia. Dose level 2 was defined as the MTD for wild-type cases. RD for wild-type cases was dose level 1, which used irinotecan at a dose of 50 mg/m².

At dose level 1 for heterotype cases, one of three cases had grade 3 neutropenia over 25 days and developed DLT. There were no DLTs in additional three cases. At dose level 2 for heterotype cases, DLTs were observed in two of three cases. One developed grade 3 diarrhea, and the other had both grade 3 febrile neutropenia and grade 4 diarrhea. Dose level 2 was determined as MTD, and RD was dose level 1.

Three cases with homotype had no DLTs at dose level 1. However, two patients developed DLTs at dose level 2: a case with grade 4 neutropenia and grade 3 febrile neutropenia, and another with grade 4 neutropenia and grade 3 non-hematologic toxicity of increased alanine aminotransferase. Dose level 2 was determined as MTD, and RD was dose level 1 for homotype patients.

RD of weekly irinotecan was 50 mg/m² for UGT1A1 wild-type patients and 40 mg/m² for hetero-type, and 30 mg/m² for homotype UGT1A1 patients.

Pharmacokinetics of irinotecan, SN-38, SN-38G, and platinum

Pharmacokinetic parameter at each dose level is shown in Table 3. AUCs of SN-38 were higher in step 2 in comparison with step 1 doses in all three groups. GR of homotype patients was significantly lower than that of wild-type/heterotype patients: 3.1 vs 8.7, p = 0.001 (Mann-Whitney U test). There were no significant differences of AUCs of platinum among three groups, and between two steps.

Response evaluation

As the present study was a dose-finding phase I study, primary endpoint did not include evaluation of response. In response evaluable cases, objective response was observed in one of the two wild-type dose level 1, one in two wild-type dose level 2, zero in five heterotype dose level 1, zero in three heterotype dose level 2, and one in two homotype patients.

Discussion

There have been reports of more than 100 polymorphisms in the UGT1A1 gene [22]. A UDP-glucuronosyltransferase (UGT) polymorphism, UGT1A1*6, is one of single nucleotide polymorphisms (SNPs) locating on the exon1 coding region of UGT1A1 gene, which was rarely seen in Caucasians but negligible in clinical studies using Asians populations. In the present study, the authors observed six cases (33%) with heterotype UGT1A1*6 allele, and one case (6%) with homotype UGT1A1*6 alleles, supporting higher abundance of UGT1A1*6 polymorphism in the Asian population. Previous report described the significant increase of basal level of serum total bilirubin in patients with UGT1A1*28 or UGT1A1*6 [17]. In the present cases, serum level of total bilirubin was not significantly elevated in homotype patients in comparison with wild-type or heterotype cases, which potentially sug-
suggested that it would be difficult to estimate UGT1A1 genotypes according to serum level of total bilirubin.

The present dose-finding phase I study revealed that RDs of irinotecan in combination with 60 mg/m² of cisplatin for phase II/III studies were 50 mg/m² for UGT1A1 wild-type patients, 40 mg/m² for hetero-type patients, and 30 mg/m² for homo-type patients. DLTs included neutropenia, febrile neutropenia, diarrhea, and increased alanine aminotransferase, which were common toxicities in patients treated with irinotecan and cisplatin. In patients with homo-type UGT1A1 alleles, significantly lower levels of GR were observed in comparison with wild-type and heterotype genotypes. Additionally, ECOG PS and a number of present chemotherapeutic regimens were not related to severe toxicities with a combination of irinotecan and cisplatin.

There might be other factors influencing pharmacokinetics of irinotecan-based chemotherapy. However, the present study demonstrated the significant association of genotyping of UGT1A1*6 and *28 in patients treated with cisplatin and low dose of irinotecan. In the clinical settings, genotyping of UGT1A1*6 in addition to UGT1A1*28 would be recommended in the patients treated with irinotecan and cisplatin, especially in the Asian population. Also, the authors recommend these RDs determined in the present study be evaluated in further phase II / III studies.

References
Analysis of the risk factors for the recurrence of cervical cancer following ovarian transposition

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Summary

Purpose: To investigate the potential risk factors related to the recurrence of cervical cancer following ovarian transposition. Materials and Methods: A total of 105 patients with cervical carcinoma were retrospectively analyzed. Each patient underwent surgical therapy in combination with ovarian transposition from September 2000 to November 2009. The potential risk factors for recurrence following ovarian transposition were analyzed. Results: The average age of the 105 patients was 38.7 years. Twelve patients were in Stage IA, 65 in IB, 12 in Stage IIA, and 16 in Stage IIB. Twenty-five patients had well-differentiated cancer (G1). Forty-eight patients had moderately-differentiated cancer (G2), and 32 patients had poorly-differentiated cancer (G3). Ninety-seven cases were squamous cell carcinoma, three were adenocarcinoma, four were small cell carcinoma, and one case was adenosquamous carcinoma. Five patients (4.8%) had a recurrence, two of whom (1.9%) had ovarian metastasis. Univariate analysis showed that the pathological type \( p = 0.005 \) and degree of differentiation \( p = 0.001 \) were potential risk factors for recurrence of cervical carcinoma following ovarian transposition. Cancer embolus in vessels or lymphatic metastasis was observed in four of the five patients who suffered a recurrence. Conclusion: Pathological type, differentiated degree, and cancer embolus in vessels or lymphatic metastasis were identified as potential risk factors for the recurrence of cervical carcinoma after ovarian transposition.

Key words: Cervical carcinoma; Ovarian transposition; Recurrence.

Introduction

Cervical carcinoma is a common tumor, with the second highest incidence rate among female tumors. Globally, the number of patients has increased over time and 560,000 females were reported to be affected in 2006 [1]. The age of onset was found to peak at two age ranges, 35-39 years and 60-64 years. Moreover, there has been a growing concern worldwide regarding an increase in affected youths. Postoperative radiotherapy is required for some patients; however, the ovaries are very sensitive to this treatment. A single dose \( \geq 4 \) Gy or ten-day doses \( \geq 15 \) Gy can induce permanent ovarian castration. When treatment is limited to one ovary, the ovary distal to the radiation field is normally unaffected [2]. In 1985, McCall first described that ovarian transposition could reserve ovarian function following radical hysterectomy. Although radiotherapy has been shown to affect transposed ovaries, subsequent studies have reported that their function could be preserved [3-5]. Therefore, for young patients that require adjuvant radiotherapy following surgical resection, ovarian transposition out of the radiation field can prevent the damage resulting in ovarian function and increase the quality of life. Studies on the recurrence rate and recurrence-related risk factors, however, are rare. In this study, 105 patients with cervical carcinoma who underwent ovarian transposition within the past ten years were retrospectively analyzed, and the recurrence-related risk factors were investigated.

Materials and Methods

A total of 105 patients with cervical carcinoma with an average age of 38.7 years (range 26-69) were enrolled in this study. All of the patients underwent surgical resection in combination with unilateral (n = 39) or bilateral (n = 66) ovarian transposition in the Department of Gynaecology and Obstetrics of Peking University People’s Hospital between September 2000 and November 2009. Based on the staging criteria set by the International Federation of Gynecology and Obstetrics (FIGO) in 2009, 12 of the patients were staged in IA (nine in IA1 and three in IA2), 54 in IB1, 11 in IB2, 12 in IIA, and 16 in IIB. According to the differentiated degree, patients were categorized as having well-differentiated cancer (G1, n = 25), moderately-differentiated cancer (G2, n = 48) and poorly-differentiated cancer (G3, n = 32). Ninety-seven cases were squamous cell carcinoma, three cases were adenocarcinoma, four cases were small cell carcinoma, and one case was poorly-differentiated adenosquamous carcinoma. All carcinomas were verified by pathological examination.

Of the 105 patients, 97 (\( \geq \) Stage IA2) underwent radical/subradical hysterectomy plus a pelvic lymphadenectomy, seven (Stage IA1) underwent exofacial hysterectomy, and one was found to have cervical adenocarcinoma by pathological examination after complete hysterectomy and underwent a pelvic lymphadenectomy three weeks later. A total of 105 patients were also treated with ovarian transposition, 39 with unilateral ovarian transposition, and 66 with bilateral ovarian transposition. A total of 29 patients received postoperative radiotherapy (external irradiation of the entire pelvic and vaginal cavities following after-loading radiotherapy) with an average dose of 6,032 Gy (4,760 - 8,000 cGy).

All patients were followed up until October 2010 in one to three-month intervals during the first year postoperatively and every three to six months thereafter. The average follow-up period was 44.3 months (range 14-81). Gynecologic examination and transvaginal color Doppler sonography were per-
formed, and serum tumor markers (CA125, CA199, and SCC) were assayed at each visit. Abdominal ultrasonography or computed tomography/magnetic resonance imaging (CT/MRI) and chest X-rays were performed annually.

SPSS 13.0 was used for all statistical analyses. Potential risk factors of recurrence of cervical carcinoma following ovarian transposition were screened using COX regression univariate analysis. Survival analysis was performed using the Kaplan-Meier method.

Results

Of the 105 patients, five (4.8%) had a recurrence, including two (1.9%) patients who were diagnosed with ovarian metastasis. A 31-year-old patient with Stage IIB small cell carcinoma self-palpated the right abdominal lump six months postoperatively and subsequently underwent exploratory laparotomy. Frozen pathology showed metastatic carcinoma in the right ovary and a normal left ovary. As a result, both of the transposed ovaries were removed. Another patient was found to suffer from undifferentiated Stage IB1 cervical squamous cell carcinoma before surgery that was upgraded to poorly-differentiated Stage IB1 cervical squamous cell carcinoma after surgery by pathology. A PET scan at ten months post-surgery, however, indicated metastasis in the right ovary. As a result, an exploratory laparotomy and a right transposed ovariectomy were performed. The postoperative pathology demonstrated right transposed ovarian metastatic cancer.

Of the remaining three patients that suffered a recurrence, one patient with Stage IB1 poorly-differentiated squamous cell carcinoma was found to have multiple pelvic metastases, supraclavicular lymph nodes metastasis, and intestinal obstruction as determined by PET at 20 months postoperatively. This patient was given a single treatment of local alleviative radiotherapy plus chemotherapy, after which she developed severe myelosuppression and died 26 months later. Another patient with Stage IIB cervical small cell carcinoma who suffered a recurrence underwent initial surgery on July 15, 2008 and was treated with bleomycin, ifosfamide, and cisplatin (BIP) chemotherapy twice. Because of IV degree myelosuppression, however, the chemotherapy was stopped. Radiotherapy was administered at the local hospital from August 23 to September 23, 2008. After this treatment, the patient was tested for tumor markers, and a CT was given every three months; no abnormalities were found. CT at 17 months postoperatively showed an enlarged solid low-echo region in the bilateral ovaries, multiple nodular shadows, and multiple retroperitoneal lymphatic and epistropheus metastasis. In addition, the patient’s CA125 also increased. After evaluation, the patient was not considered for a second surgery, and she abandoned the therapy. The last patient to suffer a recurrence was initially diagnosed with Stage IIA poorly-differentiated squamous cell carcinoma. The patient had received ten cycles of BIP chemotherapy 12 months before initial surgery. The patient refused to continue chemotherapy and is still being followed.

Discussion

The ovarian metastatic rate in patients with early-stage cervical carcinoma is very low. To evaluate the clinical pathological features of ovarian metastasis in cases of cervical carcinoma, Shimada et al. [6] studied 3,471 patients with Stage IB-IIIB cervical carcinoma from 1981 to 2000 and found that for Stage IB, IIA, and IIB carcinoma, the ovarian metastatic rate of cervical squamous cell carcinoma was 0.22%, 0.75%, and 2.17%, respectively, while that of cervical adenocarcinoma was 3.72%, 5.26%, and 9.85%, respectively. Their results showed that the ovarian metastatic rate was only 1.5%, whereas the ovarian metastatic rate of Stage IIB squamous carcinoma increased significantly. Therefore, it was determined that for patients with Stage ≤ IIA cervical squamous cell carcinoma, the ovaries could be preserved. Furthermore, they found that the ovarian metastatic rate in patients with cervical adenocarcinoma increased significantly. Nakanishi et al. [7] conducted a study in 1,064 patients with cervical squamous cell carcinoma and 240 patients with cervical adenocarcinoma. They found that the metastatic rate of cervical adenocarcinoma was much
higher than that of squamous cell carcinoma (6.3% vs 1.3%). Therefore, patients with cervical adenocarcinoma should not be encouraged to preserve their ovaries. L'ubusky et al. [8] believed that the ovarian metastatic rate was determined by pathological types and the clinical stages of the tumor. They found that the postoperative ovarian dysfunction rate was low in young patients with Stage IA-IB squamous cell carcinoma and determined that there was a better chance to preserve the ovaries.

The ovary is very sensitive to radiation. For young patients requiring postoperative adjuvant radiotherapy, the ovary can be transposed outside of the radiation field to prevent damage to its function. The relationship between ovarian function following transposition and age, however, remains a concern. Morice et al. [9] reported that of 104 patients with cervical carcinoma who underwent ovarian transposition combined with radiotherapy or not. The ovarian function was preserved in 100% of the patients who did not receive radiotherapy, 90% of the patients who received intravaginal radiation, and 60% of the patients who received intracavitary radiation combined with extracorporeal radiation. Pahisa et al. [4] performed the surgery and laparoscopic ovarian transposition concurrently in 28 Stage IB cervical carcinoma patients who were younger than 45-years-old. Of these patients, 12 were supplemented with postoperative radiotherapy and the average follow-up period was 44 months. A total of 63.6% of the patients who had received intravaginal radiation, and 93% of the patients who did not receive radiotherapy maintained normal ovarian function. Ishii et al. [10] explored the relationship between the function of transposed ovaries and age in patients with Stage IB-II cervical carcinoma and found that the incidence of menopausal symptoms was significantly lower in patients younger than 40 years of age (65/21) than in those older than 40 years of age (10/12). Ling Yan et al. [11] also observed that cervical carcinoma patients who were younger than 40 years of age had preserved ovarian function, Therefore, patients younger than 40 years of age should be considered for ovarian transposition.

Although lymph fluid of the uterine cervix can drain into the ileal and the parametrial lymph nodes, cervical carcinoma rarely spreads to the ovaries. Patients with cervical carcinoma and ovarian transposition, however, may still suffer from ovarian metastasis. Studies on the ovarian metastasis of cervical carcinoma following transposition are rare, and most are case reports. Ovarian metastasis can occur in cases of adenosquamous cell carcinoma, squamous cell carcinoma, and adenocarcinoma [12-14]. Yamamoto et al. [15] reported that the rate of ovarian metastasis was 0.4% for patients with cervical squamous cell carcinoma and 8.2% for other pathological types. Zhang Meiqing [16] indicated that only one out of 127 patients who did not opt to preserve the ovaries was diagnosed with ovarian metastatic squamous carcinoma and that the metastatic rate was 0.8%. Other studies [15, 17] also considered the pathology of cervical carcinoma and angiolymphoid infiltration to be factors affecting ovarian metastasis. In the current study, which included 105 patients with ovarian transposition, five patients suffered a recurrence (two with ovarian metastasis). Of the five cases involving recurrence, four of the patients had cancer emboli in vessels or lymphatic metastasis, suggesting that cancer emboli in vessels may be a crucial factor affecting recurrence following ovarian transposition.

Huang et al. [18] indicated that the surgical indications for ovarian transposition included (1) patients younger than 40 years of age, scheduled for radical hysterectomy due to cervical carcinoma, and that may require postoperative pelvic radiotherapy; (2) patients with a tumor diameter less than or equal to three cm; and (3) patients with no evidence of invasion into the parametrial, uterine, and/or lymph vessels. In this study, the five-year survival rate was high after ovarian transposition, but the risk of recurrence increased in patients with late-stage cervical carcinoma, non-squamous cell carcinoma, poorly-differentiated carcinoma, and cancer emboli in the vessels and lymphatic metastasis. It is unknown whether the rate of recurrence significantly changes when using 40 or 45 years as the cutoff age; however, it has been found that females older than 40 years of age are liable to have menopausal symptoms after transposition [10, 11]. Therefore, patients younger than 40 years of age are normally selected for ovarian transposition. Plante [19] followed 42 patients with Stage IAI-IIA cervical squamous cell carcinoma who were treated with extensive cervicectomy for an average of 60 months (range six to 156) and found that cervical adenocarcinoma was not a risk factor for recurrence. Therefore, young patients with cervical adenocarcinoma who wish to preserve ovarian function should be encouraged to undergo extensive cervicectomy or ovarian transposition.

Considering the present findings and those from previous studies [17, 20, 21], the authors believe that the indications for ovarian transposition may be as follows: (1) age younger than 40 years, a regular menstrual cycle before surgery, and no menopausal syndrome; (2) normal ovarian appearance during intraoperative exploration, and, when necessary, frost pathology should be performed to rule out metastasis; (3) Stage I-II highly or median-differentiated cervical squamous cell carcinoma; (4) no family history of breast or ovarian carcinoma; (5) no cancer emboli in vessels or lymphatic metastasis; and (6) patients who strongly wish to preserve ovarian function and sign an informed consent form.

Conclusion

Pathological type, differentiated degree, and cancer embolus in vessels or lymphatic metastasis were identified as potential risk factors for the recurrence of cervical carcinoma after ovarian transposition.

Acknowledgement

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References

The correlation between expression of synuclein-γ, glucose transporter-1, and survival outcomes in endometrioid endometrial carcinoma

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Summary

Purpose: To evaluate the correlation between immunohistochemical expression of synuclein-γ, glucose transporter-1, and survival outcomes in endometrioid endometrial carcinoma. Materials and Methods: A tissue microarray was constructed using formalin-fixed, paraffin-embedded tissue that included 23 early and 18 advanced cases. The intensity and area of the immunohistochemical reactions were evaluated using the semi-quantitative scoring system. Results: Synuclein-γ expression was higher in the advanced stage, although it was not statistically significant (p = 0.51). Glucose transporter-1 was overexpressed in the advanced stage (p = 0.01). Synuclein-γ (score = 0 vs > 0) and glucose transporter-1 (score ≤ 7 vs > 7) did not show any differences in overall survival (p = 0.54, p = 0.48) and disease-free survival (p = 0.61, p = 0.14). Conclusion: In this study the expression of synuclein-γ and glucose transporter-1 were not considered to be a prognostic factor and were not related with survival outcomes in endometrioid endometrial carcinoma.

Key words: Endometrial carcinoma; Synuclein-γ; Glucose transporter-1.

Introduction

Endometrial carcinoma is the most common malignancy of the female genital tract in the Western world and the fourth most common cancer in women after breast, lung, and colon cancer [1].

There are two different subtypes of endometrial cancer recognized: estrogen-related (type I, endometrioid) and non-estrogen related (type II, non-endometroid). Approximately 75% of cases are classified as endometrioid adenocarcinomas. Other histologies include uterine serous carcinoma (5% to 10%), clear cell carcinoma (5%), and a variety of relatively rare carcinomas [2].

Synucleins, a family of neuronal proteins consisting of synuclein-α, synuclein-β, and synuclein-γ, are implicated in various neurodegenerative disorders [3, 4].

A strong correlation between synuclein-γ expression and metastasis is observed regardless of the cancer type. Also, in breast cancer, synuclein-γ is causatively linked to antimitotubule drug resistance [5, 6].

Glucose transporter-1 is a member of Na+‐independent glucose transporters. This protein is largely undetectable in normal epithelium and benign tumors, but is expressed in a variety of tumors including cervix, lung, gastric, and colorectal carcinoma. It is also associated with lymph node metastasis and poor prognosis [7-10].

This study was designed to evaluate the differences in the immunohistochemical expression of synuclein-γ and glucose transporter-1 in early- and advanced-stage endometrioid endometrial carcinoma, in order to determine the correlation between the expression of synuclein-γ and glucose transporter-1 with survival outcomes. Furthermore, the relationship of the expression of synuclein-γ and glucose transporter-1 with the clinicopathological parameters of endometrioid endometrial carcinoma was investigated.

Materials and Methods

This study was performed on formalin-fixed, paraffin-embedded tissue samples obtained from 41 women who were diagnosed with endometrioid endometrial carcinoma. These slides were reviewed by two pathologists.

All patients had been treated surgically with: total hysterectomy, bilateral salpingo-oophorectomy, pelvic or/and para-aortic lymph node sampling or dissection, and omentectomy. Patient chart review was performed retrospectively. Surgical staging was restaged by revised International Federation of Gynecology and Obstetrics (FIGO), 2009 criteria. Microscopic grading was based on the FIGO grading system. The cases were divided into two groups. Group 1 was early-stage and group 2 was advanced-stage.

A microarray instrument was used. Conventional hematoxylin and eosin (H & E) slides were reviewed and representative area without necrosis or hemorrhage were marked. Cores of paraffin, two mm in diameter, were taken from the tissue blocks at sites corresponding to the previously selected areas on the H&E slides and these cores were placed in empty blocks in the
The correlation between expression of synuclein-γ, glucose transporter-1, and survival outcomes in endometrioid etc.

Table 1. — Patients’ characteristics and clinicopathologic parameters.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 23)</th>
<th>Group 2 (n = 18)</th>
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<td>Age, mean (± SD), years</td>
<td>47.4 ± 9.05</td>
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<td>BMI, mean (± SD)</td>
<td>24 ± 2.9</td>
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<td>3</td>
<td>4</td>
<td>7</td>
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<tr>
<td>Preop CA 125, mean (± SD)</td>
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<td>123.1 ± 189.1</td>
</tr>
<tr>
<td>Tumor size, mean (± SD), cm</td>
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<td>5.25 ± 2.8</td>
</tr>
<tr>
<td>Cervical stroma involvement</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Invasion depth, mean (± SD), mm</td>
<td>4.17 ± 4.5</td>
<td>14.1 ± 11.6</td>
</tr>
<tr>
<td>% of myometrium, mean (± SD)</td>
<td>16.7 ± 15.9</td>
<td>62.4 ± 36.0</td>
</tr>
<tr>
<td>Lymphovascular invasion, n</td>
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<tr>
<td>Lymph node metastasis, n</td>
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<td>7</td>
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<tr>
<td>Follow up period, mean (± SD), months</td>
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<td>26.8 ± 17.8</td>
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<tr>
<td>Recurrence or persistent disease</td>
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<td>10</td>
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<tr>
<td>Disease-related death</td>
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Table 2. — Immunohistochemical scores of synuclein-γ and glucose transporter-1.

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<tr>
<td>s</td>
<td>n</td>
<td>%</td>
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<tr>
<td>Group 1</td>
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<td>3</td>
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<td>Glucose transporter-1</td>
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<tr>
<td>7</td>
<td>3</td>
<td>16.7</td>
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<td>8</td>
<td>15</td>
<td>82.2</td>
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Figure 1. — Immunohistochemical expression of (A) synuclein-γ (B) glucose transporter-1. (A and B: brown cytoplasmic staining or membranous staining, X 400).

Results

Forty-one cases of endometrioid endometrial carcinoma, 2009 revised FIGO Stages I, III, and IV were retrieved from the Kyungpook National University Hospital cancer registry. All data is expressed as the mean ± the standard deviation. The mean age at the time of surgery was 47.4 ± 9.05 years in group 1 and 55.6 ± 10.3 years in group 2. In group 1, all 23 patients were in Stage I, grade 1. In group 2, 18 patients, 15 were in Stage III, three in Stage IV, and in histologic differentiation, five patients were grade 1, six grade 2, and seven grade 3. The preoperative levels of CA 125 were 32.3 ± 40.5 in group 1 and 123.1 ± 189.1 in group 2. The endometrial tumor sizes both groups were 1.7 ± 1.22 cm and 5.25 ± 2.8 cm, respectively. The depths of myometrial invasion were 4.17 ± 4.5 mm and 14.1 ± 11.6 mm, respectively. The percentages of myometrial invasion were 16.7 ± 15.9% and 62.4 ± 36.0%, respectively. In group 1, one patient showed LVSI and in group 2, eight patients showed the same. Postoperative pelvic or para-aortic lymph node metastasis was not observed in any of the patients in group 1 but was observed in seven patients in group 2. The follow-up periods of the two groups were 34.9 ± 20.5 months and 26.8 ± 17.8 months, respectively. Cases where the disease recurred or persisted after the operation.
included one patient in group 1 and ten in group 2. Disease-related deaths occurred in one patient in group 1 and seven in group 2 (Table 1). The immunohistochemical scores of synuclein-γ and glucose transporter-1 in two groups are shown in Table 2. The immunoreactivity values of synuclein-γ and glucose transporter-1 in the two groups are shown in Figure 2 as an error bar graph. Although synuclein-γ was not statistically significant, synuclein-γ (p = 0.513) and glucose transporter-1 (p = 0.013) showed higher immunoreactivity values in group 2. The interrelationships between synuclein-γ and glucose transporter-1 and six clinicopathological parameters: 1) histologic grades, 2) endometrial tumor sizes, 3) percentages of myometrial invasion, 4) invasion depths, 5) lymph node metastases, and 6) lymphovascular invasions were analyzed. Synuclein-γ and glucose transporter-1 showed positive relationships (p = 0.021). Glucose transporter-1 showed a positive relationship only with histological grade (p = 0.030). Synuclein-γ did not show any differences in overall survival and disease-free survival between cases where it was expressed and cases where it was not expressed (p = 0.541, p = 0.061). Glucose transporter-1 also did not show any differences in overall survival and disease-free survival between cases where its total score was higher than 7 and cases where its total score was lower than 7 (p = 0.482, p = 0.141) (Figure 3).

Discussion

Synuclein-α, β, and γ expression were not detected by immunohistochemistry in normal ovarian epithelium. Eighty-seven percent of ovarian carcinomas were found to express at least one type of synuclein, and 42% expressed all three synucleins-α, β, and γ simultaneously. The expression was different in different histological types: 45.4% in undifferentiated types, 50% in endometrioid types, 66% in mucinous types and 85.4% in serous papillary types [11]. In uterine papillary serous carcinoma (UPSC) patients, synuclein-γ expression by immunohistochemistry (IHC), correlated with advanced-stage and decreased progression-free survival [12].

Recently, as the importance of antimicrotubule drugs in the treatment of gynecological adenocarcinomas has been emphasized, the importance of synuclein-γ has been considered. Antimicrotubule drugs have been considered to be the most important drugs for the treatment of advanced-stage endometrioid endometrial carcinoma, which accounts for the majority of endometrial cancers. In this study, four of 23 cases (17%) showed expressions in group 1 and five of 18 cases (27%) showed expressions in group 2, thereby showing an entire expression rate of nine out of 41 cases (21.9%). There were no statistically significant differences between the two groups (p = 0.513). On reviewing this study and previous studies on ovary carcinoma and UPSC, it can be seen that papillary serous carcinomas are the most highly-related with the expression of synuclein-γ in two organs.

In the current study, the significant lower expression of synuclein-γ than the expression in ovarian carcinoma and insignificance as prognostic factor show the possibility that the lower drug resistance against antimicrotule drug will occur in endometrioid endometrial carcinoma, but further clinical study is needed.

The development of a malignant tumor is an energy-dependent process supported by increased glucose metabolism, which in turn produces a corresponding increase in glucose transporter proteins located in cellular membrane. Glucose transporter-1 mediates glucose uptake and thus facilitates anaerobic glycolysis. This protein is largely undetectable in normal epithelium and benign tumors but is expressed in a variety of tumors including cervix, lung, gastric, and colorectal carcinoma and is associated with poor prognosis [7, 8].

Haber et al. studied the association of glucose transporter-1 expression with the prediction of outcome for colon cancer, and they found that patients with carcinomas
with greater than 50% glucose transporter-1 positive malignant cells had a mortality rate two to three times higher than those whose tumors had less than 50% positivity.

They also showed that high glucose transporter-1 expression was also correlated with a greater frequency of lymph node metastases and concluded that the level of glucose transporter-1 expression might be an independent prognostic factor in colon cancer [10].

Similarly, in cervical carcinoma, a significant relationship between an absence of glucose transporter-1 expression and increasing the likelihood of metastasis-free survival was shown [7].

In this study, 40 of 41 cases were strongly immunostained. Immunohistochemical scores were higher with a statistical significance in group 2 (p = 0.013). Glucose transporter-1 expression was directly correlated with tumor grades, although it did not show any significant correlation with other clinicopathological parameters. The expression was not related with lymph node metastasis and survival outcomes.

Conclusion

The expression of synuclein-γ was not correlated with clinicopathological parameters, could not be shown to have the role of a prognostic factor, and was not related to survival outcomes in endometrioid endometrial carcinoma. Glucose transporter-1 was strongly expressed in both early – and advanced-stages and showed significant difference in both groups. Furthermore it showed correlation with histologic grade; however, it did not show any difference in survival outcomes. In the future, further clinical studies evaluating the relationship between antimicrotubule drug resistance and survival outcomes according to expression of synuclein-γ and glucose transporter-1 in endometrial carcinoma are needed.

References


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Differential gene expression profile in cervical cancer and parenchyma infected with human papillomavirus 16 screened by cDNA microarray

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Introduction

Cervical cancer is the second most common cancer among women [1], with about half a million new cases each year [2]. Nearly 1,315,000 cases are discovered in China annually and account for 28.8% of cases worldwide [3]. The incidence of cervical cancer has increased in young people in recent years. More than 30 kinds of human papillomaviruses (HPV) related to genital duct infection have been identified, but only a few of these viruses cause infections that develop into precancerous changes or cancers [4, 5]. The incidence of precancerous changes related to cervical cancer or cervical cancer itself is closely related to high-risk HPV [6, 7]. Among these changes, 70% are induced by infection with HPV 16 or HPV 18 [8]. Current investigations into cervical cancer include studies on telomerase, individual gene expression, some signalling pathways, research and development of vaccines, disease prevention and others, but these approaches are not sufficiently systematic or intensive. Only a few published studies can be found that describe genes that are differentially-expressed between cancerous and normal cervical tissues. Furthermore, most of these studies were carried out on samples from pathological sections after fixation, embedding or other treatments, as well as from cast-off cervical cells; autologous cervical tissues from these individuals under investigation were seldom used for further comparison.

The present study compared the differences in gene expression in cancerous tissue, and in adjacent and distal normal tissues in the surgical samples from three patients with HPV 16-positive cervical cancer in order to obtain valuable information as a basis for further investigation into the molecular mechanisms of the incidence of HPV16 type cervical cancer.

Materials and Methods

Samples

Samples were collected during surgery from five patients aged 35-55 years (mean 45 ± 10) in the Department of Gynecology, the Second People’s Hospital of Chongqing City, China. Cervical cancer was diagnosed both clinically and pathologically. All the tumors were grouped as Stage IB2 based on the International Federation of Gynecology and Obstetrics (FIGO) system. Informed consent was obtained from the subjects and the study was approved by the Ethics Committee of the Hospital. Samples were found to be HPV 16-positive in all patients using a HPV genotyping kit.

In total, 0.2 g samples of tissue were collected from the tumor center (T), from the adjacent tumor (N), and from tissues distal to the tumors (F) (Figure 1). Samples were cut into thin slices (small chunks with a thickness less than 0.5 cm and approximately the size of a soybean) in empty Eppendorf (EP) tubes. The collected tissues were firstly completely immersed in RNAlater solution within 15 min of collection to preserve the integrity of the RNA, incubated at 4°C overnight and stored at ~20°C.

Experimental methods

Total RNA isolation and purification

Total RNA was extracted from the tissues and purified using an RNA clean-up kit. RNA was then quantified using spectrophotometry and subjected to formaldehyde denaturation gel
Electrophoresis for quality inspection (Figure 2). All patient samples, with the exception of sample no. 2, had an RNA purity A260/280 ratio \( \geq 1.80 \); total RNA quantity was at least 15 μg; for RNA integrity: the ratios between the brightness of the 28S:18S rRNA bands were at least 2:1. Therefore sample purity, total quantity, and integrity all met the requirements for gene expression profile experiments, except for sample no. 2, which was not used further for these experiments. Sample no. 1 was chosen for further use.

Fluorescence labelling

Total RNA or mRNA was used as the template. T7 oligo (dT) primers that contained the T7 promoter sequence were used as the primers, and Superscript II enzyme was used for reverse transcription synthesis of the first-strand cDNA. RNase H was used to digest the RNA in the hybrid chain into shorter fragments. DNA polymerase I was used to synthesize second-strand cDNA with the short RNA fragments as the primers, and double-stranded cDNA was purified. The cDNA was used as the template and the T7 enzyme mix was used for in vitro transcription and synthesis of cRNA, next the cRNA was purified and quantified. A total of five μg cRNA, CbcScript enzyme, and random primers were used for reverse transcription, next the product of reverse transcription was purified using PCR with random primers. Primers were used for Klenow enzyme labelling, the labelled product was then purified.

Hybridization and cleaning

The labelled sample was fully mixed with hybridization buffer (3 × SSC, 0.2% SDS, 50 × Denhart’s, 25% formamide), samples were injected into the chip attached to a mixer, and hybridization was carried out using a hybridization system device at 42°C for 14-16 hours. After hybridization, the mixer was removed and the chip was cleaned using cleaning solutions I, II, and III respectively, then dried and scanned.

Chip scanning, image collection and data analysis

A scanner was used to scan the microarray chips. An appropriate software was used for image analysis and transformation of image signals into digital signals. Robust multichip analysis (RMA) normalization was carried out to calibrate the signals and significance analysis of microarrays (SAM) software was used to screen for differentially-expressed genes [9]. The criteria for screening for the differentially-expressed genes were as follows: false discovery rate (FDR) to be controlled within five percent and fold change to be controlled within two orders of magnification.

Quality control for NimbleGen chip system

The hybridization signals were uniform in terms of the images from chip hybridization, and the areas of scratch, air bubbles, and other flaws occupied no more than five percent of the lattice area. The alignment oligo was the mixture of oligo fragments of 48-mer lengths labelled with Cy3 and Cy5. The oligo was added during hybridization and hybridized with complementary sequences for quality control that were synthesized in advance, and were visualized as yellow signals in the pseudo-color image. The alignment oligo showed special alignment at the edge and in the center of the chip, and it had positioning functions during the extraction of data from the chip; the oligo was used to examine whether the quality control had been successfully carried out. Each sample tracking control (STC) was an oligosequence of 48-mer length labelled with Cy3. The oligo was added during hybridization and hybridized with complementary sequences for quality control that were synthesized in advance, and were visualized as a green signal in the pseudo-color image. The STCs showed special alignment at the edge and in the center of the chip. In total, 20 STC sequences could be found; a STC was added for each sample to determine the samples after hybridization and carry out quality control on whether cross-contamination could be detected. It could also be used to examine whether the quality control for chip hybridization was performed successfully.

Results

Differentially-expressed genes

T samples vs F samples (T vs F), N samples vs F samples (N vs F), and N samples vs T samples (N vs T) were compared, respectively. Out of the three samples from the patients (totally five cases of samples), one case was used for further use and one case did not meet the requirements on the microarray experiment. A total of 673 differentially-expressed genes were detected in the T vs F analysis; among these, 261 genes were upregulated and 412 genes were downregulated (ratio \( \geq 2, p \leq 0.05 \)). These genes were mainly proto-oncogenes, anti-oncogenes, cellular
signalling and transduction, metabolism, immunology-related genes, cellular receptors, protein translation, and synthesis-related genes and some other genes. The differentially-expressed genes that showed a three-fold change in expression levels are shown in Tables 1 and 2. For N vs F: in total 56 differentially expressed genes were detected, among these 54 genes were upregulated and two genes were downregulated. For N vs T, no differentially-expressed gene was detected. The scatter plots for the differentially-expressed genes at different positions of sample no. 4 are shown in Figure 3.

**Discussion**

The present study carried out an analysis to determine genes that were differentially-expressed in either cancerous tissue, in tissue near the tumor or in normal cervical tissue away from the tumors. Samples were taken by surgical resection from three patients who had cervical cancer and RNA was analysed by microarray chips that contained 135,000 gene probes. Screened differentially-expressed genes were representative of proteins involved in the cell cycle, cell proliferation, cell adhesion, invasion, metastasis and vascularization, plus some genes were representative of proteins with unknown function (ESTs).

The cell cycle is regulated by a series of important signalling molecules and by members of the cyclin family. Changes in the expression levels of these regulatory factors may lead, therefore, to changes in the regulation of the cell cycle that may reinforce the proliferative capability of cells, weaken differentiation, and inhibit the cell functions so that cells finally develop into tumor cells. In the present study, it was found that the expression levels of vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), glypican-1 (GPC1), and other important signalling molecules in cancerous tissues were downregulated.

Out of the differentially-expressed genes observed from this screening, the expression levels of Ras (RAB25) and Rb1 were found to be upregulated, therefore the authors speculated that the HPV E2 region of the virus genome had been cut and host inhibition of HPV E6 and E7 genes was lost when HPV DNA was integrated into the chromosome of the host. Activated HPV E6 can bind to the anti-oncogene P53 and inhibit its function to repair DNA and therefore promote cell apoptosis and further activate c-myc, H-ras, and other proto-oncogenes. E7 can activate P16 protein and cyclin E after it binds to the anti-oncogene Rb, therefore further promoting the cells to move from cell cycle stage G1 to S. These changes can promote the uncontrolled proliferation and the immortalization of cells, and finally lead to the incidence of cervical intraepithelial neoplasia (CIN) and cervical cancer; the upregulation of RAB25 and Rb1 genes confirmed this mechanism.

Loss of control of the cell cycle is one of the important reasons for excessive proliferation of cells and formation of cancerous tissue. In the present study, the expression levels of cyclin B1, CDK5, CDK8, and other genes were upregulated. Among these, cyclin B1 is an important regulatory factor for the cell cycle as it has a function at the G2/M checkpoint in cells. Chun-Ling Zhao et al. [10] found that the relationship between expression of cyclin B1 in esophageal cancer and the prognosis indicated that the expression level of cyclin B1 increased with the increase in the grading of atypical hyperplasia, which was one of the indexes for poor prognosis of esophageal cancer. CDK5 and CDK8 are both members of the cyclin-dependent kinase (CDK) family, but CDK5 expression is...
Differential gene expression profile in cervical cancer and parenchyma infected with human papillomavirus 16 screened etc.

not significantly correlated with the cell cycle; current studies indicated that it was related to degenerative disease in the nervous system and that its overactivation may lead to apoptosis of cells [7]. Tumor growth is inevitably concurrent with excessive proliferation and metastasis of tumor cells. It should be noted that in the present study, the proliferation-related antigen Ki-67 showed significantly differential expression. Ki-67 can be used as one of the indexes for determination of the growth rate of tumors, TIAN Qi et al. [11] found in their studies that increase in the expression level of Ki-67 in cells was coincident with the grade of cervical lesions; therefore this antigen can be used as one of the indicators for cervical cancer. Human leucocyte antigen (HLA), particularly HLA II, was coincident with cervical cancer, and many studies have found that the presence of DQB130603 and/or DRB1313 has protective effects on cervical cancer [12], while correlation of DQB1303, and DRB131501/DQB130602 with cervical cancer is still in dispute. In the present study, HLA2DRB showed significant upregulated expression and therefore expression of this gene may promote the progression of cervical cancer.

ICAM1 (CD54) is a member in the cellular adhesion molecule immunoglobulin superfamily and is expressed at low levels under normal conditions in human tissues. ICAM1 expression may be regulated at the transcription level and promote cellular adhesion and metastasis by the regulation of nuclear factor (NFκB) under the effects of many inflammatory factors. In the present study, the expression levels of ICAM1 and nuclear factors were all upregulated. The expression levels of other adhesion factors also changed, such as for MUC1, AKT3, AMOTL1, EPB41L3, MAGI1, JAM2, CD34, CDH2, NLGN1, CADM3, and other genes. The difference in the expression levels of adhesion molecules is the molecular basis to allow infiltration, metastasis, and other phenomena in some tumor cells. The high-expression level of MUC1 in this study confirmed the results reported by Rong Ye-Fei et al. [13].

Matrix metalloproteinase (MMP), ERBB3, and other genes also showed a tendency to be upregulated. MMPs are a group of zinc-dependent extracellular proteolytic enzymes that can degrade basal membrane, and promote invasion and metastasis of malignant tumors. As well as agreement of high expression levels of MMP3 in cervical cancer samples with the results reported by Liu Xin et al. [14, 15], MMP 10, 11, 12, and others also showed a tendency to be upregulated; ERBB3 and other EGFR members have important functions in growth, repair, survival, and other aspects of tumor cells.

It was also found that some proto-oncogenes and anti-oncogenes such as VAV3 and VAV1 showed upregulated expression. The expression levels of other adhesion factors also changed, such as for MUC1, AKT3, AMOTL1, EPB41L3, MAGI1, JAM2, CD34, CDH2, NLGN1, CADM3, and other genes. The difference in the expression levels of adhesion molecules is the molecular basis to allow infiltration, metastasis, and other phenomena in some tumor cells. The high-expression level of MUC1 in this study confirmed the results reported by Rong Ye-Fei et al. [13].

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Table 1. Genes with upregulated expression.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Full name</th>
<th>Probe set ID</th>
<th>Fold change (T vs F)</th>
<th>q value (%)</th>
<th>Chromosome no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIGR</td>
<td>Polymeric immunoglobulin receptor</td>
<td>BC110494</td>
<td>43.15861978</td>
<td>1.84544</td>
<td>1</td>
</tr>
<tr>
<td>MMP7</td>
<td>Matrix metallopeptidase 7 (matrilysin, uterine)</td>
<td>NM_002423</td>
<td>26.95478429</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>TMC5</td>
<td>Transmembrane channel-like 5</td>
<td>BC027602</td>
<td>25.51910532</td>
<td>3.53584</td>
<td>16</td>
</tr>
<tr>
<td>HSD17B2</td>
<td>Hydroxysteroid (17-beta) dehydrogenase 2</td>
<td>BC009581</td>
<td>23.86596007</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>C1orf81</td>
<td>Chromosome 10 open reading frame 81</td>
<td>BC036365</td>
<td>21.70212182</td>
<td>3.09278</td>
<td>10</td>
</tr>
<tr>
<td>MMP3</td>
<td>Matrix metallopeptidase 3 (stromelysin 1, progelatinase)</td>
<td>BC069676</td>
<td>21.15900596</td>
<td>2.68542</td>
<td>11</td>
</tr>
<tr>
<td>WFDC2</td>
<td>WAP four-disulfide core domain 2</td>
<td>NM_006103</td>
<td>20.29384258</td>
<td>1.89383</td>
<td>20</td>
</tr>
<tr>
<td>MMP3</td>
<td>Matrix metallopeptidase 3 (stromelysin 1, progelatinase)</td>
<td>BC107490</td>
<td>19.82768757</td>
<td>3.13546</td>
<td>11</td>
</tr>
<tr>
<td>C1orf178</td>
<td>Chromosome 1 open reading frame 178</td>
<td>NM_001029945</td>
<td>18.65461948</td>
<td>3.53584</td>
<td>1</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>Cyclin-dependent kinase inhibitor 2a</td>
<td>L27211</td>
<td>14.63709823</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>MMP10</td>
<td>T-complex 11 (mouse)</td>
<td>NM_002425</td>
<td>14.43021297</td>
<td>1.89383</td>
<td>11</td>
</tr>
<tr>
<td>TCP11</td>
<td>Immunoglobulin heavy constant mu</td>
<td>NM_018679</td>
<td>11.49695961</td>
<td>2.97088</td>
<td>6</td>
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<tr>
<td>IGHM</td>
<td>Mucin 1, cell surface associated</td>
<td>BC001872</td>
<td>8.92452555</td>
<td>3.53584</td>
<td>14</td>
</tr>
<tr>
<td>MUC1</td>
<td>Mucin 1, cell surface associated</td>
<td>AY327596</td>
<td>8.67884645</td>
<td>1.87956</td>
<td>1</td>
</tr>
<tr>
<td>MUC1</td>
<td>Mucin 1, cell surface associated</td>
<td>J05582</td>
<td>8.34639829</td>
<td>1.87956</td>
<td>1</td>
</tr>
<tr>
<td>MUC1</td>
<td>Keratin 7</td>
<td>J05581</td>
<td>6.38861818</td>
<td>3.04621</td>
<td>1</td>
</tr>
<tr>
<td>KRT7</td>
<td>Mucin 1, cell surface associated</td>
<td>NM_005556</td>
<td>6.30436226</td>
<td>4.65563</td>
<td>12</td>
</tr>
<tr>
<td>MUC1</td>
<td>Mucin 1, cell surface associated</td>
<td>AY327592</td>
<td>5.68949588</td>
<td>3.04621</td>
<td>1</td>
</tr>
<tr>
<td>MUC1</td>
<td>Apolipoprotein b mRNA editing enzyme, catalytic polypeptide-like 3d</td>
<td>AY327600</td>
<td>5.626257325</td>
<td>3.04621</td>
<td>1</td>
</tr>
<tr>
<td>APOBEC3D</td>
<td>(putative)</td>
<td>NM_152426</td>
<td>5.48962436</td>
<td>3.09278</td>
<td>22</td>
</tr>
<tr>
<td>MMP11</td>
<td>Matrix metallopeptidase 11 (stromelysin 3)</td>
<td>NM_005940</td>
<td>5.28973576</td>
<td>3.53584</td>
<td>22</td>
</tr>
</tbody>
</table>
expression levels of the immunity-related genes CD4 and CD96 were also upregulated. While the expression level of BCA P29 was downregulated, the expression level of platelet-derived growth factor receptor-alpha (PDGFRA) was upregulated and the expression level of transforming growth factor beta receptor 2 (TGFBR2) was downregulated. The correlation of some tumor-related genes with tumors still requires further study.

Smad7 (SMAD, mothers against DPP homolog 7) gene is potentially related to the incidence of tumors [16, 17]. The expression level of Smad7 was downregulated in the present study, which was not in agreement with the results reported by Yu Xingping et al. [18]. This finding may be related to differences in sample stages, the experimental control or the source of samples, but still needs further confirmation.

Invasion and metastasis are complex processes composed of multiple steps. These steps involve the infiltration of tumor cells to tissues adjacent to the primary tumors, ingestion of lymphatic vessels and blood vessels, arrival of new organs for further growth and vascularization [19]. It was found in the present study that the expression levels of some key genes, such as STAT, MAPK3, AKT3, PAK, and others showed significant changes between cervical cancer and normal cervical tissues. Moreover, MMPs are a group of extracellular proteolytic enzymes that can degrade basal membrane, and promote the invasion and metastasis of tumors. Vascularization in tumors is a very important process that is closely-related to growth, metastasis, and other aspects of tumors. The expression levels of VEGF, hypoxia inducing factor 1A (HIF1A), tumor necrosis factor alpha induced protein 1 (TNFAIP1), and angiopoietin 1 (ANGPT1) were not enhanced, while the expression level of angiogenesis inhibitive factor thrombospondin (TSP) did not decrease, which may be attributed to the inactivation of signalling molecules for vascularization as the three patients were in the middle or early stages of cancer.

Significant difference was not detected in the present study in the genes between the tumor tissues and the tissues near the tumors, which may be related to the differences in positions during sample collection and in tumor stages.

### Conclusion

In conclusion, detection was carried out on tissues from different positions in the samples of HPV 16 type cervical cancer from surgical resection. It was found that the expression levels of many genes showed significant differences between the normal tissues far from the tumors and the cancerous tissues, while certain differences could be detected in the expression levels between the tissues near to the tumors and the normal tissues. The differentially-expressed genes found to be present included proto-oncogenes, anti-oncogenes, metabolism, signalling transduction, cellular receptor, and other genes, which indicated that the incidence of cervical cancer was very complex and resulted from the effects of multiple factors and genes. This type of cancer is a continuous and gradual process, and the interregulation among genes indirectly or directly leads to incidence and progression of cervical
cancer. The present study provided valuable data for further investigations into the molecular mechanisms for the incidence of cervical cancer in the future.

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Nodal involvement evaluation in advanced cervical cancer: a single institutional experience

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Gynecologic Oncology Unit, La Paz University Hospital, Madrid (Spain)

Summary

Purpose: To assess the usefulness of different imaging techniques in the detection of nodal involvement in patients with advanced cervical carcinoma. Moreover, to analyze the correlation between the presurgical (FIGO) and postsurgical (pTNM) staging classifications.

Materials and Methods: All patients diagnosed with advanced cervical cancer (FIGO Stages IIB-IV) from 2005 to 2012 were selected. The medical charts of 51 patients that underwent presurgical assessment with posterior surgical staging by means of para-aortic lymphadenectomy, were reviewed. Nodal status assessment by computed tomography scan (CT scan), magnetic resonance imaging (MRI), positron emission tomography (PET), and sonography was compared, as well as the size given in imaging techniques compared to the final pathologic report information.

Results: Presurgical analysis by CT scan, MRI, PET, and sonography showed pelvic nodal involvement in 51.3% of patients, and para-aortic involvement in 30.8% of cases. CT scan showed positive pelvic nodes in 35% of cases, but pathologic confirmation was observed in just 17.6% of cases. However, MRI resulted in higher rates of up to 48.8% of cases. Concerning para-aortic nodal involvement, CT scan showed positive nodes in 25% of cases, MRI in 3.2% of cases, and the pathologic report in 15.6% of cases. The authors found significant differences between staging groups among both classifications (FIGO vs. pTNM; \( p < 0.001 \)). Eight cases (15.7%) were understaged by FIGO classification.

Conclusions: Despite all imaging techniques available, none has demonstrated to be efficient enough to avoid the systematic study of para-aortic nodal status by means of surgical evaluation.

Key words: Cervical cancer; Staging; Imaging techniques; Lymphadenectomy; Nodal involvement; Extraperitoneal.

Introduction

Cervical cancer is the second malignancy according to frequency around the world [1]. The incidence of cervical cancer is even greater in developing countries, and it is the first cause of death due to cancer [2]. Prognosis is directly related to the tumor stage at diagnosis, and despite the efforts for an early diagnosis, 25% of cases are diagnosed at an advanced stage.

The accepted classification for cervical cancer is the one proposed by FIGO [3], although it does not consider imaging tests and nodal involvement. Postsurgical staging or pTNM [4] is the most accurate approach for valuing the stage, since it determines nodal involvement more accurately. Up to 26% of women without evidenced disease in the presurgical study show postsurgical para-aortic involvement [5]. This underestimate of tumor stage could modify the planned treatment in up to 40% of patients [6], thus a correct assessment of the spread of the disease is essential.

Five-year overall survival is estimated at 85%-90% when nodal involvement is negative, dropping to 20%-75% when positive. This is the main reason to consider nodal metastasis the most important prognostic factor for these patients [7]. The gold standard to assess nodal involvement is to perform a para-aortic lymphadenectomy, and the extraperitoneal approach seems to be the most appropriate when nodal involvement is suspected [8, 9]. However, if the pathologic study of para-aortic nodes is not feasible, imaging tests will be chosen. Available choices include magnetic resonance imaging (MRI), abdominal/pelvic computed tomography (CT) scan, and positron emission tomography (PET). Each one presents different advantages and disadvantages.

The aim of this study was to assess the usefulness of different imaging techniques in the detection of nodal involvement in patients with advanced cervical carcinoma, and their correlation with actual involvement by means of their pathological assessment. The second objective was to analyze the correlation between presurgical (FIGO) and postsurgical (pTNM) staging classifications.

Materials and Methods

After obtaining approval from the Institutional Review Board approval, the medical charts of all patients diagnosed with advanced cervical cancer (FIGO Stages IIB-IV) [3], from January 2005 until December 2010 at the gynecologic oncology unit of La Paz University Hospital in Madrid, were reviewed. Pathologic confirmation was required. The authors excluded FIGO Stage IVB cases, since no follow-up of these patients was available.

Data collected included: patient’s age, presurgical FIGO Stage, postsurgical TNM Stage, pathological details, physical exam findings, and metastatic assessment through CT scan, MRI, PET, and sonography.

Patients with suspected advanced cervical cancer underwent presurgical assessment, and later on, the surgical staging was determined by means of para-aortic lymphadenectomy. Surgical staging was systematically conducted in all patients, but especially those with high morbidity. The authors found 40% of patients with surgical staging, since the present department only undertakes this procedure routinely since 2009.

Surgical technique consists of the removal of all the lymph nodes from the common iliac artery to the renal vessels, includ-
Nodal involvement evaluation in advanced cervical cancer: a single institutional experience

Results

Among the 155 patients diagnosed with cervical cancer between January 2005 and December 2010 in the present center, only 51 cases corresponded to locally-advanced FIGO Stages. The average patient age was 54.9 ± 14.6 years.

Most of them (80.4%) were epidermoid carcinomas, 17.6% were adenocarcinomas, and two percent were adenosquamous carcinomas. Regarding the grade of differentiation, the authors observed 10.8% of grade 1, 32.4% of grade 2, and 56.8% of grade 3.

Ten patients (19.6%) underwent pelvic lymphadenectomy, which resulted positive just in one case (10%). The most common surgical route was laparoscopy (77.7%), with an average of 9.1 ± 4.9 pelvic nodes obtained and an average positivity of two ± 2.4 nodes. Moreover, 48.8% of cases. Concerning para-aortic nodal involvement, CT scan showed positive nodes in 25% of cases, but pathologic confirmation was observed just in 17.6% cervical atypical vascularization was detected. Incidences of vaginal and bladder infiltrations were found, respectively.

CT scan showed positive pelvic nodes in 35% of cases, but pathologic confirmation was observed just in 17.6% of cases. However, MRI informed of higher rates of up to 48.8% of cases. Concerning para-aortic nodal involvement, CT scan showed positive nodes in 25% of cases, MRI in 3.2% of cases, and the pathologic report resulted in 15.6% of cases (Figure 1).

Discussion

Cervical cancer staging is often based on clinical characteristics through FIGO classification. Although this strategy is usually valid in the assessment of local spread of the disease, it is not valid for nodal involvement or distant assessment. Clinical exam and primary tumor evaluations are the decisive factors to determine if a patient will undergo primary surgery or chemo-radiation therapy as curative treatment. Surgical staging seems to improve the treatment adjustment in comparison to the underestimation caused by FIGO classification [6].

In the present study, the authors observed significant differences (p < 0.001) between both staging systems, showing that FIGO classification was understaged in over 15% of patients. Available data published report differences from 25% to 90% of understaging when comparing clinical to surgical staging [10]. On the other hand, FIGO staging does not take into account the use of imaging tests, and thus, it does not consider nodal involvement. Nevertheless, the most important predictor of relapse in patients with cervical cancer is para-aortic lymph node involvement [11]. This could lead to incomplete or incorrect treatment.
Regarding para-aortic adenopathies, CT scan once again overestimated nodal involvement, but MRI showed a higher false-negative rate (MRI just detected 3.2% of positive para-aortics while real involvement was 15.6% of cases). The present data contrasts with the published data where MRI outperformed CT scan in the assessment of para-aortic region. Statistical differences ($p < 0.05$) were found among CT scan and MRI.

Besides nodal status, some factors may be taken into account, such as local spread of the disease, tumor size, parametral infiltration, etc. In this series, MRI outperformed CT scan in parametrical evaluation. Among 25 positive parametria, CT scan detected seven cases while MRI detected 14 of them. Similar results were reported by Yang et al. [14], with a sensitivity of 74% and 55% for MRI and CT-scan, respectively. The authors also observed that MRI was the most precise test to assess the size of the uterine cervix, showing a significant Pearson correlation of $r = 0.7$; similar to data reported in literature [13].

Globally, MRI seems to offer more accuracy in detecting nodal involvement of the disease [13], but it has the great inconvenience of being unsuitable for the virtual simulation and 3D-dosimetry for radiation therapy planning, which only uses CT-scan slides.

Recently, PET has emerged as a technique that allows the assessment of lymph node involvement in patients with uterine cervix carcinoma and does not present the inconvenience of MRI regarding the radiation therapy treatment planning. Since PET is based on physiological processes of cell metabolic activity, it seems more effective than CT scan and MRI for the detection of metastasis in the retroperitoneal area, showing a greater sensitivity and specificity than MRI in the detection of adenopathies [12]. Choi HJ et al. [15], compared PET to MRI in the presurgical detection of nodal metastases in patients with FIGO Stages IB-IV A cervical cancer. They observed a precision rate of 72.6% for MRI compared to 85.1% for PET. Sugawara [16] also studied 21 patients with FIGO Stages IB-IVA and observed higher sensitivity for PET compared to CT scan (86% vs 57%, respectively), in the detection of pelvic and para-aortic metastases. On the other hand, both PET and CT scan allow to assess the response to treatment, but PET also seems to be correlated to disease-free survival and overall survival. In fact, some authors consider PET more useful in prognosis than to evaluate lymph node involvement [17, 18].

The authors only performed PET in seven (13.8%) cases due to economical restrictions and to the high work-load on the radiology department. They observed an uptake of 100% at cervical level, and although in literature it is presented as one of the most sensitive and specific tests for the study of adenopathies, the small sample size did not allow them to analyze the metastatic node uptake. Nonetheless, given its high cost and availability only in third-level centers, its use as a routine screen in the study of cervical cancer spread may be questioned.

In a survey conducted among members of the Society of Gynecologic Oncologists in the USA, most of them

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Figure 1. — Comparison between presurgical pelvic and para-aortic nodal involvement through CT scan, MRI, and final histological study.
did not routinely use PET for the study of presurgical metastasis, although they upheld its great importance in monitoring the response to treatment and follow-up [19]. In conclusion, FIGO classification, in spite of being a valid method for the staging of cervical cancer, showed a higher rate of understaging compared to pTNM classification. Moreover, it does not take into account nodal involvement, which is the most important prognostic factor; but, it could be more useful in developing countries where the lack of resources does not allow a more precise evaluation.

In the authors' opinion, MRI seems to be the most suitable technique for the presurgical evaluation of cervical carcinoma, despite its higher percentage of false negatives in the study of para-aortic area. Moreover, it offers lower cost compared to PET and greater accuracy in pelvic and cervical evaluation compared to CT-scan.

Despite all imaging techniques available, none has demonstrated to be efficient enough to avoid the systematic study of para-aortic nodal status by means of surgical evaluation.

Acknowledgments

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References


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Mifepristone sensitizing cisplatin for cervical adenocarcinoma HeLa cell sensitivity to chemotherapy and its mechanism

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Introduction
Cervical cancer is a common gynecological malignancy, although its overall morbidity and mortality is declining, but the incidence of cervical adenocarcinoma has increased significantly in recent years and worldwide detection rate reached 10% to 22% [1]. Surgery and radiotherapy are the traditional treatments of cervical cancer and cisplatin-based concurrent chemoradiation became the standard mode of treatment for more than Stage IIb in advanced cervical cancer [2-4]. Some patients will be cured or alleviated with these treatments. However, the mortality rate of patients with cervical adenocarcinoma is 50% due to early metastasis, high recurrence, no sensitivity to chemotherapy, and poor prognosis. Therefore, it is particularly important to improve the sensitivity of cervical adenocarcinoma to chemotherapeutic drugs. Mifepristone is a progesterone and glucocorticoid hormone receptor antagonist. An in vitro study found that mifepristone inhibited the growth of human cervical squamous cell carcinoma [5], but was still in laboratory stage. However, there is no two-drug combination treatment of cervical cancer reported. This study was designed to investigate the effect of cisplatin combined with mifepristone to treat cervical adenocarcinoma HeLa cells including the sub-cell cycle and apoptosis of HeLa cells, explore its possible mechanism, and provide a theoretical basis for clinical application.

Materials and Methods

Cells and their culture
The human cervical adenocarcinoma HeLa cells were supplied by the Wuhan University Type Culture Preservation Center and were then sent to the Institute of Biology, the Three Gorges University. Monolayer culture were established in RPMI 1640 medium with 10% dialyzed fetal calf serum. The cells were grown for three days (showing approximate density of 1 × 10^6 cells/ml) before they were used in the experiment.

Drug and reagents
Cisplatin was supplied by Shandong Qilu Pharmaceutical Co., Ltd. (batch number: 031106), paired with saline two mg/ml stock solution, and diluted to the desired concentration with complete culture solution when used. Mifepristone was provided by the Shanghai Hualian Pharmaceutical Factory. Mifepristone was added from an ethanol stock to the cell culture medium to obtain a final concentration of 10 mg/ml, was diluted to the desired concentration with culture medium when temporarily used. The final ethanol concentration in the culture medium was lower than 0.2%. MTT, propidium iodide (PI) was obtained from Sigma. Mouse anti-human p53 monoclonal antibody, rabbit anti-human survivin, HPV16 E6 protein antibody,

Summary
Objective: The study was designed to investigate proliferation inhibition for cervical adenocarcinoma HeLa cell treated with cisplatin combined with mifepristone and access its possible mechanism. Materials and Methods: HeLa cell was processed by different concentrations of mifepristone, cisplatin, and their combination respectively. Cell’s proliferation inhibition rate and induction apoptosis ability were detected by MTT assay, FCM; the expression of P53, survivin and HPV E6 protein were measured by Western Blot. Results: The results showed that cisplatin inhibits proliferation of HeLa cells in different concentrations (p < 0.01). Mifepristone had no effect on HeLa cell proliferation inhibition rate during 24 and 48 hours (p > 0.05). Mifepristone at low concentrations (≤ 10 μmol/l) combined with cisplatin can significantly enhance the inhibitory effect of cisplatin on HeLa cell line. Flow cytometry showed that mifepristone at low concentrations (≤ 10 μmol/l) combined with cisplatin can induce apparent apoptosis of HeLa cell line in concentration dependent manner. Western blotting demonstrated that the expression of P53 protein increased and the expression of HPV E6 survivin protein decreased in HeLa cells treated with MIF at low concentrations (≤ 10 μmol/l) combined with cisplatin. Conclusion: Mifepristone at low concentrations (≤ 10 μmol/l) can enhance chemosensitivity and capability of inducing apoptosis of cisplatin to HeLa cells. The strengthening effect of growth inhibition and chemosensitivity to cisplatin of mifepristone are associated with down-regulating HPV E6 survivin protein and upregulating p53 protein.

Key words: Mifepristone; Cervical cancer; Cisplatin; Chemotherapy sensitivity.
Mifepristone sensitizing cisplatin for cervical adenocarcinoma HeLa cell sensitivity to chemotherapy and its mechanism

Mifepristone: a single-agent dosing group was as follows: mifepristone: 0.625, 1.25, 2.5, 5, 10, 20 μg/ml; cisplatin: 0.625, 1.25, 2.5, 5, 10, 20 μg/ml. The final concentration of mifepristone and cisplatin in combined group were accordance with the experimental results of single-agent program as follows: 0, 0.625, 1.25, 2.5, 5, 10 μg/ml + 2.5 μg/ml of l/m. Indwelling zero holes (containing only culture medium used in the absorbance of zero), and control well (without any intervention agent) and the vehicle group (cell culture system by adding the drug dissolving media). Set 37.5% CO2, humidified incubator to continue to foster 24, 48, and 72 hours. Each interval adding a final concentration of 0.2 g/ml of MTT solution 100 μl, cultured for four hours, discard supernatant, add 200 μl hole dimethyl sulfoxide (DMSO), mixed on the vortex oscillator for 30 min, and detected on a microplate reader at 570 nm with the measured absorbance (A) values. The inhibition rate of tumor cells to each drug with different concentrations was calculated as follows: inhibition rate = 100% × [(Adrug treated - Ablank)/(Acontrol - Ablank)]. The IC50 value resulting from 50% inhibition of cell growth was calculated. Each concentration of drugs was measured in triplicate wells on the same plate in three independent experiments.

**MTT assay**

Digesting the HeLa cells in logarithmic growth phase, percutting into a single cell suspension to adjust cell concentration to 3 × 10⁴ Ge/ml, to 100 μl/well were seeded in 96-well plates. The culture medium was replaced with the medium containing serial dilutions of various chemotherapeutic drugs. Experimental groups were as follows: each concentration of the groups or between the groups

**Statistical analysis**

Using SPSS 13.0 software for statistical analysis. The Student’s t-test was used within the group. Data between the two groups were compared using ANOVA analysis. Statistical significance occurred if \( p < 0.05 \).

**Results**

This present experiment showed that different concentrations of cisplatin inhibited the growth of the cervical adenocarcinoma cells HeLa and the contrast of growth inhibition rate had statistical significance whether within the groups or between the groups \(( p < 0.05, p < 0.01 )\). The inhibitory effect of cisplatin to HeLa cells strengthened with increased drug concentration and the extension of time (Figure 1).

Mifepristone single drug results showed that mifepristone inhibits the proliferation of HeLa cells when the concentration was greater than 10 μg/ml less than 48 hours with a time-dependent manner \(( p < 0.05 )\). The inhibition rate of mifepristone to HeLa cells was not significant \(( p > 0.05 )\) in HeLa cell vehicle group, low concentrations of mifepristone, \(( 0.625, 1.25, 2.5, 5, 10 \mu g/ml )\) and blank control group. HeLa cell proliferation inhibition rate enhanced with the concentration of mifepristone increased in 72 hour group in concentration-dependent manner \(( p < 0.05 )\). The relationship between different doses of mifepristone, duration of action, and cell inhibition rate are shown in Figure 2.

The authors obtained the values of IC50 of cisplatin to HeLa cells for 24, 48 and 72 hours and were 9.86, 2.95, and 0.96 μg/ml, respectively, based on the concentration of cisplatin and the corresponding inhibition rate with cisplatin in HeLa cells. Therefore the authors chose 2.5 μg/ml as cisplatin fixed concentration in combined group and less than or equal to 10 μg/ml as mifepristone follow-up experimental drug dose in accordance with inhibitory effect of single-agent mifepristone to HeLa cells. The authors observed the effect of 2.5 μg/ml cisplatin with different concentrations of mifepristone to HeLa cells in 24, 48, and 72 hours. It was found that the inhibitory effect on HeLa cells became increasingly apparent \(( p < 0.05 )\) while the mifepristone concentration was gradually increased \(( 1.25 - 10 \mu g/ml )\). The combined effects of cisplatin with different concentrations of mifepristone enhanced the inhibition of cisplatin on the proliferation of HeLa cells (Figure 3).

The authors detected an apoptosis rate by measuring sub-G1 peak and chose 2.5 μg/ml of cisplatin combined with different concentrations of mifepristone \(( 2.5, 5, 10 \mu g/ml )\) in the following experiments according to the result of MTT’s results in HeLa cells for 24 hours. The results showed that the apoptotic rate differences had a statistical significance compared to the combination, monotherapy, and control groups. The statistic values are shown in Table 1. There were no significant differences in cell cycle in each group (Figure 4).

The authors observed the expression level of HPV E6,
Caihong Li, Hong Ye

p53, and survivin protein in HeLa cells after incubation with 2.5 μg/ml cisplatin combined with different concentrations of mifepristone (2.5, 5, and 10 μg/ml) for 24 hours by Western Blot. The detection showed that the expression of HPV E6 and survivin protein decreased whereas the P53 expression gradually increased (Figure 5).

Discussion

Cisplatin is the most-widely used single-agent chemotherapy in cervical cancer and its efficacy is the most positive. Cisplatin is the main drug program in combination chemotherapy for cervical cancer, but the results
Figure 4. — Apoptosis rates and cell cycle distribution of HeLa cells after treatment with cisplatin and different concentrations of mifepristone evaluated by PI staining.
are not effective because its adverse effects and the resistance of tumor cells to cervical cancer. Therefore, it is extremely necessary to find an efficient chemosensitizer and study its anti-tumor mechanism in combination with chemotherapeutic drugs. Chemosensitizer is a class of drug which can improve the efficacy of chemotherapeutic drugs through synergy with chemotherapeutic drugs whereas its effect on tumor treatment is relatively weak.

Mifepristone, a potent progesterone and glucocorticoid antagonist, can bind progesterone receptor, prevent the receptor complex with DNA progesterone response element binding, and stop DNA transcription. Few observations have demonstrated that progesterone and sex hormone regulated HPV gene expression, and concerned with the conversion of the cancerous cell [6-8]. In this experiment, the inhibitory rate of mifepristone on cervical cancer HeLa cells indicated that mifepristone had no effect on cell obvious proliferation inhibition in low-dose (concentration lower than 10 μg/ml) and in a short time (less than 24h). However, mifepristone showed the trend of inhibition of proliferation of HeLa cells if extending the duration of action and increasing its concentration. This result indicates that mifepristone possesses a potential to become a chemosensitizer.

The killing effect of chemotherapeutic drugs on tumor is divided into the direct result of cell necrosis and induction of apoptosis. Analysis results of FCM showed that cisplatin, cisplatin combined with low concentrations of mifepristone had the ability of inducing apoptosis of Hela cell. Presumably, mifepristone’s sensitizing effect to cisplatin may be mainly manifested in its ability to enhance the ability of cisplatin-induced apoptosis. In addition, the antiglucocorticoid properties of mifepristone have not only been effective in treating acute psychotic depression, but also helpful in high stress-related conditions including HIV. Mifepristone has helped in other conditions with progesterone receptors such as uterine leiomyomas, endometriosis, and some breast cancer [9]. Cisplatin combined with mifepristone does not cause adverse reactions superimposed on the body. The combination treatment may provide a new idea for cervical cancer chemotherapy.

In the study of apoptotic mechanism of mifepristone-reinforced cisplatin’s inhibitory effect on HeLa cells showed that expression of HPV E6 protein gradually reduced with an increasing concentration of mifepristone. The occurrence of cervical adenocarcinoma with high-risk HPV infection is inseparable. The main mechanism of HPV carcinogenic effects is generally believed to be a transformation of the genes E6 and E7 genes encoding the protein to induce cell oncogene activation and tumor suppressor gene inactivation; therefore, E6 and E7 oncogene expression regulation and its variation become the focus of the carcinogenic mechanism of HPV. Lee et al. [6,10] found that the E6 and E7 affected tumor suppressor factor p53 and Rb respectively, inhibited their apoptosis, led to the oncoprotein expression of increased high-risk HPV, resulting in cell cycle disorders. This is the most important step in the carcinogenic process. Bartholomew et al. [11] found that progesterone, glucocorticoids, and other steroid hormones may down-regulate the level of expression of type I leukocyte antigen (HLA) on cervical cancer cell surface in HPV (+). The regulation of HLA-I expression of steroid hormones on tumor cells was dependent on the integration and transcription of the HPV genome, but HPV can be blocked by mifepristone. Prompt mifepristone may play a sensitizing role through inhibition of HPV E6 protein interactions in cervical cancer.

Survivin gene is the strongest inhibitor of apoptosis IAP family, which is an important factor for the contact interface of the cell cycle and apoptosis with dual function of inhibition of apoptosis and regulation of mitosis. The Beardsmore and Temme [12, 13] studies’ results showed that high expression of survivin in various tumors are not only associated with poor prognosis and resistance to chemotherapy. Branca et al. [14] study had shown that survivin is a sign of an early cervical cancer, and its anti-apoptotic function is achieved by blocking the normal transcription of the wild-type p53 by HPV E6. In this present study, the authors detected that the expression of HPV16 E6, survivin decreased and P53 protein was gradually increased as the concentration of mifepristone increased in combined group compared with the control group and the monotherapy group. The authors presume that mifepristone impacted the synthesis of DNA, affected the integration of HPV16 E6 gene and the expression

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cell Cycle</th>
<th>Apoptosis</th>
<th>( F )</th>
<th>( p )</th>
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<tr>
<td>Control group</td>
<td>G1 ~ G0</td>
<td>S</td>
<td>G1 ~ G0</td>
<td>( F )</td>
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<tr>
<td>Mifepristone (10)</td>
<td>77.27 ± 2.49</td>
<td>12.93 ± 0.31</td>
<td>14.26 ± 0.39</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td>Cisplatin (2.5)</td>
<td>77.27 ± 0.47</td>
<td>10.47 ± 0.25</td>
<td>8.67 ± 0.16</td>
<td>3.59 ± 0.18</td>
</tr>
<tr>
<td>Cisplatin (2.5) + Mifepristone (2.5)</td>
<td>77.33 ± 0.76</td>
<td>8.62 ± 0.29</td>
<td>9.23 ± 0.28</td>
<td>4.82 ± 0.15</td>
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<tr>
<td>Cisplatin (2.5) + Mifepristone (5)</td>
<td>80.87 ± 0.85</td>
<td>6.57 ± 0.25</td>
<td>7.55 ± 0.13</td>
<td>5.01 ± 0.12</td>
</tr>
<tr>
<td>Cisplatin (2.5) + Mifepristone (10)</td>
<td>76.97 ± 0.80</td>
<td>6.31 ± 0.18</td>
<td>10.63 ± 0.15</td>
<td>6.09 ± 0.80</td>
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\( p \) values < 0.05 and corresponding \( F \) values are highlighted vs the former group.

![Figure 5.](image)
of HPV16 E6 protein reduced, reducing the inhibition of p53 protein so that the expression of p53 protein increased, and consist with the Reedy [15] results. Of course, this is only a relative preliminary study of cisplatin combined with mifepristone mechanism of action. Therefore further experiments in the future are required, such as detection of the gene expression of these proteins, transcriptional activity, the changes of target DNA binding capacity, and related protein expression levels.

References

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Diagnostic value of CA125 as a predictor of recurrence in advanced ovarian cancer


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Summary

Purpose: The aim of this study was to establish the guidelines for detecting early recurrences of advanced epithelial ovarian cancer by use of the CA-125 level. Materials and Methods: Eighty-five of the patients who met the inclusion criteria were enrolled in this study. The authors examined 25 incremental changes of CA125 from one to 25 IU/ml and compared the CA-125 value with other prognostic factors. Increases in the CA-125 level from the nadir level were expressed as CA-125-increments. Results: Among the 25 increments, a CA-125-8 (eight IU/ml) was selected as the predictor that was the most efficient and time-effective. CA-125-8 had a sensitivity of 91.5%, a specificity of 84.6%, a positive predictive value of 93.1%, a negative predictive value of 81.5%, an efficiency of 89.4%, and a median lead-time of 68.5 days (p < 0.0001). Conclusion: The authors suggest the incremented CA-125-8 as a predictor of recurrent advanced ovarian cancer.

Key words: Advanced ovarian cancer, CA-125; Early detection of recurrence.

Introduction

Ovarian cancer has the highest mortality rate of all gynecologic cancers. The majority (nearly 75%) of patients with newly-discovered ovarian cancer have advanced disease at the time of diagnosis. Nevertheless, complete clinical remission can be achieved in 80% of advanced disease with the use of optimal cytoreductive surgery followed by adjuvant chemotherapy [1, 2]. However, advanced ovarian cancer ultimately recurs within the first two years after diagnosis in up to 80% of cases, consequently the expected five-year survival rate of advanced ovarian cancer does not exceed 30%-50% [3, 4]. Recently, one study group announced that early treatment for recurrent ovarian cancer based on a rising CA-125 does not appear to improve the overall survival compared with treatment that is commenced upon presentation of symptoms [5]. The clinical study was based on chemotherapy alone. However, it has also been reported that secondary cytoreductive surgery achieves survival benefits in the management of patients with recurrent ovarian cancer [6-11]. It is well-known that localized recurrent disease is more treatable with secondary cytoreductive surgery [12, 13]. Therefore, it is important to inspect the early state of recurrence in order to detect localized or small volume recurrence before dissemination [6, 9]. Currently, recurrence of ovarian cancer is diagnosed by consideration of several factors, including an increase in the level of CA-125, radiologic findings, and clinical symptoms. Generally, among these factors, the CA-125 level changes most rapidly and emerges two to six months earlier than the appearance of new lesions identified by imaging [14-16]. To date, the CA-125 level as a criterion for recurrence is considered to be a two-fold increase in the upper limit of normal (35 IU/ml) [17, 18]. The present study was undertaken to determine the early state of recurrence of advanced epithelial ovarian cancer by analysis of serially-measured CA-125 levels.

Materials and Methods

Patient population

Between January 1995 and May 2008, 571 patients were diagnosed with ovarian cancer at Seoul St. Mary’s Hospital. The medical records of all patients were analyzed retrospectively. Eighty-five cases satisfied all of the following criteria: (1) FIGO Stage III-IV; (2) initial serum CA-125 level > 35 IU/ml at the time of diagnosis; (3) complete remission (CR) after proper primary treatment (cytoreductive surgery followed by adjuvant chemotherapy (platinum + taxane) and a normal CA-125 level (< 35 IU/ml); (4) recurrence confirmed with radiographic documentation or surgery; and (5) in patients with no recurrence, sustained CR for at least two years. After the completion of proper primary treatment, patients underwent follow-up every three months for the first two years, every six months thereafter for three years, then annually. A history, physical examination, CA-125, and a radiologic examination were performed in routine follow-up, and chest/abdominal/pelvic computed tomography (CT) or positron emission tomography (PET) scans and chest X-rays were ordered if determined to be clinically necessary. The Institutional Review Board approved this study.

CA-125 level

Serial CA-125 levels were determined during the interval from CR to recurrence or last day of follow-up. Increments of CA-125 levels were calculated by subtracting the baseline nadir
from subsequently measured values, with the nadir defined as the level within the normal range (< 35 IU/ml) at the time of completion of primary chemotherapy and radiographic CR. In this study, the authors evaluated 25 one-unit increments from 1 - 25 IU/ml.

Statistical methods

The distribution of patient characteristics is presented as a frequency and percentage, and Fisher's exact test was used to compare the differences between patients with recurrence and patients with sustained CR. The median values of the disease-free interval, initial CA-125 level, and nadir were compared using the Wilcoxon rank-sum test. A two-sided p value of < 0.05 was considered to be statistically significant.

Results

Characteristics of patients

Table 1 shows the patient characteristics. Fifty-nine (69.4%) patients had an ovarian cancer recurrence and 26 patients had a sustained CR. The majority (71) of the patients (83.5%) had serous tumors and 40 patients (56.3%) had grade II tumors. Of the 85 patients, 63 (74.1%) underwent optimal surgery (no visible residual lesions), with 41 patients (69.5%) in the recurrent group and 22 patients (64.6%) in the sustained CR group (p = 0.1838). Fifty-five patients (64%) underwent second-look surgery, with negative results for 37 patients (67.3%). Specifically, 23 patients (60.5%) in the recurrent group and 14 (82.3%) patients in the sustained CR group had negative results on second-look surgery (p = 0.133). The CA-125 nadir level was similar for both groups (6.0 IU/ml in the recurrent group and 6.17 IU/ml in the sustained CR group; p = 0.973; Table 1). Ten of 85 patients had another pattern for the CA-125 level; specifically, the CA-125 level increased > 35 IU/ml at the time of diagnosis, but had not increased at the time of recurrence. The CA-125 level increased > 35 IU/ml at the time of diagnosis, but had not increased at the time of recurrence. The CA-125 level increased > 35 IU/ml at the time of diagnosis, but had not increased at the time of recurrence. The CA-125 level increased > 35 IU/ml at the time of diagnosis, but had not increased at the time of recurrence. The CA-125 level increased > 35 IU/ml at the time of diagnosis, but had not increased at the time of recurrence.

Multivariate logistic regression for recurrence

Table 2 shows the prognostic factors for the prediction of recurrence, CA-125-8, disease stage, optimal surgery, histologic type, initial CA-125 level, and nadir CA-125 level. Based on univariate analysis, CA-125-8, histologic type, and the initial CA-125 level were significant factors for recurrence; however, based on multivariate logistic regression analysis, CA-125-8 was statistically the most significant predictor of recurrence in advanced ovarian cancer (OR for recurrence = 127.9; 95% CI, 17.1-955.9; p < 0.0001).

Discussion

Recurrent ovarian cancer is a lethal disease. Furthermore, the optimal timing and modality of second-line therapy for recurrent disease is still a matter of debate. Systemic chemotherapy is generally offered to women with recurrent ovarian cancer and secondary surgical cytoreduc-
tion is considered for patients with several good prognostic factors, such as an extended progression-free interval of at least 12 months, the potential to eradicate all gross residual disease, response to first-line therapy, and good performance status. There are many reports that optimal secondary cytoreductive surgery followed by chemotherapy provides a significant improvement in the median survival time for select patients [6, 9, 10, 17]. It was suggested that platinum-sensitive patients with seemingly resectable masses should be considered for surgery [6, 10, 17, 18]. CA-125 plays an important role in ovarian cancer, including screening, assessing the response to therapy, and follow-up after completion of initial therapy [19, 20]. A number of studies have attempted to define the CA-125 increment that can predict recurrence of ovarian cancer [15, 16]. A relative increment of 50% or 100% of the CA-125 level from the reference level (25 IU/ml) was introduced as a predictor of recurrence during follow-up after primary therapy [13]. Recent studies have analyzed the CA-125 levels at the time of follow-up after CR in patients with CA-125 levels that remained within the normal limit (< 35 IU/ml) at the time of recurrence. Patients with an absolute increment of five and ten IU/ml or relative increments of 100% from the nadir during follow-up experienced recurrences; thus, a small change in the CA-125 level could be a predictor of disease recurrence [21, 22]. These are notable results for clinicians, but there are several limitations to apply in the clinic. First, these studies enrolled the only patients with CA-125 levels that remained within a normal range after recurrence. It is impossible to predict whether or not a CA-125 level will be elevated after recurrence. Consequently, the studies have not proven useful to predict recurrences; rather a small increment in CA-125 can be a sign for recurrence. Furthermore, there is no rationale to define an absolute increment (five or ten IU/ml) or a relative increment (100%). For these reasons, the authors analyzed the CA-125 levels of all patients and compared diagnostic values and median lead times of 25 increments to define the most effective increment. As shown in Figure 2, the diagnostic values from CA-125-8 to CA-125-15 showed similar effective values. The authors considered median lead-times of each increment, and consequently CA-125-8, with the longest median lead time in this range (from CA-125-8 to CA-125-15), was selected as the best predictor. Furthermore, as compared with the other prognostic factors for recurrence, CA-125-8 was also statistically the most significant predictor, with an odds ratio of 127.9 (95% CI, 17.1 - 955.9) and a p < 0.0001. It is difficult to consider CA-125-8 as an early signal for recurrence due to the limited sample size and retrospective design of the study. In addition, most of clinicians may hesitate to accept the results that CA-125-8 is a very small increment. However, if it is considered that the CA-125 levels of the CR group were sustained in a fixed field without fluctuation, a small change of the CA-125 level could also be a significant indicator of a recurrence. Clinicians should only recognize the risk of recurrence and decide the next examination or follow-up date according to the change in the CA-125 level, such as CA-125-8. An early state of recurrence, such as a small volume or localized lesion, can yield options for treatment and a favorable prognostic factor. Therefore, the early detection of recurrence is important and an effort should be made to detect an early state of recurrence, which is a more curable state.

Conclusion

The current study has described the novel concept that an earlier indicator can be applied before the CA-125
level reaches an abnormal range (> 35 IU/ml) for recurrent disease. The authors suggest an increment of eight IU/ml for the CA-125 level among the assessed CA-125 increments as the best predictor. A larger study should be performed to evaluate the validity of these results in a prospective and controlled clinical setting.

References


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Livin and caspase-3 expression are negatively correlated in cervical squamous cell cancer

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Summary

Objective: Overexpression in cancer cells of inhibitor of apoptosis proteins like livin appears to promote tumorigenesis by regulating expression of proteins involved in apoptosis signaling. Here, the authors investigated expression of livin and an apoptosis protein that is known to inhibit, caspase-3, in cervical squamous cell carcinoma. Materials and Methods: Their expression was assessed for correlation with tumor invasiveness. Immunohistochemistry for livin and caspase-3 was used in 36 normal cervical tissues and in 98 samples of cervical squamous cell carcinoma. The percentage of cells expressing these proteins was compared between normal and cancer samples. Their expression rates in cancer samples were subsequently compared with one another and with the clinical and pathological characteristics of the samples. Results: Livin was more commonly expressed in tumor samples than in normal tissues, while the opposite pattern was observed for caspase-3. Expression of livin was significantly associated with advanced clinical stage, higher pathological grade, and lymph node metastasis (p < 0.05). Expression of caspase-3 was significantly associated with lower clinical stage, lower pathological grade, and lack of lymph node metastasis (p < 0.05). Finally, expression of livin was negatively correlated to caspase-3 expression in cervical squamous cell carcinoma tissue (r = -0.57, p < 0.05). Conclusions: Livin may inhibit apoptosis in cervical squamous cell carcinoma by downregulating caspase-3, thereby promoting disease progression.

Key words: Livin; Caspase-3; Cervical squamous cell carcinoma; Immunohistochemistry.

Introduction

Although the incidence has declined in recent years, cervical cancer remains a common malignant tumor in women. Cervical cancer occurs in two main pathological types, squamous cell carcinoma and adenocarcinoma; 80%-90% of cervical cancers present as squamous cell carcinoma [1]. The disease is typically caused by the human papillomavirus (HPV), which can activate oncoproteins such as p16 [2] and p53 [3], thereby causing cell proliferation and cell apoptosis disorders [4] that promote tumorigenesis.

Research efforts have been devoted to understanding how proteins involved in apoptosis become dysregulated in cancer cells. In particular, inhibitors of apoptosis (IAPs) appear to play important roles in preventing apoptosis by binding to caspases, the activators of programmed cell death, allowing tumor cells to avoid elimination. Expression of several IAPs is higher in tumor cells than in normal tissues. One IAP, livin (also known as ML-IAP), is expressed in normal fetal kidney and liver and adult testis and thymus, but is also highly-expressed in lymphoma [5], melanoma cell lines, breast [6], and esophageal carcinoma [7]. Livin inhibits apoptosis by binding caspases 3, 7, and 9 [8]. Caspase-3 is a significant executioner in the caspase family because it hydrolyzes a number of substrates [9]. This protein initiates cell death following activation by either the mitochondrial or the death ligand pathway, after which the caspase cascade is triggered [10].

Given the interaction between livin and caspase-3 and the role of IAPs like livin in tumorigenesis, the authors sought to determine whether expression of livin is dysregulated in cervical squamous cell carcinoma, whether its expression effected a change in caspase-3, and whether these changes may correspond to disease severity. To determine the relationship between livin and caspase-3, immunohistochemistry was applied to these two proteins in tissue samples from normal and cancerous cervixes. Protein expression was also correlated to tumor pathology.

Materials and Methods

Specimens

Cervical tissues were collected from 134 patients who received biopsy or surgery treatment in Department of Obstetrics and Gynecology in the first People’s Hospital of Yancheng City from June 2009 to June 2011 and had not received any radiotherapy and chemotherapy. These samples included 98 cases of cervical squamous cell carcinoma and 36 normal cervical tissues. Of the cancer patients, 45 women were less than 45 years old, and 53 were greater than or equal to 45 years old. Samples were clinically classified according to the International Federation of Gynecology and Obstetrics (FIGO): 61 cases were Stage I, and 37 cases were Stage II. Pathological classification [11] indicated 14 well-differentiated cases (low-grade), 46 moderately-differentiated cases, and 38 poorly-differentiated (high-grade) cases. Additionally, 31 cases had lymph node...
Livin and caspase-3 expression are negatively correlated in cervical squamous cell cancer

**Results**

**Expression of livin and caspase-3 in cervical carcinoma**

Expression of livin was compared between 98 squamous cell carcinoma and 36 normal cervical tissues. In normal cervix, only 4/36 samples (11.1%) exhibited livin expression in more than 5% of cells. However, 77/98 (78.6%) cervical carcinoma samples were positive for livin expression (Table 1). This difference in livin expression between normal and squamous cell carcinoma cervical tissues was significantly different ($\chi^2 = 50.1$, $p < 0.05$). In contrast, caspase-3 was more likely to be detected in normal cervix than in cervical cancer: 26/36 (72.2%) of normal tissue samples were positive for caspase-3 expression and just 26/98 (26.5%) cervical squamous cell cancer tissues were positive for caspase-3 (Table 1). Thus, the differences in expression of caspase-3 between normal and cancerous cervical tissues were statistically significant ($\chi^2 = 23.2$, $p < 0.05$).

**Livin and caspase-3 expression are differentially correlated with clinico-pathological parameters of cervical squamous cell carcinoma**

Although expression of livin in cervical squamous cell cancer was not correlated with patient age, its expression was more prevalent in Stage II vs Stage I tumors ($p < 0.05$; Table 2). Similarly, livin expression was lower in well-differentiated tumors than in moderately- and poorly-differentiated tumors, and thus its expression was correlated with higher tumor grade ($p < 0.05$). Livin expression was also higher in tumors with lymph node metastasis than those without lymph node metastasis ($p < 0.05$).

Age was also not a factor for caspase-3 expression in cervical squamous cell cancer. In contrast to livin expres-
sion, caspase-3 expression was more common in Stage I cervical squamous cell cancer than in Stage II (p < 0.05; Table 2). Additionally, correlations of caspase-3 with pathological grade were identified: expression of caspase-3 was more common in well-differentiated than in moderately- or poorly-differentiated tumors and therefore, caspase-3 expression was correlated with lower tumor grade (p < 0.05). Finally, caspase-3 expression in cervical squamous cell cancer was correlated with not having lymph node metastasis as it was more commonly detected in tumors that had not metastasized (p < 0.05).

Livin and caspase-3 expression correlation in cervical squamous cell cancer

In cervical squamous cell cancer tissues, livin was expressed in 78.6% of tumors, while caspase-3 was expressed in 26.5%. Spearman rank correlation test indicated that the expression of these proteins in cervical squamous cell tumors was negatively-correlated (Table 3).

Discussion

The occurrence of cervical squamous cell cancer is not only related to HPV infection, but also to the imbalance of carcinogen-induced cell proliferation and apoptosis that follows HPV infection. Cell apoptosis initiates in response to intrinsic or extrinsic factors via a signaling program [12]. Several important players in this program are the caspase family proteins [13], Bcl-2 family proteins [14], p53 [15], and survivin [16].

The inhibition of apoptosis can allow cancer cells to escape elimination, promoting tumorigenesis. IAP proteins have therefore become central to the investigation of cancer mechanisms. In particular, the IAP protein livin is important for its ability to inhibit apoptosis by interacting with caspases, especially caspase-3. Recent work has demonstrated that livin expression is dysregulated in tumors, such that it is more abundantly expressed in the tumor cells [6,7]. Additionally, Gazzaniga et al. showed that, in bladder cancer, the postoperative recurrence time of patients with expression of livin in their tumors was shorter compared to patients lacking livin expression; thus, livin expression is correlated with poorer prognosis [17]. The present research results were consistent with those obtained by Gazzaniga et al. Livin was significantly more commonly expressed in cervical squamous cell carcinoma than in normal cervical tissues. Further, expression of livin in tumors was significantly correlated with higher cancer Stage (clinical classification, p < 0.05), worse pathological grade (differentiation degree, p < 0.05), and lymph node metastasis (p < 0.05). Thus, expression of livin protein can predict poorer prognosis in patients with cervical squamous cell carcinoma.

Of the caspase family proteases involved in apoptosis, caspase-3 is one of the most significant. In cases in which caspase-3 expression is dysregulated (i.e., expression is reduced), apoptosis may not occur at a normal level. Indeed, in tumor cells, caspase-3 expression is down-regulated. The authors investigated whether caspase-3 expression is dysregulated in cervical squamous cell carcinoma. They found that caspase-3 appears to be down-regulated in these tumors; expression was more common in normal cervical tissues than in the carcinoma tissues. Additionally, caspase-3 expression was correlated with lower cancer Stage (clinical classification; p < 0.05), lower pathological grade (differentiation degree, p < 0.05), and lack of lymph node metastasis (p < 0.05). Thus, reduced or absent caspase-3 expression can predict a poorer prognosis in patients with cervical squamous cell carcinoma. These results are consistent with reports from ovarian cancer [18] and breast cancer [19].

Finally, the authors determined whether livin and caspase-3 expression were correlated with one another. Indeed, these proteins displayed a negative correlation of expression in cervical squamous cell cancer, suggesting that livin may regulate and control cell apoptosis by inhibiting expression of caspase-3. In fact, recent work indicates that livin reduces apoptosis by directly decreasing expression of caspase-3 and inhibiting death acceptor pathways. Furthermore, livin can inhibit mitochondrial apoptosis pathways by combining with caspase-9 [20].

In summary, the authors found that that livin is highly-expressed in cervical squamous cell cancer tissues, and its expression level is related to disease severity. In contrast, caspase-3 is minimally expressed in cervical squamous cell cancer tissues, and its expression level is inversely related to disease severity. The negative correlation between livin and caspase-3 expression suggests that livin expression can promote cervical squamous cell cancer by inhibiting cellular apoptosis.

Table 3. — Correlation of livin and caspase-3 expression in cervical squamous cell cancer.

<table>
<thead>
<tr>
<th>Livin</th>
<th>Caspase-3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>11</td>
<td>66</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>72</td>
</tr>
</tbody>
</table>

Significance: *p* < 0.05.

References


Livin and caspase-3 expression are negatively correlated in cervical squamous cell cancer


Laparoscopically-assisted radical vaginal hysterectomy with five years follow-up: a case control study

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Summary

Objective: To compare a novel surgical approach, laparoscopically-assisted radical vaginal hysterectomy (LARVH) with abdominal radical hysterectomy in women with cervical cancer, and to investigate whether selected women benefit from the minimally-invasive approach without high recurrence rate and complications. Materials and Methods: Forty women undergoing LARVH were included and compared with 40 women undergoing abdominal radical hysterectomy. The control group was matched for age and disease stage. Retrospective chart review was performed and patients were followed for an average of 2.5 years. Results: Blood loss was significantly increased in the control group (343.3 vs 606.3 ml, p = 0.012). Transfusions were given in 42.5% of women in the control group and 17.5% in the LARVH group. Mean operative time was longer in the control group (151 vs 240 minutes p = 0.0001). Mean nodal counts did not show a significant difference (27.3 in control vs 21.4 in LARVH, p = 0.886). Recurrence group was 7.5% at mean follow up of 30.1 months in LARVH group and in 30.8 months follow-up. Conclusions: The LARVH procedure was comparable in terms of safety (recurrence and complication rates) meanwhile LARVH showed minimally-invasive advantages in terms of blood loss, operative time, and shorter hospital stay.

Key words: Cervical carcinoma; Laparoscopic surgery; LARVH.

Introduction

The technical feasibility of laparoscopically-assisted radical vaginal hysterectomy (LARVH) as treatment for early-stage cervical cancer has been well-established through a series of retrospective reports [1-5]. These reports suggest that LARVH may have an intraoperative reduction in blood loss, transfusion requirement, and hospital stay, but surgical time may be prolonged. Furthermore, a growing evidence-based study indicates that adequate lymphadenectomy can be achieved laparoscopically. Indeed the inherent advantages of laparoscopic surgery, improved visualization of tissues, and magnification may improve accuracy of disease staging and lymphadenectomy [6, 7].

Laparoscopic management of cervical carcinoma was advocated by some authors as an alternative to open radical hysterectomy and pelvic node dissection. A number of case-control studies supported the hypothesis that preoperative morbidity, blood transfusion, and hospital stay can be reduced without compromising outcomes [8-10]. Pelvic lymphadenectomy, as an integral part of surgical management of cervical carcinoma, could be possibly achieved by laparoscopic approach.

This study aims to evaluate LARVH in terms of preoperative and postoperative complications, outcome (mortality and recurrence rates), and consumption of medical resources (hospital stay, operative duration). These parameters were compared with the standard open radical hysterectomy.

Materials and Methods

Between December 2003 and December 2008, 40 patients with cervical carcinoma of International Federation of Gynecology and Obstetrics (FIGO) Stage IA to IIA. The control group consisted of 40 open procedures, matched for age, stage of disease, and magnetic resonance imaging (MRI) outcomes, in the regional cancer center. This study was conducted in accordance with the declaration of Helsinki. This study was also conducted with approval from the Ethics Committee of Tumor Hospital of WuXi City affiliated to Soochow University. Written informed consent was obtained from all participants. All women in both groups underwent preoperative pelvic MRI scans and computed tomography (CT) scans. Results from two groups were compared for matching age and disease stage before operated by the same team over the same time period.

Date were collected retrospectively from the charts as follows: age, clinical stage, histopathological stage, blood loss, conservation of ovaries, duration of surgery, nodal counts, number of nodes involved, intraoperative complications, postoperative complications, hospitalization days, and recurrence of disease.

LARVH is performed through four-port laparoscopy with ligation of pedicles. Excision of the uterus, fallopian tubes, and ovaries was conducted when indicated and excision of parametria was carried out laparoscopically, with formation of vaginal cuff and extraction of specimen completed vaginally. Laparoscopic dissection of the ureteric tunnels and removal of pelvic node was performed within the same anatomical margins in the open procedure. Postoperative hospital stay was recorded.

Results were analyzed using SPSS software. Age, blood loss, hospital stay, duration of surgery, and node counts were compared with the two-tailed t-test.

Results

From December 2003 until December 2008, 40 LARVHs were carried out for cervical carcinoma. All procedures were included in the study, including two cases converted to laparotomy due to ureteric injury and

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bladder injury, which gave a conversion rate of five percent. Results of these two cases were included within the laparoscopic group. Over the same time period, 40 open radical hysterectomies with pelvic node dissection were identified and matched for age, clinical FIGO Stage, and histological results (Table 1). Significant differences were found including the following: mean blood loss was significantly reduced in the LARVH group (p = 0.012) at 343.3 ml compared with 606.3 ml in the open group (Table 2). Seventeen women received blood transfusion in the open group, while seven women in LARVH group required blood transfusion, two of whom were converted to laparotomy. Duration of surgery differed significantly between the two groups (240 min in LARVH and 151 min in the open group, p = 0.0001). Postoperative stay was significantly longer in the open group, with a mean of 11.0 days (range 7-25) compared with 7.2 days (range 5-12) in LARVH (p < 0.001). No significant difference was found when comparing age, preoperative hemoglobin, and node counts. No women in the LARVH group had positive nodes, with five women in the open group having positive nodes (Table 2).

Recurrence rates were equal, with three recurrences in each group. Mean follow-up in the LARVH group was 30.8 months (range 6-60). Recurrence was found to be located in vault and pelvic sidewall in two patients with preoperative Stage IB in LARVH group. The other women developed lung metastases at 28 months follow-up postoperatively and was managed with chemoradiation and adjuvant radiotherapy, but died 40 months postoperatively (Table 3).

**Discussion**

This study lacked the power to prove that LARVH procedure was comparable to open radical hysterectomy in terms of complications and clinical outcomes, but provides further data to support LARVH. This procedure was not conducted in a randomized manner, which weakened the evidence power. The FIGO Stage in the LARVH group was not matched with that in the control group, which presented a lower Stage and might impact final outcomes. However, this is inevitable when learning a new complex technique. Mean follow up in this study was 30.8 months (range 6-60) in the LARVH group and 30.8 months (range 6-60) in the open group. Three recurrences resulted in each group (7.5%). Sites of recurrence were vault and pelvic sidewall in two women in LARVH group, one of which had a squamous cell tumor staged as pT1b1 histopathologically, with clean margins and no adjuvant treatment. Suspicious signs of recurrence were discovered by clinical examination 20 months postoperatively, which was confirmed by MRI, arising from right pelvic sidewall. The patient underwent chemoradiation and received surgery for resection of residual disease. The second recurrence case in the LARVH group was in a woman with adenocarcinoma pT1b1N0 who received adjuvant chemoradiation, and pulmonary metastatic disease was detected 28 months postoperatively on CT scan and treated surgically. The third recurrence in the LARVH group was adenocarcinoma staged at pT1b1N0 histopathologically, whose margins were clean and no adjuvant treatment was adopted. Suspicious signs of recurrence were discovered by clinical examination 30 months postoperatively, which was confirmed by MRI, arising from right pelvic sidewall, who underwent chemoradiation and resection of residual disease.

In the open group, there were three recurrences. One woman resulting in death had an adenocarcinoma staged at pT1b1 histopathologically with positive lymphovascular invasion but negative nodes and received adjuvant chemoradiation. Relapse was detected by clinical examination 16 months postoperatively. MRI revealed a four cm lesion between bladder and rectum. She received further chemoradiation but died 26 months following detection of relapse. The second recurrence in the open group was adenocarcinoma pT1b1N0 with lymphovascular invasion, who received adjuvant radiotherapy. Recurrence was detected by clinical examination 17 months postoperatively. MRI demonstrated bilateral sidewall masses and right hydronephrosis. She received chemoradiation and adjuvant radiotherapy. Suspicious signs of recurrence

**Table 1. — Age, clinical FIGO Stage, and histological results.**

<table>
<thead>
<tr>
<th></th>
<th>Open (n = 24)</th>
<th>LARVH (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age years (range)</td>
<td>39.1 (28-57)</td>
<td>44.9 (30-61)</td>
</tr>
<tr>
<td>Stage I A2</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Stage I B1</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Stage I B2</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Stage II A</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Squamous</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table 2. — Comparison of hemoglobin, nodal counts, operative time, and hospital stay.**

<table>
<thead>
<tr>
<th></th>
<th>Open (n = 44)</th>
<th>LARVH (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood loss (ml)</td>
<td>606.3</td>
<td>343.3</td>
</tr>
<tr>
<td>Mean operating time (minutes)</td>
<td>151</td>
<td>240</td>
</tr>
<tr>
<td>Mean nodes retrieved (range)</td>
<td>27.3 (19-32)</td>
<td>21.4 (18-28)</td>
</tr>
<tr>
<td>Number of women with positive nodes</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Mean hospital stay</td>
<td>11.0</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* na: not applicable.

**Table 3. — Comparison of complications.**

<table>
<thead>
<tr>
<th>Complications</th>
<th>Open (n = 40)</th>
<th>LARVH (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrexia (requiring antibiotic therapy)</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Bladder dysfunction (requiring ISC post-discharge)</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Bowel dysfunction</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Wound infection</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Lymphoedema</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Fistula formation</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Bladder injury</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>24</td>
</tr>
</tbody>
</table>
were discovered by clinical examination 30 months postoperatively, which was confirmed by MRI, arising from right pelvic sidewall. She underwent chemoradiation and surgical resection of residual disease.

As expected, morbidity of wound complications in the open group was high and two (5%) required antibiotic therapy. Bowel dysfunction requiring laxative treatment occurred in two women who had open surgery, while none occurred in the laparoscopic group. Bladder morbidity, as measured by the necessity for intermittent self-catheterization (ISC) at post-discharge, was equal in the two groups, and temporary for all women. It has been suggested that an inherently less radical parametric dissection in LARVH results in less bladder morbidity [2], but was not found in this study. Unsafe margins requiring adjuvant radiotherapy were reported in one woman in LARVH group and two women in open group. This indicates that the operative approach did not affect radical dissection in these women. One uretero-vaginal fistula was managed successfully by stenting of the affected ureter, which was the only ureteric complication. Reported rates for ureteric fistula in the literature range from one to three percent intraoperatively [11, 12] and relative ureteric injury rates were 0%-3.5% [12]. The incidence of which is up to 10% in published series [2], which was not compared with this study, as no cystotomies were performed. Complications in women in LARVH group were distributed throughout the series with no discernible pattern, suggesting that earlier cases had higher morbidity. Analyzing operative time across the series shows no downward trend and node counts in women in LARVH group show no correlation with increasing experience. It is possible that with greater numbers, a discernible improvement in surgical time and complication rate would occur, as in the prospective study of 200 women by Hertel et al. [1].

There are some women, for whom open surgery is more suitable. As observed by Roy et al. [10], a relatively new group of women, who wish to preserve fertility, benefited from the techniques of LARVH. Demand for this operation is likely to grow in the future, as published data re-confirm favorable obstetric outcomes and efficacy [13-15] LARVH was applicable to radical tracheectomy with skills developed in surgeons, which may even be used for the management of two endometrial carcinoma stages. Published series imply that LARVH may be a valid alternative in endometrial carcinoma [16, 17], especially in obese women with co-morbidities. Tozzi et al. [18] found morbidity following LARVH decreased compared with open radical hysterectomy in women with BMI greater than 30, diabetics, hypertensives, and those with cardiorespiratory failure. They proposed that LARVH could be used for treatment of choice for all such women with endometrial carcinoma, a disease with significantly greater incidence than cervical carcinoma. This perhaps represents a paradigm shift for anesthetists as well as gynecologists.

This study provides further data to suggest that LARVH is a safe and alternative approach of open radical hysterectomy, by reducing operative complications without comprising the recurrence rate.

References


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Analysis of one year follow-up of women with cervical cytology report of atypical squamous cells and the diagnostic role of high-risk HPV infection

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Summary

Objective: To investigate the risk of developing cervical intraepithelial neoplasia grade 2 (CIN2) or greater disease in patients with cytology report of atypical squamous cells of undetermined significance (ASC-US) or cannot exclude high-grade atypical squamous cells (ASC-H) in one year follow-up. Study design: Analysis of colposcopy-directed multiple cervical biopsies in all patients. Patients without CIN2 or greater diseases were tested for human papillomavirus (HPV) DNA at the enrollment and at 12th month and followed up by cytology at the 6th and 12th month. Patients with repeated abnormal results were subjected to colposcopy-directed biopsy. Results: A total of 894 ASC-US and 101 ASC-H patients were enrolled. The rate of CIN2 or greater disease was 14.2% in ASC-US group and 46.5% in ASC-H group, at the first test respectively. A total of 65.0% of patients in ASC-US have completed the study and 47.5% repeatedly showed abnormal cytology, while the same rates in ASC-H were 62.7% and 50%. Only four cases were diagnosed with CIN2 in ASC-US group. The rate of HPV DNA becoming negative was 54.9% and 51.5% for ASC-US and ASC-H, respectively. Conclusions: The diagnosis rate of CIN2 or greater lesions in ASC-US and ASC-H patients was about 15% and 46.5%, respectively, within one year.

Key words: Cervical intraepithelial neoplasia; Atypical squamous cell; HPV; Follow-up.

Introduction

The number of deaths worldwide in women with cervical cancer is just after that in women with breast cancers. Each year, new occurrence of cervical cancers is about 466,000 cases globally, among which one-third are from China. As the incidence of cervical cancer has risen in recent years, it shows a trend of affecting younger patients [1, 2]. Cervical smear is the widely-used method in screening precancerous lesions and cervical cancers. The highest proportion of cells in the Bethesda system (TBS) is atypical squamous cells, which show certain atypia and can be divided into two subtypes: atypical squamous cells of undetermined significance (ASC-US) and high-grade atypical squamous cells (ASC-H) [3]. ASC-US accounts for a huge number of patients but has lower chance to develop cervical intraepithelial neoplasia (CIN2) or greater lesions (about 10%) [4, 5]; although it is rare in cytology report, ASC-H shows higher potential of developing CIN2 or greater lesions (about 40%) [6]. The current concern is how to effectively detect cancer from these atypical squamous cells without over-diagnosis and over-treatment of the disease. After the establishment of relationship between high-risk human papillomavirus (HR-HPV) infection and cervical precancerous lesion and cancer, HPV detection has been applied in the primary screening of cervical cancer and precancerous lesions, the ASC-US triage management, and the follow-up management after treatment of CIN disease [7]. Although there are extensive forward-thinking studies with a large number of samples regarding the application of HPV detection in patients with cytology report of positive atypical squamous cells, few data are from China. This study analyzed 995 patients who were admitted to the Hospital from June 2007 to December 2008 with ASC in liquid-based cytology test and went through colposcopy-directed cervical biopsy (894 cases of ASC-US; 101 cases of ASC-H). The patients who were not primarily diagnosed with CIN2 or higher-grade lesions were subjected to one-year follow-up to observe the prognosis, to further explore the reasonable approach to ASC management, and to investigate the significance of application of HPV test in performing reasonable diagnosis.

Materials and Methods

Patients who were admitted into outpatient for cervical lesions to the Peking University Third Hospital from June 2007 to December 2008, diagnosed with ASC-US or ASC-H in cervical cytology report, and accepted colposcopy-directed biopsy, were included in this study and recommended for HPV DNA detection. Patients who were positive for both ASC-US and HPV DNA were recommended for colposcopy; HPV DNA negative cases were selected for colposcopy or cytological follow-up based on patients’ and clinical needs. ASC-H patients were subjected to colposcopy-directed cervical biopsy and endocervical curettage. Patients who were pregnant, could not be followed up, had history of CIN lesion, or went through hysterectomy, were all excluded from this study. A total of 995 patients were enrolled with an average age of 39.3 years (range 23 - 73), an average number of births of 1.07 (range 0 - 6). The first colposcopy-directed multiple cervical biopsies and pathological analysis were carried out in no less than two quadrants of the cervix in ASC-US group and no less than three quadrants in ASC-H group. Patients failed to be diagnosed with...
DNA positive patients in ASC-H group was 86.7% was 54.9% (107/195). During follow-up, 195 were first-time diagnosed as HPV positive; the rate of patients turning into HPV negative completed follow-up, 195 were first-time diagnosed as HPV DNA positive. During follow-up, no cases of CIN2 or greater diseases in the ASC-H group were HPV DNA positive. Among the 54 patients who were without CIN2 or greater diseases during the initial diagnosis but completed follow-up, 33 were first-time diagnosed as HPV DNA positive; during one year follow-up, the rate of patients turning into HPV negative was 51.5% (17/33). The ratios of patients whose HPV DNA turned negative showed no significant difference between ASC-US and ASC-H groups (p = 0.72).

Discussion

The recognition and definition of atypical squamous epithelial cells in cervical epithelia exactly reflect the process of modern understanding of cervical cancer and precancerous cervical lesions. A particular category of “atypical cells” was introduced in 1988 [8] and became the most common type of abnormalities. A total of 10% - 17% of the patients in this category have been diagnosed with CIN2/3 [4, 5, 9]; while cervical cancer accounts for 0.1% - 0.3% of the patients in this category [10].

Based on TBS 2001 criteria, patients with atypical cells are divided into two subgroups: ASC-US and ASC-H; ASC-US accounted for 3.6% in the cytology report [11]; while in the literature, ASC-H is rare in cytology report and accounts for only 0.27% - 0.56% of patients [12]. Since most of these patients do not develop CIN2 or greater lesions, the authors require a more sensitive and specific way to perform the subsequent diagnosis in order to decrease the burden on these patients, both psychologically and economically.

The controlled forward-thinking ASC-US/LSIL triage study (ALTS) has been carried out in order to better guide the choice of clinical measures for diagnosis and treatment of ASC-US/LSIL. ALTS has evaluated the follow-up cytology, colposcopy, and HPV triage management, and validated these measures as well [13]. The authors hope that the data of this study can be used to elaborate the clinical significance of these abnormal cytological reports. The outpatient cervical and vaginal cytology specimens in this study are collected from the Department of Gynecology and Obstetrics of the Hospital. In recent years, the different detection rate of ASC-US reflects, to a certain extent, the particularity of a group of patients, especially those without regular periodic physical examinations. Therefore, a reasonable treatment of these patients who are first-time diagnosed with

### Table 1. Follow-up of patients who had not been diagnosed with CIN2 or greater lesions.

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>Abnormal</th>
<th>Normal</th>
<th>Abnormal</th>
<th>Normal</th>
<th>Abnormal</th>
<th>Normal</th>
</tr>
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<tbody>
<tr>
<td>Cytology</td>
<td>92</td>
<td>103</td>
<td>51</td>
<td>55</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Colposcopy*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN1, warts, inflammation</td>
<td>90</td>
<td>49</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ CIN2</td>
<td>2**</td>
<td>2**</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>301</td>
<td></td>
<td></td>
<td>248</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Colposcopy and cervical biopsy were performed when the cytology was abnormal.
** Two cases in HPV DNA positive group and two cases of CIN2 in HPV DNA negative group were diagnosed during follow-up, and all the four cases became HPV DNA positive at the end of follow-up. One case was histologically diagnosed with VaIN2.

CIN2 or higher-grade lesions were cytologically followed-up after six and 12 months and recommended for further HPV detection after 12 months. Patients with recurrent abnormal cytology were subjected to colposcopy-directed cervical biopsy. Follow-up ended when the patient completed two consecutive cytology tests, diagnosed with CIN2 or greater lesions in repeated colposcopy-directed cervical biopsy, or received cone biopsy. This study used the Bethesda System (TBS) 2001 criteria in cervical cytology diagnosis. Liquid-based tests such as SurePath and TriPath (BD Diagnostics) were used in cervical cytology; HR-HPV was detected by hybrid capture 2 high-risk HPV DNA Test system (HC-II, Digene Corporation).

Data were analyzed with SPSS12.0 software, and significant differences were determined when p < 0.05 in χ² test.
abnormal cytology, is particularly important in reducing the future incidence of cervical cancer. This study has for the first time shown that among patients who were not diagnosed with CIN2 or greater diseases by colposcopy in the initial examination, the chance to be diagnosed with CIN2 or greater diseases during one year follow-up was 1%. Facing such a minor rate of abnormality, comprehensive colposcopy examination becomes important, especially when the rate of loss to follow-up is high.

Based on recent literatures, the diagnosis rate of CIN2 and higher-grade lesions in ASC-H patients is 36% - 88%, higher than that in ASC-US patients, but lower than that in HSIL patients; therefore, the treatment principle of ASC-H patients also differs from that of the latter two [14-16]. The American Society for Colposcopy and Cervical Pathology (ASCCP) 2006 guidelines recommend colposcopy and multiple biopsies for those cytologically diagnosed ASC-H patients and closer follow-up for patients who are first-time diagnosed with CIN2 or higher-grade lesions but without histological confirmations. In this study, the ratio of first-time diagnosis of CIN2 or higher-grade lesions was 46.5%, but there were no CIN2/3 cases detected during follow-up. This is consistent with the existing reports and may be related to the less number of patients and shorter period of follow-up. All cervical lesions in this study were diagnosed by using this method during the first examination, which suggests that in combination with endocervical curettage, colposcopy is the most effective way for detecting CIN2 or higher-grade lesions.

Using HPV in the screening of cervical lesions has also experienced a process of gradual acceptance, and initially the methods have been recommended only for “those who know their usefulness” [17].

During the continuous investigation of ASC-US, further specification of the morphology has encountered difficulties. Some studies, which are attempting to further define the ASC-US, have shown that the cellular changes are highly subjective; even professional pathologists face the same problem of poor reproducibility [18-21]. For the difficulties in dealing with the large number of ASC-US patients, HR-HPV testing plays a critical triage role for further diagnosis [22, 23]. The relationship between persistent HR-HPV infection and the incidence of cervical cancer have been well-recognized since 1990 and HPV testing has also begun to be used in the screening of cervical cancer and precancerous lesions and in post-treatment follow-up [19]. ALTS and other studies show that triage through detection of HPV DNA is objective, economic, and can almost immediately detect CIN2 or higher-grade lesions; the rate of accurate negative predictions achieves 98%-100% [24]. This study has also shown that the rate of accurate HPV negative predictions in first-time colposcopy was 99%. During follow-up, no CIN2/3 patients had been detected in HPV negative patients. For cytologically-diagnosed ASC-H patients, physicians should pay more attention to the first comprehensive examination; in addition to colposcopy-directed multiple biopsies, HPV testing has a high value for negative prediction of the disease.

Due to the detection of CIN2 or greater diseases during follow-up, more attention should be paid to patient management after the primary diagnosis [25]. Unfortunately, because the high-rate of patient loss to follow-up is a common problem, appropriate manpower and resources are needed for reducing patient loss during follow-up, and the individualized patient treatment should be performed during the tracking process.

After effective training, even in developing countries such as China, the diagnostic quality in some areas can approach the level of that in developed countries, where new cases have been reduced by 90% because of effective implementation of cervical cancer screening. However, the follow-up strategy in developed countries is difficult to implement by developing countries due to the lack of medical resources [26]. To improve the follow-up rate, various efforts may be required, such as to educate patients with medical knowledge, effective follow-up from the hospital, and financial support from the country to relieve patients’ medical and economic burdens.

This study has shown that during one-year follow-up after initial colposcopy, in both ASC-US and ASC-H groups, the detection rate of CIN2 and greater lesions was very low. Although ASCCP has preferred HPV DNA triage management for ASC-US patient, due to the high-rate of loss to follow-up in developing countries, the authors recommend that once diagnosed with abnormal cytology, the patient needs to undergo an immediate colposcopy with cervical biopsy, which will timely and effectively detect cervical cancer, as well as precancerous lesions.

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The value of mesothelin in the diagnosis and follow-up of surgically treated ovarian cancer

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Introduction

Ovarian cancer is a severe disease that threatens the health and lives of many women; in the United States, for example, the incidence and mortality rates of ovarian cancer are ranked fifth and fourth among cancers, respectively [1]. The five-year survival rate after a diagnosis of ovarian cancer is about 30%, but 85% or more of those who survive > five years are diagnosed with Stage I ovarian cancer [2]. To increase the survival rate and quality of life of women with ovarian cancer, it is necessary to improve the rate at which early-stage ovarian cancer is diagnosed.

In recent decades, some serum biomarkers such as CA 125, HE4, CA12-4, CA15-3, glycolidem, MMP7, SLP1, Plau-R, and Muc-1 have been studied in the diagnosis of ovarian cancer. Of these, CA 125 is the most extensively-examined predictive marker [3-5], but it is elevated only in about 50%-60% of patients with early-stage ovarian cancer. Furthermore, it has a low specificity [6], and its positive predictive value is < 10% as a single marker; the addition of ultrasound screening to measurements of CA 125 improves the positive predictive value to approximately 20% [7]. HE4 is effective for ovarian cancer detection [8, 9] and has received approval from the US Food and Drug Administration (FDA) as a recurrence-monitoring marker. Limited information suggests that rising HE4 could detect a recurrence earlier than CA 125 [9, 10].

Because of the limited sensitivities and specificities constraining the use of CA 125, HE4, and other biomarkers, new technologies for the detection of early-stage ovarian cancer are needed. One possible candidate is mesothelin, a plasma membrane differentiation antigen that is strongly-expressed in mesothelial cells and has been suggested as a marker for ovarian cancer diagnosis [11, 12] or remission monitoring [9]. The goal of this study was to evaluate if mesothelin is independently effective in monitoring disease diagnosis and remission.

Materials and Methods

Subjects

The Ethics Committee of the Shandong University Qilu Hospital approved the research protocol. Informed consent was obtained from each of the patients and control participants. A total of 126 women were hospitalized for an “ovarian tumor” from January 2011 to March 2012 who intended to undergo surgical intervention were randomly selected as study subjects. Of these, 49 women were diagnosed with ovarian cancer, 64 were diagnosed with a benign ovarian tumor, and 13 women were diagnosed with other diseases.

Women with ovarian cancer and benign ovarian tumors were excluded if they had received hormone therapy or chemotherapy or their condition occurred in combination with other malignancies. After screening, the ovarian cancer group included 42 patients: eight (19%) FIGO Stage I cases, 12 (29%) FIGO Stage II cases, 15 (36%) FIGO Stage III cases, and seven (17%) FIGO Stage IV cases. All participants were Asian Chinese. The cancers had different histological types, as follows: serous papillary carcinoma (n = 29), endometrioid carcinoma (n = 2), mucinous carcinoma (n = 4), clear cell carcinoma (n = 6), and mixed cystadenocarcinoma (n = 1). Another 48 women with benign ovarian tumors were recruited for the benign ovarian tumor group. These patients also had different histological types, as follows: serous cystadenocarcinoma (n = 18), mucinous cystadenocarcinoma (n = 9), mixed cystadenocarcinoma (n = 2), and simple ovarian cysts.

Summary

Objective: To assess the value of serum mesothelin concentration for diagnosis of ovarian cancer and for monitoring the therapeutic effect of surgical treatment. Materials and Methods: The study consisted of 42 patients with ovarian cancer undergoing surgery, 48 with benign ovarian tumors, and 49 healthy controls. Blood was drawn pre-operatively and one month post-operatively to test serum mesothelin levels. Results: Mesothelin values were higher in the ovarian cancer group compared to controls and higher pre-operatively vs post-operatively in the ovarian cancer group. For the diagnosis of ovarian cancer, the positive predictive value of serum mesothelin was 80.5%, the negative predictive value was 81.6%, sensitivity was 78.6%, and specificity was 83.3%. Conclusion: Serum mesothelin is increased in ovarian cancer, has high-specificity, and can be used in the pre-operative diagnostic evaluation for ovarian cancer.

Key words: Mesothelin; Ovarian cancer.
The value of mesothelin: a diagnostic marker in ovarian cancer

Table 1. — Demographics and clinical characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Ovarian cancer Stage I (n = 20)</th>
<th>Ovarian cancer Stage III (n = 15)</th>
<th>Ovarian cancer Stage IV (n = 7)</th>
<th>Normal (n = 49)</th>
<th>Ovarian benign tumor (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.1 (2.8)</td>
<td>57.7 (2.4)</td>
<td>58.9 (1.4)</td>
<td>53.2 (2.6)</td>
<td>51.9 (3.1)</td>
</tr>
<tr>
<td>Range</td>
<td>47-59</td>
<td>52-61</td>
<td>55-62</td>
<td>46-58</td>
<td>44-60</td>
</tr>
<tr>
<td>Age distribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 55</td>
<td>11 (55%)</td>
<td>5 (33%)</td>
<td>1 (14%)</td>
<td>30 (61%)</td>
<td>40 (83%)</td>
</tr>
<tr>
<td>&gt; 55</td>
<td>9 (45%)</td>
<td>10 (67%)</td>
<td>6 (86%)</td>
<td>19 (39%)</td>
<td>8 (17%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>15 (35.7%)</td>
<td>10 (23.8%)</td>
<td>4 (9.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
<td>3 (7.1%)</td>
<td>1 (2.4%)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>0</td>
<td>4 (9.5%)</td>
<td>2 (4.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>2 (4.8%)</td>
<td>1 (2.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed cystadenocarcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(n = 19). Finally, 49 healthy, age-matched individuals who had undergone medical examinations in this hospital were recruited for the healthy control group. Table 1 gives the demographics and clinical characteristics of the study population. Diagnoses of ovarian cancer and ovarian benign tumors were made by pathologists after surgery.

Serum samples

Blood samples from patients were collected before surgical intervention and at one month after surgery. Blood was collected in a clotting tube, and within four hours of collection, clotted blood was centrifuged at 2,000 × g for ten minutes, then serum was aliquoted and stored at −80°C until assayed.

Determination of mesothelin

Soluble mesothelin concentrations were determined in duplicate following the manufacturer’s instructions using a double determinant ELISA assay. Mesothelin concentrations were determined from a standard curve performed on each plate and expressed as nM. Dilution of samples was carried out if necessary using the diluent supplied by the manufacturer. All assays were performed on coded samples by technical staff unaware of each patient’s diagnosis. A serum mesothelin value greater than or equal to 2.5 nM was considered to be positive [13, 14].

Statistical analysis

Laboratory measurements of mesothelin were analyzed and are presented as means ± standard deviation. Comparisons between groups were performed using one-way ANOVA. Comparisons between the ovarian cancer pre-operative values and ovarian cancer post-operative values were made using paired t-tests. Homogeneity of sample variances was assessed using the homogeneity test of variances. All statistical analyses were performed using GraphPad Prism 3.0. A p value of <0.05 was considered statistically significant.

Results

Comparison of serum mesothelin among different groups

Serum mesothelin was significantly elevated in malignant cases (3.91 ± 1.08 nM) compared to healthy controls (0.43 ± 0.35 nM), and in malignant compared to benign cases (0.99 ± 0.52 nM) (p < 0.05). In addition, post-operative mesothelin values (2.82 ± 0.64 nM) were significantly lower than pre-operative values (3.91 ± 1.08 nM) in the ovarian cancer group (p < 0.05).

Analysis of the diagnosis value of serum mesothelin

The positive-negative cutoff for serum mesothelin was 2.5 nM; any value greater than or equal to 2.5 nM was considered positive. In the benign tumor group, eight of 48 patients were mesothelin-positive; in the ovarian cancer group, however, 33 of 42 met or exceeded the cutoff value. The positive predictive value of serum mesothelin was 80.5%, the negative predictive value was 81.6%, sensitivity was 78.6%, and specificity was 83.3%.

Discussion

Here the authors investigated whether a newly-discovered cell-surface glycoprotein, mesothelin, can be independently effective in monitoring disease diagnosis and remission in ovarian cancer. Pre-operative serum mesothelin values for the ovarian cancer group were significantly higher than in healthy controls while post-operative serum mesothelin was significantly higher in the ovarian cancer group than in healthy controls. Values for the benign tumor group did not differ from those of healthy controls. For the diagnosis of ovarian cancer, the positive predictive value of serum mesothelin was 80.5%, the negative predictive value 81.6%, the sensitivity 78.6%, and the specificity 83.3%. The results indicate that serum mesothelin is increased in ovarian cancer and can be used in diagnostic evaluation.

In the re-examination at one month post-operatively for ovarian cancer patients, serum mesothelin was significantly lower than pre-operative values in the cancer patient group. The result indicates that serum mesothelin has a significant value in the surveillance of surgical therapeutic effect. Because of the limited number of cases and short follow-up, further study is ongoing regarding the use of this marker as an early warning in relapsed patients.

In summary, serum mesothelin measurement has a significant value in diagnostic distinction of epithelial ovarian benign and malignant tumors, and dynamic surveillance of serum values is a convenient way to monitor the surgical effect.

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How to prevent the iatrogenic diffusion of gynecological malignant tumors?

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Summary
An analysis of the causes for iatrogenic diffusion of gynecological malignant tumors during their diagnosis and treatment, through clinical experience and a comprehensive review of both Chinese and international literatures was carried out. When a curettage, hysteroscopy or laparoscopy is performed, an iatrogenic diffusion of malignant tumors can be caused. Therefore this phenomenon needs to be prevented and reduced during the diagnosis and treatment of gynecological malignant tumors, and to improve the curative effect and survival times of tumor patients.

Key words: Gynecological tumors; Iatrogenic diffusion; Prevention.

Introduction
The iatrogenic diffusion of tumors refers to improper or inaccurate treatment at some point during the diagnosis and treatment of malignant tumors [1]. As early as the 19th century, some reports state that surgery might promote the diffusion of cancer cells. Nowadays, the incidence of gynecological malignant tumors increases year after year and the metastasis and diffusion of them is one of the main causes of death in tumor patients. The attempt to analyze the causes for possible iatrogenic diffusion during the diagnosis and treatment of gynecological tumor patients formulates the preventive measures so as to reduce the diffusion itself, enhance the survival rate among patients, improve the prognosis, and reduce the death rate as much as possible.

Iatrogenic diffusion in diagnosis of gynecological tumors

Diagnostic curettage. Although diagnostic curettage is the routine diagnostic method to diagnose vaginal bleeding, it may promote the diffusion of cancer cells of patients with endometrial cancer [2]. This is mainly due to the scratching of the tissues during curettage that may cause metastasis through lymphatic vessels or blood vessels for the pressure exerted on the muscular layer; the larger the surface area is, the more possible the diffusion is [3]. If it is necessary to expand the neck of uterus because the orifice of uterus is too tight, cervical injury is most likely, causing the diffusion of tumor cells. Therefore, avoiding uterine neck expansion as much as possible, the curettage force will be adequate, and no blind or invasive curettage will occur; when the sample amount is sufficient or the scraped matter is suspicious, surgery should be stopped in order to avoid repeated curettage of the tumor area [4]. Targeted curettage may be carried out under ultrasound guidance. Before curettage, the surgeon must be aware of the thickness of the endometrioid mucosa with suspicion of cancer. In the case of fractional curettage, the cervical mucosa may be scraped before the endocavitary mucosa in order to diagnose the site of the tumor.

Hysteroscopy
At present, hysteroscopy has been widely utilized in the etiologic diagnosis for irregular vaginal bleeding, intrauterine abnormal echo, sterility, etc. Its main advantages include: direct viewing of changes of the uterine cavity and well-targeted indication of biopsy; however, as to whether the patients with endometrial cancer should be examined with hysteroscopy, there is much dispute at present, and the main focus is whether hysteroscopy promotes the diffusion of cancer cells or not. Since hysteroscopy requires medium distention, and when the distention pressure exceeds a certain range [5], the endometrial cancer cells may enter the abdominal cavity through uterine tube together with distention medium. Some researchers find that: after carrying out hysteroscopy on endometrial cancer, a collection of the abdominal lavage cell examination results in a positive rate for cancer in 9.7%, while after examining the abdominal cancer cells of patients with clinical phase I endometrial cancer, but without conducting hysteroscopy, the positive rate is 12.7%, and the abdominal cytologic positive rate of operation phase I is 7.3% [6]; there is no statistical significance in their comparisons. However, Zerbe conducted a retrospective research on 222 cases with endometrial cancer; among them, 64 cases had carried out hysteroscopy before surgery and 158 cases in the control group had not. According to the results of cytologic examination of abdominal lavage in the hysteroscopy group, the positive patients account for 17.2%, higher than 6.3% of the control group, and there is sig-
The current literature defining the role of laparoscopy in tumors or ovarian cancer remains controversial [15, 16]. Iatrogenic diffusion during the management of women with borderline ovarian and there was no statistical difference. Laparoscopy in surgeries of all patients, in comparison to the laparotomy no tumor cells found in the abdominal lavage, CO2 fil-

MMP-2, VEGF proteins; he discovered that there were also detected the E-cadherin, sion of full uterus, plus pelvic lymphadenectomy, and

or endometrial cancer conducting the laparoscopy, exci-
mation lavage obtained from patients with cervical cancer and isolated PSMs were rare. Fu Chun, 0.97%, often connected to the wide abdominal diffusion and tissues around the port site may reduce the implantation of tumor cells. (3) during surgery, special attention should be paid to the following: shorten the CO2 pneumoperitoneum duration as much as possible; avoid repeated punctures to reduce the tissue injuries; reduce the times of instruments entering into and out of the sleeve holes during surgery as much as possible; maintain the CO2 pneumoperitoneum pressure stable during surgery and use heated and wetted CO2 to reduce the aerosolized state of tumor cells; place the uterine manipulator under laparoscopy to avoid piercing through the uterus; before surgery of endometrial cancer, clamp the gorse section of uterine tube with Ti clamps to avoid the diffusion of tumor cells while cutting off the tumor tissues; avoid breakage or direct operations on the tumor; before removing the cut tissue, place it into the sample bag to avoid polluting the wounds; while completing surgery, exhaust the gas inside the abdominal cavity, and then pull out the sleeve to avoid the chimney effect, causing diffusion of tumor cells after the laparoscopic surgery; close the peritoneum, fascia, and skin layers of the wound carefully and completely; cutting off the tissues around the port site may reduce the implantation and metastasis at the puncture locations; rinsing of wound surfaces and puncture holes at the pelvic and abdominal cavity with the diluents of drugs such as taur-
olidine, povodine iodine, fluorouracil, amethopterin, etc., may reduce the peritoneal diffusion and incisional implantation and metastasis; (4) if indicated, selecting gasless laparoscopy may reduce the incidence of implantation and metastasis [18, 19].

Laparoscopy

The rapid development of minimally-invasive surgery, as laparoscopy, is very useful for surgical-pathological staging and surgical treatment of the gynecological malignant tumors. On the other hand, there is the risk of celiac tumor diffusion, port site metastasis (PSM), etc [11]. Some researchers think that the frequent changes of devices during the laparoscopic surgery increases the incidence rate of implantation in the area of entry. Other researchers think that performing pneumoperitoneum may cause a large amount of tumor cells to fall-off with gas from the sleeve side and cause PSM. Although laparoscopic surgery of malignant gynecological tumors has been carried out for over 20 years [12], its advantages and disadvantages in the therapy of malignant tumors are still under exploration and disputed. According to a retrospective analysis of 2,593 patients with gynecological malignant tumors conducting laparoscopic surgery in 1991-2003 by Nadeem, the incidence rate of PSM was 0.97%, often connected to the wide abdominal diffusion and isolated PSMs were rare. Fu Chun, et al. [13, 14] carried out the tumor cytological examination on the abdominal lavage, CO2 filtered liquid, surgical instrument lavage obtained from patients with cervical cancer or endometrial cancer conducting the laparoscopy, excisi-

cion of full uterus, plus pelvic lymphadenectomy, and also detected the E-cadherin, β-catenin, P-selectin, MMP-2, VEGF proteins; he discovered that there were no tumor cells found in the abdominal lavage, CO2 filtered liquid, surgical instrument lavage before and after surgeries of all patients, in comparison to the laparotomy and there was no statistical difference. Laparoscopy in the management of women with borderline ovarian tumors or ovarian cancer remains controversial [15, 16]. The current literature defining the role of laparoscopy in the diagnosis and treatment of ovarian cancer is limited to case reports, case series, and cohort studies Liu et al. reported equal efficacy of laparoscopy compared with laparotomy in both early and advanced-stage ovarian cancer. Fauvet reported that laparoscopic management of borderline ovarian tumors is associated with a higher rate of cyst rupture and incomplete staging [17]. Although there is proof that laparoscopy can change the biological behaviors of gynecological malignant tumors, there are no consistent results about the diffusion, PSM incidence, and mechanism after the laparoscopic surgery of malignant tumors; however, the following points must be recommended: (1) perfect the normalized preoperative examination and routine diagnostic programs, and grasp the indication for laparoscopy of gynecological malignant tumors; (2) surgeries should be performed by skilled doctors; (3) during surgery, special attention should be paid to the following: shorten the CO2 pneumoperitoneum duration as much as possible; avoid repeated punctures to reduce the tissue injuries; reduce the times of instruments entering into and out of the sleeve holes during surgery as much as possible; maintain the CO2 pneumoperitoneum pressure stable during surgery and use heated and wetted CO2 to reduce the aerosolized state of tumor cells; place the uterine manipulator under laparoscopy to avoid piercing through the uterus; before surgery of endometrial cancer, clamp the gorse section of uterine tube with Ti clamps to avoid the diffusion of tumor cells while cutting off the tumor tissues; avoid breakage or direct operations on the tumor; before removing the cut tissue, place it into the sample bag to avoid polluting the wounds; while completing surgery, exhaust the gas inside the abdominal cavity, and then pull out the sleeve to avoid the chimney effect, causing diffusion of tumor cells after the laparoscopic surgery; close the peritoneum, fascia, and skin layers of the wound carefully and completely; cutting off the tissues around the port site may reduce the implantation and metastasis at the puncture locations; rinsing of wound surfaces and puncture holes at the pelvic and abdominal cavity with the diluents of drugs such as taur-
olidine, povodine iodine, fluorouracil, amethopterin, etc., may reduce the peritoneal diffusion and incisional implantation and metastasis; (4) if indicated, selecting gasless laparoscopy may reduce the incidence of implantation and metastasis [18, 19].

Laparotomy

The scalpel is a kind of double-edged sword while the doctors resect tumors boldly and resolutely. Carelessness or inaccuracies during surgery often increase the iatrogenic diffusion of tumor cells. As early as 1954, researchers presented the notion regarding tumor-free operation technique during surgery. The so-called tumor-free surgical technique refers to a series of measures taken during surgery of malignant tumors in order to reduce or prevent the fall-off, implantation, and spread of tumor cells. Its purpose is to prevent the metastasis and

Iatrogenic diffusion during gynecological tumor therapy

Iatrogenic diffusion during gynecological tumor therapy may cause the implantation of tumor cells at the cutting location, as well as the diffusion and implantation of tumor cells in the pelvic and abdominal cavity; this may occur during laparotomy and laparoscopy.
diffusion of tumor cells through the blood or lymph, as well as prevent the abdominal spreading and implantation of tumor cells. Nowadays, a large amount of local and international research has proved that the tumor-free surgical technique can effectively reduce local relapse and remote metastasis of tumor after radical surgery, improving patient prognosis and extending the patient’s tumor-free survival time [20].

Tumor metastasis due to laparotomy, mainly occurs in the following cases: (1) during surgery of malignant ovarian tumors, in some benign tumors (such as ovarian mucinous cystadenoma) and borderline tumors, if the tumor size is big or heavily adhered to surrounding tissues and with unclear border, during dissection or its removal [21], the liquid inside the tumor may leak, causing the tumor cell to implant in the incisional and surgical fields, i.e., the tumor cell implantation in the incision and abdominal cavity occurs; (2) for tumor patients with ascites, during surgery, if there are no incision protective measures, too many ascites, untimely treatment, or improper treatment, incision implantation may occur postoperatively; (3) while performing radical surgery of endometrial or cervical cancers, the cancer cells may fall off in the vagina, causing vaginal implantation and relapse [22]; (4) less invasive treatment concept prompts many doctors to select small incisions during surgery; however, while performing the surgery of malignant tumors, small incisions suffer multiple defects, such as insufficient exposure of surgical field, difficult removal the tumor, and celiac implantation caused by tumor breakage during removal [23].

For these reasons, the tumor-free technique during surgery should be adopted: (1) for malignant tumors, small incisions should be limited as much as possible, and the surgical field should be sufficiently exposed to reduce the stimulation and pulling towards tumors, as well as facilitate the handling of fortuitous events such as bleeding during surgery, etc; (2) suturing the peritoneum protective tissue to the peritoneum on both sides, and then securely suturing the peritoneum protective tissue to the upper and lower corners of incision, to protect the peritoneum and incision; (3) during surgery, the tumor should be detected from far to near, it should be gently treated and not squeezed, and special attention should be paid when adhesion is extensive. The touching and squeezing of tumor will increase the fall-off phenomenon and implantation of cancer cells into the abdominal cavity [24]; (4) avoid re-using polluted instruments by tumor as much as possible; if the surgical instruments cannot be changed, they should be dipped into distilled water for five min before use; some reports say that after being dipped with normal saline solution, the tumor cells can still maintain certain activities; (5) for tumor patients with a large amount of ascites, a small incision should be opened on the peritoneum, the liquid should be aspirated in order to maintain the same as dry as possible by patiently absorbing the peritoneum as much as possible, and then carry out surgery to avoid in this way the overflow of the peritoneum and tumor cell implantation; (6) during tumor excision, protect the serosal surface of tumor as much as possible; if the tumor has invaded the serosal layer, apply sealant glue for cancer serosal layer on the serosal surface, or dress and separate with gauze or surgical towel [25]; (7) before resecting off the tumor, treat the tumor blood vessels first, to reduce the probability of spreading of tumor cells along with blood; (8) while carrying out endometrial cancer surgery, it is advisable to perform the eight-shaped suture of the orifice of uterus or fill gauze into the fornix of vagina first, then clamp the side of uterus, and then detect the pelvic cavity and touch the uterus, to avoid the fall-off of tumor cells, causing vaginal and pelvic implantations and metastasis along the vessel; (9) during surgery, use an electric scalpel as much as possible, since it does not only reduce bleeding, but can also cauterize small lymphatic vessels or blood vessels, reducing the probability of cancer cells entering the vessel system [26]; (10) surgical toilette after resecting the tumor plays an important role in the prevention of infection and residue of tumor cells, as well as in the prevention of tumor cell implantation and spreading. During surgical toilette of the various gaps of wound surface, wait for 3-5min before absorbing the lavage; carry out the rinsing twice or thrice; absorb the liquid completely with absorber, and do not use gauze to wipe and absorb, in order to avoid tumor cell implantation [27, 28]. Before closing the abdominal cavity, both gloves and instruments should be substituted; after closing the abdominal cavity, dip the incision with distilled water or rinse it with normal saline solution repeatedly, to avoid incisional implantation of tumor cells.

Whether the iatrogenic diffusion during diagnosis and treatment of gynecological tumors can be reduced, as well as the curative effect and survival time of tumor patients improved, are dependent to a considerable degree on the specialist’s sense of responsibility, knowledge, surgical normalization, and skill [29]. The tumor-free operation is the fundamental principle the authors advocate and it should be followed during the whole process of diagnosis and treatment. Iatrogenic tumor diffusion caused by abnormal acts of medical treatment is contrary to the ethical service directed to the patient, and the recognition and regard of this issue should be considered.

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The regulation network and network motif analysis in ovarian cancer

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Summary

**Objective:** Several gene alterations have been identified associated with ovarian cancer (OC) development. However, how these genetic elements are coordinated in transcription network during OC initiation and progression is poorly understood. Thus, the objective of this study was to interpret the transcription regulation network of OC. **Materials and Methods:** The GSE14407 microarray data was used for analysis of the transcription regulation network of OC. **Results:** The results showed that the TP53 (tumor protein p53) was the most crucial transcription factor in the transcriptome network. P53 could down-regulate CDC14A (CDC14 cell division cycle 14 homolog A [S. cerevisiae]) and FAS (TNF receptor superfamily, member 6) expression, but up-regulate SFN (stratifin)

**Conclusion:** This transcriptional regulation may provide a better understanding of molecular mechanism and some potential therapeutic targets in the treatment of OC.

**Key words:** Regulation network; Network motif analysis; Ovarian cancer.

Introduction

Ovarian cancer (OC) is the leading cause of death from gynecologic cancer for women worldwide. OC comprises of four main histological subtypes. 1) serous cystadenocarcinoma, 2) mucinous, 3) endometrioid, and 4) clear cell [1]. The majority of patients are diagnosed with advanced disease, which is cured with surgery and postoperative chemotherapy [2]. However, some patients exhibit resistance to chemotherapy, resulting in an overall five-year survival of 10%-30% [3].

A number of gene alterations have been identified and frequently encountered during ovarian tumorigenesis, including KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog), BRAF (v-raf murine sarcoma viral oncogene homolog B1), TP53 (tumor protein p53), RB (retinoblastoma), PTEN (phosphatase and tensin homolog) [4], MYC (v-myb myelocytomatosis viral oncogene homolog avian) [5], E2F-1 (E2F transcription factor 1), EGFR (epidermal growth factor receptor), etc. Approximately 60%-70% of low-grade serous carcinomas carry KRAS or BRAF genes mutations [6], but deregulation of the tumor-suppressing pathways p53 and BRCA1/2 is more common in high-grade serous tumors [7]. Expression level of E2F-1 is elevated in all the OC cell lines studied when compared with control cells. High expression of E2F-1 is found to be associated with histopathologic grade 3 tumors and residual tumor over two cm in diameter in OC patients [8, 9]. EGFR is reported over-expression in the majority of OC [10] and has been implicated in both the growth and progression of this disease. Targeting the EGF receptor via antisense transfection or tyrosine kinase inhibitor in OC reduces the expression of EGFR and suppresses OC cell to grow and responsiveness to exogenous EGF [11, 12]. EGF activated in vivo binding of E2F1 to the B-Myb promoter and subsequent activation of B-Myb gene expression [13], whose expression is also involved in OC [14, 15]; however, relatively little is known about how these genetic elements are coordinated in transcription network during OC initiation and progression.

Therefore, the objective of this study was to identify potential transcription regulation relationships between transcription factors and differentially expressed target genes in OC by using the microarray data and transcriptional network analysis. Network motif was used to represent the different interaction type. Moreover, the authors characterized their underlying molecular mechanisms by KEGG pathway enrichment analysis.

Materials and Methods

**Data source - affymetrix microarray data**

The transcription profile of GSE14407 was obtained from NCBI GEO database (http://www.ncbi.nlm.nih.gov/geo/) which is based on the Affymetrix Human Genome U133 Plus 2.0 Array. A total of 24 chips, purchased from Cancer Development and Evolution in Georgia Institute of Technology, were used for this analysis. Twelve healthy ovarian surface epithelia samples (OSE) were compared to twelve laser-captured microdissected serous OC epithelia samples (CEPI) via Affymetrix 3’ expression array.

**Regulation data**

A total of 774 pairs of regulatory relationship between 219 transcription factors (TFs) and 265 target genes were collected from TRANSFAC [16] (http://www.gene-regulation.com/pub/ databases.html). A total of 5,722 pairs of regulatory relationships between 102 TFs and 2,920 target genes were collected from TRED [17] (http://rulai.cshl.edu/TRED/). By integrating the

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above two regulation datasets, a total of 6,328 regulatory relationships between 276 TFs and 3,002 target genes were ultimately obtained.

**Differentially-expressed genes (DEGs) analysis**

For the GSE14407 dataset, the limma method [18] was used to identify DEGs. The original expression datasets from all conditions were extracted into expression estimates and then to construct the linear model. The DEGs only with a fold change value larger than 2 and p value less than 0.05 were selected.

**Co-expression analysis**

For demonstrating the potential regulatory relationship, the Pearson correlation coefficient (PCC) [19] was calculated for all pair-wise comparisons of gene-expression values between TFs and the DEGs. The regulatory relationships whose absolute PCC were larger than 0.75 were considered significant.

**Regulation network construction**

Using the regulation data that have been collected from TRANSFAC [16] database and TRED [17] database, the authors matched the relationships between differentially-expressed TFs and their target genes.

Based on the above two regulation datasets, the significant relationships (PCC > 0.6 or PCC < -0.6) between TFs and their target genes, the authors constructed the regulation networks by Cytoscape [20].

**Network motif**

Fanmod [21] is a tool for finding so-called networks motifs in a network, that is, it locates small vertex-induced subgraphs that occur significantly more often than in random networks.

Enumeration algorithm was applied to search for five sizes of subgraphs, which were found more than five times, |Z-Score| ≥ 5, and p value ≤ 0.05. The Z-Score is the original frequency minus the random frequency divided by the standard deviation. The higher the Z-Score, the more significant the motif is. The p value of a motif is the number of random networks in which it occurred more often than in the original network, divided by the total number of random networks. Therefore, p values range from 0 to 1; the smaller the p value, the more significant the motif is.

**Pathway analysis**

DAVID [22], a high-throughput and integrated data-mining environment, analyzes gene lists derived from high-throughput genomic experiments. DAVID was used to identify KEGG pathway analysis.

**Results**

**Microarray data analysis**

Publicly-available microarray data set GSE14407 was obtained from GEO. A total of 10,836 DEGs with the fold change value > 2 and p value < 0.05 were selected using the limma method. All of these genes are positive expression genes.

**Regulation network analysis**

To obtain regulation network, based on the significant relationships (PCC > 0.6 or PCC < -0.6) between TFs and their target genes, 160 expression relationships including 44 TFs and 138 target genes were selected. By integrating expression relationships above, a regulation network was built between TFs and their target genes (Figure 1). TP53 (tumor protein p53), E2F1 (E2F transcription factor 1), NFKB (nuclear factor of kappa light polypeptide gene enhancer in B-cells 1), MYC (v-myc myelocytomatosis viral oncogene homolog [avian]), and SPI1 (spleen focus forming virus [SFFV] proviral integration oncogene spi1) were hub nodes in the transcriptional network. Among them, TP53 had the most interaction regulation relationships with its target genes, such as p53 could down-regulate CDC14A (CDC14 cell division cycle 14 homolog A [S. cerevisiae]) and FAS (TNF receptor superfamily, member 6) expression, but up-regulate SFN (stratifin) and THBS1 (thrombospondin 1) expression.

**Network motif analysis**

To search for five sizes of subgraphs, which were found more than five times, |Z-Score| ≥ 5, p value ≤ 0.05 between TFs and their target genes, 175 expression relationships including 42 TFs and 114 target genes were selected. Network motif subgraphs were built between TFs and their target genes (Figure 2). Figure 2 lists the top five motif subgraphs. From these results, the authors also suggest the more important role of TP53 in regulation network.

**Function analysis of the network**

Using the KEGG pathways to describe the function of the regulation network, several KEGG pathways were enriched among these pathways in the regulation network, including pathways in cancer (hsa05200), acute myeloid leukemia (hsa05221), small cell lung cancer (hsa05222), cell cycle (hsa04110), p53 signaling pathway (hsa04115), and apoptosis (hsa04210). Table 1 only lists the top ten enriched KEGG pathways.

<table>
<thead>
<tr>
<th>Term Description</th>
<th>Count</th>
<th>p value FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa05200 Pathways in cancer</td>
<td>32</td>
<td>8.23E-12 9.57E-09</td>
</tr>
<tr>
<td>hsa05221 Acute myeloid leukemia</td>
<td>12</td>
<td>7.12E-08 8.28E-05</td>
</tr>
<tr>
<td>hsa05222 Small cell lung cancer</td>
<td>12</td>
<td>3.46E-06 0.004024</td>
</tr>
<tr>
<td>hsa04110 Cell cycle</td>
<td>13</td>
<td>3.18E-05 0.036994</td>
</tr>
<tr>
<td>hsa05212 Pancreatic cancer</td>
<td>10</td>
<td>4.10E-05 0.047633</td>
</tr>
<tr>
<td>hsa04115 p53 signaling pathway</td>
<td>9</td>
<td>1.72E-04 0.200131</td>
</tr>
<tr>
<td>hsa04210 Apoptosis</td>
<td>10</td>
<td>1.83E-04 0.213119</td>
</tr>
<tr>
<td>hsa05215 Prostate cancer</td>
<td>10</td>
<td>2.19E-04 0.253811</td>
</tr>
<tr>
<td>hsa05218 Melanoma</td>
<td>9</td>
<td>2.34E-04 0.271445</td>
</tr>
<tr>
<td>hsa05220 Chronic myeloid leukemia</td>
<td>9</td>
<td>3.43E-04 0.397652</td>
</tr>
</tbody>
</table>

The smaller the FDR is, the higher the correctness is.
Discussion

In this study, the authors systemically investigated the regulation network of OC between TFs and target genes and their underlying molecular pathways. The authors have shown that the gene TP53 was a crucial TF in the transcriptome network. The gene TP53 that encodes the tumor suppressor protein p53 is amongst the most commonly altered genes in human cancer, including OCs [23]. The loss of tumor-suppressor function of the TP53 protein, subsequent to a mutation in the coding sequence (missense or nonsense mutations) [24, 25], seems to be a common feature in a majority of epithelial OCs. A mutation of p53 in early stage of OCs is associated with a short-term improvement in overall survival and progression-free survival [26, 27]. However, in advanced-stage cancers, p53 mutation seems to confer a more aggressive biology related to metastasis and poor clinical outcome [28]. Missense TP53 mutations have been reported to result in p53 accumulation. Therefore, high-level expression of nuclear p53 protein is detected in malignant or benign epithelial OC [29]. A positive correlation was observed between PAX8 and p53 expression in endometrial carcinomas [30]. TP53 expression could also be regulated by PAX8 in the present study, which is highly expressed in benign and malignant epithelial OC when compared to normal ovarian samples [31, 32].

In response to DNA damage, p53 is imported into the nucleus, binds to target genes, and alters their transcription to involve in cell cycle arrest and apoptosis [33]. In this study, the authors found TP53 could down-regulate CDC14A and FAS expression, but up-regulated SFN and THBS1 expression. These genes were proposedly involved in cell cycle, P53 signaling pathway, and apoptosis pathway based on previous reports.

CDC14A protein is a member of the dual specificity protein tyrosine phosphatase family, which plays pleiotropic roles during the cell cycle, including the initiation of DNA replication [34] and the exit from mitosis [35]. Cdc14A is found differentially-expressed in human tissues with high-protein expression in brain, heart, small intestine, and skeletal muscle, moderate expression in spleen, and low or undetectable expression in kidney, liver, lung, testis, and pancreas. Low expression of Cdc14A occurs in cancer cell lines harboring wild-type p53 [36]. This protein has been proved to interact with, and dephosphorylate tumor suppressor protein p53 at ser315 site, and is thought to regulate the function of p53 in cancer [37]. In this study, the authors predicted that Cdc14A was also of low expression in OCs.

Fas (CD95/APO-1), a type I transmembrane cell surface protein with a molecular weight of 48 kD, is generally regarded as the prototypical cell death receptor of tumor necrosis factor family. It initiates apoptosis following engagement by Fas ligand, FasL, which has been
described as trigger molecules of apoptosis, such as caspase-8, resulting in downstream cell death caspase cascades [38]. Serum soluble Fas levels are significantly increased in women with OC compared with healthy controls. Increased pretreatment serum soluble Fas levels were associated with shortened disease-free and overall survival [39]. Decreased sensitivity to Fas-mediated apoptosis could contribute to ovarian tumorigenesis [40]. There is a p53-responsive element within the first intron of the Fas gene, as well as three putative elements within the promoter [41, 42]. Wild-type p53 binds to and trans-activates the Fas gene, whereas mutant p53 fails to induce apoptosis via activation of the Fas gene [42, 43], this is, down-regulated by mutant p53.

SFN protein, also known as 14-3-3σ, plays a crucial role in the G2 checkpoint by sequestering the mitotic initiation complex, cdc2-cyclin B1, in the cytoplasm after DNA damage [44]. This prevents cdc2-cyclin B1 from entering the nucleus in which the protein complex would normally initiate mitosis. In this manner, 14-3-3σ induces G2 arrest and allows the repair of damaged DNA. Recently, the expression of 14-3-3σ protein has been reported to be frequently methylated and inactivated in ovarian carcinoma tissues [45]. Treatment of ovarian cell with demethylating agent resulted in the demethylation of the promoter CpG islands and restored the expression of 14-3-3σ gene. Decreased 14-3-3σ expression was significantly associated with positive p53 immunoreactivity, that is, an inverse correlation between 14-3-3σ and p53 expression [46].

TSP-1, a 420 kDa extracellular matrix-bound adhesive glycoprotein, is the first protein recognized as an endogenous inhibitor of angiogenesis. Several studies have indicated that TSP-1 has tumor suppressive properties in vivo and overexpression of TSP-1 in human various cancer cells blocks tumor progression. TSP-1 is found to be up-regulated upon THY-1 induction (a tumour suppressor gene) in human OC [47]. TSP-1 mRNA and protein levels are significantly decreased by hepatocyte growth factor (HGF) induction through MAPK signaling pathways, leading to the induction of MMP-9 and subsequent invasion of OC cell [48]. P53 expression is found inversely-correlated with TSP-1 staining in OC cases. The reduction of TSP-1 expression associated with overexpression of p53 may be coupled with the development of a pro-angiogenic environment and malignant phenotype [49].

In conclusion, the present findings shed new light on the regulation network of OC. The authors showed that TP53 TF could play an important role in OC via up-regulating or down-regulating target genes, such as SFN, THBS1, CDC14A, and FAS genes which are all associated with breast cancer progression.

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An analysis of Turkey’s scientific contribution in ovarian cancer research

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Summary

Aim: Bibliometric studies can be used to evaluate the performance of a country in contributing to the accumulation of medical evidences on a specific topic. The authors aimed to evaluate the contribution of Turkey to the scientific repertory in the field of ovarian cancer. Methods and Materials: The authors retrospectively searched papers published in the field of ovarian cancer between 1980 and 2012 by using the Web of Science software. Results: Turkish authors published 400 papers in Science Citation Index (SCI) Expanded indexed journals ranking Turkey 25th globally. Turkey’s first publications in this field appeared at the beginning of the 1990s and showed a distinctive acceleration after 2000. Overall, publications from Turkey were cited 2,987 times and the trend of citations increased in 2000. Most of the papers from Turkey in this field were published in the European Journal of Gynaecological Oncology. Conclusion: The authors observed a significant improvement in the scientific activity of Turkey in the field of ovarian cancer during last decade.

Key words: Bibliometric analysis; Ovarian cancer; Research activities; Turkey.

Introduction

Bibliometric analysis is a method for evaluating the quantitative parameters of a topic or field in scientific literature. However, the results of such analyses should not be considered as solely quantitative because citation parameters and improvement trends may also give clues regarding the qualitative features. Therefore, medical bibliometric studies can be used to evaluate the performance of a country in contributing to the accumulation of medical evidences on a specific topic [1].

In general, if scientists have something important to say, they want to publish it in peer-review journals. Constituting databases facilitates the accessibility of these journals and publications. The Science Citation Index (SCI), which provides a large database for journals and publications, has been used since 1961 as a tool for bibliographic retrieval [2]. The Web of Science is comprised of a software application, which can be used for bibliometric assessment of scientific publications indexed in the SCI Expanded via several parameters and refinements [3].

In this study, the authors aimed to make a quantitative analysis of the scientific publications related to ovarian cancer originating from Turkish institutions and authors, which are published in SCI Expanded covered medical journals during the last three decades. The authors’ main point of interest was to evaluate the amount and trend of this contribution to the literature. To their knowledge, this is the first bibliometric study in English literature, investigating Turkey’s contribution to the field of scientific repertory on ovarian cancer.

Materials and Methods

The authors conducted a bibliometric analyses in June 2012 using the Web of Science (WOS) software to investigate the scientific publications about ovarian cancer. They retrospectively searched papers published in the field of ovarian cancer between 1980 and 2012. The authors evaluated papers that are published in the journals covered by SCI Expanded. The following search entries were used in the search field: “ovarian cancer”, “ovarian neoplasm”, and “cancer of the ovary”. All matched results were first refined in English language. “Analyze” function of the software was then used to investigate the contribution of the countries, distribution of the publications among years, type of the documents, name of the journals, institutions, and the authors. This analysis was also performed for investigating Turkey’s contribution separately. Publications and cited papers from Turkey were also analyzed with respect to the last three decades (between 1980 and 2009).

Results

WOS based search using the defined entries through the database of SCI Expanded from 1980 to the date of the study (June 29, 2012), revealed 41,770 scientific documents. Of these, 40,527 were published in English; further analysis was made in this group of publications. Among 40,527 English publications, 18,346 (45.3%) were from United States, followed by England (8.2%), Italy (7.1%), Japan (6.6%), Germany (6.4%), and Canada (5.8%). All others had a contribution smaller than 5%. Turkey had 400 (1%) publications and ranked 25. Table 1 presents the contribution of the first 20 countries in the field of ovarian cancer.

The international scientific repertory on ovarian cancer was relatively poor in the beginning of the 1980s. In example there were only 58 publications concerning ovarian cancer in 1980. On the other hand, the authors observed an international acceleration with respect to the
publication number after the 1990s. Between 1980 and 1989, there were only 1,458 (3.6% of the total) publications in the field of ovarian cancer, however that number increased in the 1990s and reached 9,094 (22.4%) between 1990 and 1999. Publications from Turkey on the other hand appeared only after 1990. Turkey’s contribution to international repertory of ovarian cancer research seems to speed up after 2000. There were 35 (8.8%) and 267 (66.8%) papers published by Turkish authors between 1990-1999 and 2000-2009, respectively.

Number of publications from all over the world and Turkey are presented in Figure 1.

The types of the documents between 1980 and the date of the study (June 2012), classified by WOS in English SCI-Expanded based literature were as follows: article (n = 30,435, 75.1%), meeting abstract (n = 3,683, 9.1%), review (n = 3,631, 9%), proceedings papers (n = 1,900, 4.7%), and others (n = 878, 2.2%). The types of the documents from Turkey are summarized in Table 2. When the ranking of the authors with respect to the number of publications was evaluated, Markman M. was found to be the first, followed by Berchuck A. and Scambia G. The ranking of the authors worldwide according to the number of their publications is shown in Table 3. Ayhan A. (gynecologic oncologist) with 43 publications ranked first among Turkish authors with respect to the number of scientific papers.

Overall, publications from Turkey were cited 2,987 times until June 2012. The first citation came from 1992. The number of cited papers from Turkey increased dramatically over the last decade. Distribution of the citation number of the publications from Turkey with respect to time is shown in Figure 2.

The top four journals publishing papers of Turkish authors are presented in Figure 3. Between 1980 and June 2012, most of the papers in the field of ovarian cancer from Turkey were published in European Journal of Gynaecological Oncology (n = 54, 13.5%).

The main source of the publications from Turkey was from the universities and their hospitals. There were three main cities in Turkey, which produced these publications: Istanbul, Ankara, and Izmir. The ranking of the Turkish universities with respect to the number of publications is shown in Table 4.

**Discussion**

In this study, the authors have evaluated the contribution of Turkey to the scientific repertory in the field of ovarian cancer. Between 1980 and June 2012, Turkish authors published 400 papers in SCI - Expanded indexed
An analysis of Turkey's scientific contribution in ovarian cancer research

177

Figure 1. — Distribution of the publications in journals included in Science Citation Index Expanded, between 1980 and June 2012 in the field of ovarian cancer. (A) Global publications with respect to years. (B) Global publications with respect to decades. (C) Publications from Turkey with respect to decades.

Figure 2. — Citation report of the publications from Turkey in the field of ovarian cancer.

Figure 3. — The top four medical journals in Science Citation Index Expanded list publishing papers from Turkey in the field of ovarian cancer, between 1980 and June 2012.

journals ranking Turkey 25th globally. Turkey's first publications on this topic began at the beginning of the 1990s and showed a distinctive acceleration after 2000. Overall, publications from Turkey were cited 2,987 times until June 2012 and the trend of citation increased by 2000. Most of the papers from Turkey in this field were published in European Journal of Gynaecological Oncology, which was founded in 1980 as the second gynecologic oncology hyperspecialization journal in the world. The main source of the publications from Turkey was the universities and their hospitals; Istanbul University, Hacettepe University and Ege University, ranked as first three.

Evidence-based practice or evidence-based medical applications is an invaluable approach aiming to evaluate the best available evidence produced by medicine or related medical sciences and to perform the optimal practice by making the best medical decisions. Scientific publication inherently, is one of the most efficient and convenient modalities for disseminating the evidences gained from medicine. Therefore, monitoring the scientific publications is an important way to assess the quality and quantity of the dissemination of medicine-based evidences.

With respect to the number of publications, Turkey ranked 25th globally in the field of ovarian cancer, being inferior to several European countries. Ovarian cancer incidence in Turkey seems to be comparable with that of other European countries [4]. Although, international scientific repertory on ovarian cancer was relatively poor in the beginning of the 1980s, there was an international acceleration with respect to the publication number after the 1990s. Glynn et al. reported a similar increase in activity of publications regarding breast cancer beginning with 1990 [5]. However, publications from Turkey appeared only after 1990. Turkey's contribution to international data on ovarian cancer research seems to accelerate only after 2000. An article in the field of ovarian
cancer from Turkey was first cited in 1992 and citation statistics showed an increasing trend especially after 2000. Ayhan A., one of the pioneers of gynecologic oncology in Turkey, contributed with the greatest amount of publications to this repertory. Chua et al. similar with the present findings, in their bibliometric analysis of surgical oncology research in Australia, reported an increasing trend in the number of publications from 2002 [6]. The most probable explanation of this trend seen in Turkey is that the number of experienced gynecological oncology centers and specified physicians has been increasing rapidly since 1990. In Turkey, gynecologic oncology has just been recently recognized as a subspecialty, and after that the first fellowship programs acknowledged by Ministry of Health began. The authors believe that formal fellowship programs will contribute to further accelerate this increasing trend in Turkey.

European Journal of Gynaecological Oncology ranked first for publishing papers from Turkey in the field of ovarian cancer.

The ultimate goal of scientific research and publication in medicine is to make a clinical impact, which can be called “patient impact factor” [7]. This aim can be achieved by efficient dissemination of medicine-based evidences especially via publication. Bibliometric analysis allows to monitor scientific activity in a specific topic. The present results indicate that there is a positive trend in Turkey for disseminating the findings in the field of ovarian cancer. However, it can be estimated that domestic expenditure of Europe in the field of cancer research has been lagging behind North America during the last decade and may drop back from China in the future [8]. Therefore, when planning the future, these findings should be taken into account and policies aimed at improving resources in cancer research and to support researchers should be considered in a more efficient way.

Conclusion

In conclusion, the authors observed a significant improvement in the scientific activity of Turkey in the field of ovarian cancer during last decade. The authors believe that emerging of acknowledged fellowship programs with hyperspecialization, increasing facilities on cancer research, and encouragement originating from the remarkable achievements of Turkish pioneers of gynecologic oncology will help to further increase Turkey’s contribution in ovarian cancer research.

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Case Reports

Endometrial adenocarcinoma in a young woman

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Summary

Background: Endometrial carcinoma usually occurs in post-menopausal women, but in three to five percent of cases, it affects patients under 40 years of age, who wish to preserve their fertility. Patients with polycystic ovarian syndrome (PCOS) and the features of this syndrome (including obesity, hyperinsulinemia, and hyperandrogenism) are at great risk of developing endometrial carcinoma. Case report: The authors report a case of endometrial adenocarcinoma at Stage I in a 37-year-old obese woman with PCOS who underwent surgical staging. Discussion: In young women with obesity and PCOS, a periodic evaluation of the endometrium and implementation of risk-reducing measures for the development of endometrial cancer are needed.

Key words: Endometrial adenocarcinoma; Atypical endometrial hyperplasia; Obesity; Polycystic ovarian syndrome; Surgery; Conservative treatment.

Introduction

Endometrial carcinoma is the most common cancer among women and the most common gynecological malignancy in Western countries, with an incidence of 15-20 per 100,000 women per year [1, 2]. Although it is primarily a disease of postmenopausal women, 25% of patients are in premenopausal age, with three to five percent 40 years of age or younger [3]. According to the international literature, the majority of cases of endometrial adenocarcinoma in young women were well-differentiated (Grade 1) and at early Stages with a superficial invasion (Stage I) [4]. Young patients with endometrial carcinoma tend to have a history of estrogen use or hormone-related disorders such as ovarian dysfunction, chronic anovulation, infertility, obesity, and polycystic ovarian syndrome (PCOS) [5]. These conditions are associated with unopposed estrogen status, which induces endometrial proliferation resulting in increased risks of endometrial hyperplasia and carcinoma.

The standard treatment of endometrial carcinoma is surgical staging, which would destroy the reproductive function. The classic treatment consists of total hysterectomy and bilateral salpingo-oophorectomy, with a pelvic and aortic lymphadenectomy, if required. However, in young women with low histological grade and early stage of the disease, conservative hormonal therapy has also been attempted with close follow-up.

In this report, the authors present the case of a 37-year-old patient with endometrial cancer diagnosed at Stage IA, grade 1 according to the American Joint Committee on Cancer (AJCC) 2010 classification of endometrial cancer.

Case Report

A 37-year-old woman was referred to this present hospital for menorrhagia. The patient’s past medical and surgical histories were uneventful. Her gynaecological history revealed that she was nulliparous and had used an oral contraceptive for the last two years for treating oligomenorrhea. Her menarche had occurred at the age of 16 years, her menstrual cycles were irregular, and her last menstrual period occurred 45 days prior to the first visit to this hospital. Her recent pap smear was negative.

On physical examination, she was hirsute (Ferriman Gallwey classification Grade III) and obese, with a body mass index (BMI) of 35.2 kg/m² and a waist circumference of 110 cm. A speculum examination showed an apparently healthy-looking cervix. Pelvic examination was normal.

Blood tests for thyroid function and prolactin concentrations were normal. Her oral glucose tolerance test values were within normal range. She was normotensive (blood pressure of 110/70 mmHg). Her fasting lipid profile was normal (total cholesterol 198 mg/dl, triglycerides 99 mg/dl, HDL 50 mg/dl, and LDL 128 mg/dl). The urine analysis, renal function test, coagulation tests, liver function test, and electrocardiography were all normal.

A transvaginal ultrasound demonstrated a normal-sized uterus and an endometrial thickness of 20 mm in secretory phase of cycle. The ovaries were micropolycystic. Right ovarian volume was 7.7 ml, left ovarian volume was 7.2 ml. Sonohysterography showed a hyperplastic endometrium and the presence of an endometrial polyp.

A hysteroscopy was performed to investigate the suspected intrauterine lesion, and it revealed the presence of a polypoid mass of ten mm in diameter protruding from the fundus of the uterus and two polyps of five and three mm in diameter, respectively, in proximity to the left tubal ostium. The polyps were removed using an electrical loop. The histological examination diagnosed the presence of a well-differentiated adenocarcinoma in the context of atypical complex endometrial hyperplasia.

The patient was informed about diagnosis and prognosis. Conservative medical treatment including high-dose progestin, follow-up endometrial biopsy, and the benefits and demerits of surgery were explained in detail to the patient. The patient chose to proceed with surgical treatment and an informed consent was obtained. During laparotomy the uterus appeared normal and the...
ovaries were micropolycystic. There was no free liquid in the pelvis. She underwent a total hysterectomy and bilateral salpingo-oophorectomy with iliac lymphadenectomy. Peritoneal washing was performed by rinsing the cavity with 100 cm³ of physiological saline.

At histological examination the uterus measured 8.5 × 7 × 4.5 cm and it was attached with unremarkable fallopian tubes and with ovaries of brown colour and irregular profile (Figure 1). The adenocarcinoma originated in the context of complex atypical hyperplasia (Figure 2). On sectioning, the tumor was limited to the endometrium. The superficial myometrium was unremarkable. In both ovaries, multiple subserosal cystic follicles were observed. Histopathology diagnosis was consistent with endometrioid adenocarcinoma at Stage IA, grade 1 according to the AJCC 2010.

The patient was discharged on the fifth postoperative day. At the two-week postoperative follow-up visit, the patient no longer had vaginal bleeding and her abdominal wound was well-healed. She was advised to continue her follow-up care at the present hospital; hence she did not require any further treatment.

Discussion

The risk factors associated with endometrial adenocarcinoma include obesity, nulliparity, unopposed estrogen, chronic anovulation, early menarche, late menopause, diabetes mellitus, and hypertension.

Endometrial carcinoma was the first cancer to be recognized as being related to obesity. The percentage of endometrial cancer cases attributed to excess body weight is increasing, and recent estimates suggest that up to 90% of type 1 endometrial cancer patients are obese [6]. Alterations in endogenous hormone metabolism may provide the main links between endometrial cancer risk and obesity. Obese women have high levels of endogenous estrogen due to the conversion of androstenedione to estrone and the aromatization of androgens to estradiol in peripheral adipose tissue. High serum estrogen and androgen levels have been correlated with risk for endometrial cancer [6]. In obese postmenopausal women, the increased risk of this malignancy is mainly due to elevated circulating estrone levels and to decreased sex hormone binding globulin levels. Conversely, in premenopausal women, obesity may affect endometrial cancer risk through its tendency to cause anovulation and luteal phase deficiency [7].

Obesity is strongly associated with PCOS, in which prolonged anovulation and consequent exposure of the endometrium to estrogen, unopposed by progesterone, could increase the risk of endometrial cancer by inducing endometrium proliferation [8].

PCOS is considered to be a common endocrine disorder in women of reproductive age, with population prevalence estimates of six to ten percent of women of reproductive age [9]. An international consensus group proposed that at least two of three criteria have to be met in order to diagnose PCOS. These criteria are oligo-anovulation (usually manifested as oligomenorrhea or amenorrhea), elevated levels of circulating androgens (hyperandrogenemia) or clinical manifestation of androgen excess, and polycystic ovaries as defined by ultrasonography [10]. In addition to reproductive and hyperandrogenic concerns, women with PCOS are more likely to be insulin resistant, overweight, and obese, and several studies have demonstrated that PCOS is associated with an increased risk of glucose intolerance and type 2 diabetes mellitus, independent of BMI [11]. PCOS has also been associated with an increased prevalence of lipid-related abnormalities, hypertension, subclinical atherosclerosis, and vascular dysfunction [12].

A recent systematic review showed that women with PCOS were almost three times more likely to develop endometrial cancer [13]. Women with PCOS have several risk factors for endometrial cancer, including unopposed estrogen stimulation of the endometrium in anovulatory women, obesity, diabetes, insulin resistance, insulin-like growth factors, nulliparity, cyclin D1, glutathione-S-transferase, and progesterone resistance [14]. Several studies demonstrated that the incidence of PCOS was significantly higher in young patients with endometrial cancer than in older patients [15, 16].

In the present case report, the patient was severely obese, hirsute, and nulliparous, and she complained of oligomenorrhea. Chronic anovulation due to unopposed

Figure 1. — Surgical specimen: uterus with unremarkable fallopian tubes and polycystic ovaries.
Figure 2. — Histopathologic image of endometrial adenocarcinoma at x100 magnification (Hematoxylin-eosin stain).
estrogens represents a factor which increases the risk of endometrial adenocarcinoma by inducing endometrium proliferation.

At the histological examination, the adenocarcinoma had developed in an area of endometrial atypical hyperplasia. Endometrial hyperplasia with cellular atypia is considered a precancerous lesion. Endometrial cancer has been reported a concurrent condition in 42.6% of women who have been diagnosed with atypical endometrial hyperplasia by endometrial biopsy [17]. Endometrial hyperplasia occurs in 35% of women with PCOS who are not receiving either contraceptive steroids or periodic progestin withdrawal. Those at higher risk of endometrial hyperplasia are women who have intermenstrual thickness of more than seven mm [18]. Several studies have appeared to support this association, and it is common practice among gynaecologists and physicians to prescribe hormonal treatments to reduce this perceived risk, although there is no consensus on the subgroup of patients with PCOS in whom this treatment is required [19].

The most important prognostic factors of endometrial cancer are histological grade, cancer stage, and myometrial invasion. Fortunately, most cases of endometrial adenocarcinoma in young women are at early stages with a superficial invasion (Stage I) and 90% of all cases are well-differentiated (grade 1) [4].

Surgery is the classic treatment for endometrial cancer. It consists of total abdominal hysterectomy and bilateral salpingo-oophorectomy, with a pelvic and aortic lymphadenectomy, if required. For clinical Stage I tumors, cell type, histologic grade, depth of myometrial invasion, peritoneal cytology, vascular invasion, and age are all significant independent risk factors. Since only cell type and grade can be determined without performing hysterectomy, the International Federation of Gynaecology and Obstetrics (FIGO) has defined endometrial cancer as a surgically-staged disease.

There is a therapeutic alternative for young women wishing to become pregnant in the future, but this is not standard management and should not be routinely recommended. Only strictly selected patients with early-stage disease should be indicated for long-term medical treatment and careful evaluation before and after treatment should be performed. Patients must be carefully informed of the oncological risks.

The authors did not consider the conservative management in the present patient because she did not desire to preserve fertility, had poor compliance, and chose surgical treatment.

Conclusion

The association between obesity, PCOS, atypical endometrial hyperplasia, and endometrial cancer has relevant implications for clinical practice as it calls for the implementation of risk-reducing measures, including the potential of introducing a screening programme for early cancer detection.

Based on the data presented in this report, the authors would carefully suggest the greater monitoring of premenopausal women with PCOS and/or the associated symptoms of obesity and irregular periods, by performing accurate physical examination and diagnostic studies.

Regarding treatment, although there are several examples of successful medical therapies in the literature, surgery remains the gold standard, whereas medical treatment should not be routinely recommended, but it must be reserved for selected patients who understand and accept that it is not a standard treatment.

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Sustainable complete remission in recurrence yolk sac tumor patient treated with tandem high-dose chemotherapy and autologous stem cell

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Summary
A 21-year-old lady diagnosed with Stage 3 ovarian yolk sac tumor (YST) underwent primary cytoreductive fertility sparing surgery, followed by conventional courses of platinum-based chemotherapy and etoposide. Recurrence at cul-da-sac was noted after a short period of remission and secondary debulking performed followed by four cycles of conventional chemotherapy. The patient’s disease progressed despite courses of treatments. A joint team management including a hematologist was commenced following the failure of conventional chemotherapies. Two cycles of high-dose chemotherapy (HDCT) with ifosfamide/cisplatin/etoposide (ICE) regimen, followed by autologous stem cell transplantation (ASCT) were given. With this salvage treatment, she remained in complete remission and disease-free for more than 30 months, while maintaining her reproductive function. These approaches appear to be effective as a salvage treatment in selected cases of patients with ovarian germ cell tumor, especially those who failed primary conventional chemotherapy.

Key words: High-dose chemotherapy; Germ cell tumor; Autologous stem cell.

Introduction
Malignant ovarian germ cell tumors (MOGCT) comprise five percent of all ovarian malignancies. Dysgerminoma and yolk sac tumors (YST), account for approximately 20% of MOGCT [1]. YST are aggressive tumors with high mortality and recurrence rates, before the era of effective chemotherapy. The combination regimen with bleomycin/etoposide/cisplatin (BEP) has improved the survival of ovarian YST from 13% to 95% for Stage 1 and 75% for advanced stage [2].

However, the prognosis for chemotherapy-refractory YST is still dismal where 20% of advanced stages may experience progressive disease after BEP chemotherapy [3]. The introduction of combination high-dose chemotherapy (HDCT) and autologous stem cell transplantation (ASCT) in solid tumors appears to have promising favorable outcome for this chemo-refractory tumor. Here, the authors present a case of advanced stage YST, which was chemo-refractory and received combination HDCT and tandem ASCT.

Case Report
A 21-year-old lady presented with sudden onset of lower abdominal pain. Her past medical and gynecologic history was unremarkable. Physical examination revealed a pelvic mass. Ultrasonography and computer tomography (CT) showed complex right ovarian tumor measuring 12 cm, minimal ascites and no enlarged lymph nodes. Tumor markers were as follows: alpha-fetoprotein (AFP): 120423, CA 125: 58.8.

She underwent exploratory laparatomy, right salpingo-oophorectomy, omentectomy, appendectomy, and pelvic lymphadenectomy. The intraoperative findings included large right ovarian tumor with multiple tumor deposits at right pelvic peritoneum, uterine surface, rectal serosa, and omentum. An optimal debulking surgery was performed and staged as FIGO Stage 3C. Final pathology revealed YST. Adjuvant chemotherapy with PE (cisplatin/etoposide) regimen was given. AFP levels reduced during six courses of chemotherapy (including two courses of consolidation chemotherapy). After completed adjuvant chemotherapy, AFP was elevated, thus courses of PE regimen commenced. She was disease-free for only two months, evidenced by normalization AFP and negative CT scan findings.

An elevation of AFP level was noted after short remission and CT scan showed abnormal soft tissue lesion at pre-sacral region (Figure 1). Secondary debulking performed with intraoperative findings of pelvic recurrence at cul-de-sac. Histopathology confirmed metastatic YST. Salvage chemotherapy was commenced after surgery. She had four cycles of carboplatin (AUC 5) and etoposide. Unfortunately, her disease progressed with persistent elevation of AFP.

In view of her incurable disease, HDCT and ASCT was suggested after discussion with the hematologist. Whole body fluoride-18 2-fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET) scan was performed for tumor assessment which revealed no evidence of recurrence. Chemotherapy regimen (carboplatin/etoposide) commenced and recombinant human granulocyte colony stimulating factor (subcutaneous RhG-CSF five ug/kg) was given every 12 hours, after completing chemotherapy. This is crucial for stem cell mobilization.

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Stem cell collection was performed on day 16 when the total white cell count reached > 10,000/μl. A double lumen catheter was inserted and Baxter-Fenwal CS 3000 cell separator was used for stem cell apheresis. A total of ten liters of whole blood was processed and CD34+ cells dose yielded which was separated into two bags and cryopreserved for future uses. Pre-transplant evaluation included cardiac blood pool image study and pulmonary diffusion capacity.

The first course of HDCT with ifosfamide/carboplatin/etoposide (ICE) was given for five days. Thawed autologous stem cells were transfused after completed ICE regimen. Recombinant human granulocyte-colony stimulating factor (RhG-CSF) (5µg/kg) was given to accelerate myeloid recovery. Leucocyte depleted blood components were transfused as indicated. The patient developed grade ¾ febrile neutropenia. The neutrophil engraftment documented by absolute neutrophil count > 500/μl was achieved at day ten. The sustained platelet recovery with transfusion-independent and platelet count > 20,000/μl was achieved at day 13. Her AFP level dropped to 11.73 ng/ml two weeks after ASCT.

Her general condition and hematological profile recovered rapidly. In terms of her highly-chemosensitive disease with short duration of response, second ASCT as tandem treatment was commenced two months later with the exactly similar conditioning regimens. The use of growth factor and supportive care was similar to the first ASCT. The myeloid and platelet engraftments were achieved on days ten and 13, respectively. She was then discharged and followed up regularly as an outpatient. She had normalization of performance status and resumed her regular menstrual cycle. She did not have any premature menopausal symptoms and normal AFP level throughout the post-transplant period (Figure 2). She has now been disease-free for three years.

Discussion

YST commonly presents within the second and third decades of life. It is highly-aggressive and frequently shows early intra-abdominal dissemination and metastasis. Most of YST carry worst prognosis when compared with other MOGCT. They usually secrete AFP, which can be reliably used in monitoring effectiveness of treatment commenced and detection of recurrence [1, 3].

With the advent of platinum-based chemotherapy since the 1970’s, the extent of surgery required has been progressively reduced. Whenever possible, preservation of fertility is considered. Similar menstruation and fertility rates are seen in those with ovarian YST after combination of fertility-sparing surgery and adjuvant chemotherapy when compared with the healthy population. An optimal cytoreduction surgery, presence of ascites at diagnosis, intra-peritoneal dissemination lesion, and normalization of AFP half-life were noted to have a significant effect on overall survival [4-6].

Approximately 20%–30% of the MOGCT that receive primary therapy for advanced stage will relapse or have incomplete response. Options of salvage treatment that may show curative potential in these groups are either by conventional chemotherapy or HDCT. Conventional chemotherapy only achieves 10%–40% of long-term remission, however these patients may ultimately succumb to the disease [7].

Aside from using of HDCT for patients with relapse, it is also being evaluated as first line treatment in poor prognosis disease of testicular germ cell tumor. Early HDCT studies reported longer survival rates of 15%–25%, but treatment related toxicity was formidable and treatment related death occurred in approximately ten percent of the patients [7]. While administration of HDCT alone showed severe and lethal myelosuppression, combination with bone marrow transplantation (BMT) or ASCT, and availability of hemopoietic growth factors were used to overcome severe adverse effects of HDCT. ASCT is more favorable compared with BMT as it avoids surgical procedures and convenience for the patient.

Helw et al. in his review on disease-free survival for germ cell tumors treated with HDCT, revealed that long-term disease-free survival rate for refractory or heavily pre-treated GCT, first relapse GCT, and those poor-risk groups was respectively 13% (range 0%–35%), 45% (range 21%–67%), and 52% (range 36%–84%) [3]. This is supported by a German group that compared GCT patients treated with or without HDCT. The analysis showed that the benefit of HDCT was slightly more pro-
nounced and that the hazard ratio was more favorable in terms of event-free survival and overall survival [8].

The usage of two or more HDCT drugs is widely investigated. The rationale for this approach is an assumption that upfront uses of multiple HDCT may induce cell death in higher fraction of sensitive tumor cell before drug resistance develops. The choices of chemotherapeutic agents used for HDCT in treating MOGCT are still focused on the usual chemotherapy agents use in ovarian cancer. Among the evaluated regimens are TICE (paclitaxel/ifosfamide/cisplatin/etoposide) where complete response was seen in 56%, half of patients are free of disease at the median follow-up at > 40 months [7, 9]. This is supported by the retrospective review of advanced GCT where disease progressed after platinum-based chemotherapy. Two main active high-dose density drugs used in this setting were carboplatin and etoposide. Complete remission was observed in 63% at a median follow-up of 48 months. At two years, 90% of these patients remained free of disease [7, 10].

Phase 3 trials comparing single and sequential HDCT in relapsed or refractory disease failed to show the superiority of single HDCT regimen. One-year event-free survival rate for sequential HDCT was 55% compared with single HDCT of 37%. Further strategies to incorporate ≥ two high-dose cycles of chemotherapy may yield promising results in the future, thus becoming the preferable option for those with a second relapse [7]. In the present patient, a second cycle of HDCT with ASCT was administered as she was in the high-risk group of relapse, and these prolonged her remission.

Late complications of multiple courses of chemotherapy are of great concern as GCT patients are within reproductive age. Risk of hypothyroidism, infertility, early menopause, and secondary malignancies should be assessed during surveillance. The risk of secondary myeloid leukemia reported is two to three percent of cases treated with HDCT. Improvement of supportive care and less pre-treatment may help to reduce these morbidities [7]. The present patient did not experience any late adverse effects after treatment.

There is growing evidence on the role of combination HDCT and ASCT supported with hematopoietic growth factor benefits in YST who failed conventional primary chemotherapy. Most of the studies and guidelines on GCT management with HDCT concentrate on testicular germ cell malignancy and further studies on recurrence MOGCT failed primary treatment managed with HDCT should be implemented in order to prolong survival and disease-free in these reproductive-aged women.

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Coexistence of three benign and a borderline tumor in the ovaries of a 52-year-old woman

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Summary

Purpose of investigation: In this paper, the authors describe a rare case of four simultaneous ovarian tumors. Materials and Methods: A 52-year-old postmenopausal woman underwent total hysterectomy and bilateral salpingo-ophorectomy. Multiple slides from the ovaries were examined. Results: Histological examination revealed the presence of three ovarian tumors on the right ovary, of the following types: cystadenoma, mucinous borderline ovarian tumor and mature teratoma, and also a benign Brenner tumor on the left ovary. Conclusion: Pathologists must examine multiple sections of both ovaries, regardless of the macroscopic or clinical specimen’s appearance, in order to exclude the presence of malignancy, which could alter the surgical approach. Particular attention should also be paid to the frozen section of the contralateral ovary, as depending on the result, it could change the surgical approach. From the surgeon’s perspective, bilateral salpingo-ophorectomy with total hysterectomy should be the treatment of choice in postmenopausal women with multiple ovarian tumors. The diagnosis of a malignant or borderline tumor on a normal-appearing ovary changes the radicality of the surgical approach. In such a case, staging surgery, including omentectomy, multiple peritoneal biopsies, and washes are required.

Key words: Borderline; Brenner; Mucinous; Ovarian tumor; Teratoma.

Introduction

Ovarian tumors are frequent and usually benign. Borderline tumors are also relatively frequent and mainly occur in middle-aged women [1]. Although two different ovarian tumors can co-exist, the presence of four simultaneous tumors is an extremely rare fact.

Mucinous cystadenomas are benign ovarian tumors, representing about 15% of all ovarian neoplasms [2]. These tumors may become large, and cystomas weighing over 136 kg have been reported. It is believed that they derive from simple metaplasia of the germinal epithelium [3]. On the other hand, besides the fact that mucinous borderline tumors have almost similar microscopic appearance to benign mucinous cystadenomas, they present microscopic differences such as nuclear abnormalities, intermediate mitotic activity between benign and malignant tumors, irregular hyperchromatic nuclei, and enlarged nucleoli [3, 4].

Brenner tumor is a rare type of epithelial ovarian neoplasm. Although it was initially believed that Brenner tumors were uniformly benign, a number of malignant tumors of this type have already been described. They arise from diverse sources as in surface epithelia, rete ovarii, and ovarian stroma [3].

Finally, mature teratomas are common (10% to 25% of all ovarian neoplasms) ovarian benign tumors with smooth, rounded, or ovoid shapes. Although ectodermal elements usually predominate, endodermal or mesodermal derivatives can also be found. Malignant transformation of these tumors is very rare (1% to 3%) [5].

Materials and Methods

A 52-year-old postmenopausal woman, nulliparous, with four different synchronous ovarian tumors with multiple hematoxylin and eosin (H&E) stained slides, from both ovaries, were examined.

The patient presented at this hospital with abdominal inflation and an ovarian mass, diagnosed with ultrasound. The imaging characteristic of the tumor included both cystic and solid parts. There was no fluid in the pouch of Douglas.

The patient underwent total abdominal hysterectomy, bilateral salpingo-ophorectomy, omentectomy, and staging. During surgery, a 26 x 20 x 9.5 cm tumor of the right ovary was found while the other on left ovary measuring 3 x 2.5 x 2 cm. Frozen section of the specimen from the right ovary, confirmed the presence of mucinous cystadenoma and mature teratoma.

Results

Macroscopic pathological examination revealed a smooth tumor of the right ovary, consisting of both unilocular and multilocular cystic areas. Multilocular area was covered by a fibroelastic wall and contained jelly fluid, while a four cm white-yellow colored area of localized growth, containing sebum and hairs, protruded into the unilocular cavity. Histopathology revealed three different histological tumors on the right ovary. Half of the tumor partially consisted of both mucinous cystadenoma (Figure 1) and of a mucinous borderline tumor of intestinal type (Figure 2), and the other half consisted of a mature teratoma (Figure 3). The latter contained a great range of mature tissues from all three germinal layers (epidermis, skin appendages, gland cells, bone, cartilage, intestinal and respiratory epithelium, and thyroid tissue). There was no invasion or immature neuroepithelial tissue. Neoplasm did not extended out of the ovary.

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Coexistence of three benign and a borderline tumor in the ovaries of a 52-year-old woman

On the other hand, pathological examination on the left revealed an atrophic ovary with microscopic elements of benign Brenner tumor (Figure 4). There was also uterine leiomyomas, atrophic endometrium and an endometrial polypoid. Histological examination of the omentum and cytological examination of the peritoneal lavage indicated no malignancy.

Postoperative clinical situation was normal and patient was discharged home on postoperative day seven without any major complications. She received no additional treatment. At one year postoperatively, the patient still remains well with no residual symptoms.

Discussion

Despite the rarity of the phenomenon, a connection between mucinous cystomas and mucinous borderline tumors has already been described. Actually, according to some reports, mucinous borderline tumors present a frequently heterogeneous composition with coexisting benign, borderline, and malignant elements [4].

Moreover, although simple metaplasia of the germinal epithelium is considered as the most accepted histogenetic pathway of mucinous cystadenomas, some 30 years ago, the teratomatous hypothesis of mucinous cystomas’ histogenesis was expressed. According this theory, these tumors derive from monophyletic endodermal development of a teratoma [6].

Conclusion

From a pathological view, the presence of a histological ovarian tumor does not exclude the coexistence of other tumors of various histological types on one or both ovaries. For these reasons the pathologist must examine multiple sections of both ovaries, regardless of the macroscopic or clinical specimen’s appearance, in order to exclude the presence of malignancy. In postmenopausal women, particular attention should be paid to the frozen section not only of the clinically suspicious ovary, but also of the contralateral, as the surgical approach could be altered depending on the result.

On the other hand, preoperative clinical and imaging examination of the ovaries cannot always determine with certainty the benign or malignant nature of the tumors. Therefore, bilateral salpingo-oophorectomy with total hys-
Hysterectomy should be the treatment of choice in post-menopausal women independently of frozen section’s result. However, the diagnosis of a malignant or borderline tumor on a non-suspicious ovary, regardless of the benign nature of the contralateral and more suspicious ovary, changes the radicality of the surgical approach. In this situation, instead of a conservative operation, a staging surgery, including omentectomy, multiple peritoneal biopsies, and washes is needed.

In premenopausal women, the decision of the radicality of the surgery, considering the need of a possible fertility-sparing approach, is more complicated. Simple unilateral salpingo-ophorectomy, excision of any suspicious peritoneal lesion, multiple biopsies, washes, appendectomy, and pelvic and para-aortic lymphadenectomy could be applied in well-selected patients, depending on the histological type, grade, and stage of the disease [7]. In this case, the decision of single or multiple “blind” biopsies of the other normal-appearing ovary is controversial. On the other hand, in patients with borderline ovarian tumors, a fertility-sparing procedure could include the wedge resection or the ovarian cystectomy of the contralateral ovary [8].

The probability of multiple ovarian tumors in both ovaries, dictates the realization of a multicenter study, in order to determine with accuracy the best treatment approach of these patients, especially in premenopausal ones.

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Borderline ovarian tumor - a case report with genetic testing

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Summary
Despite an accurate classification, the borderline category of ovarian tumors is one of the most controversial topics in gynecologic oncology and is confusing to both clinicians and patients. The treatment is often confronted with the necessity of a fertility-sparing approach, although under-treatment increases the risk of disease recurrence. The clear definition of a group of patients with low or high risk of relapse requiring more or less extensive treatment is lacking. Currently, the main criteria affecting the treatment extent include histopathological features, particularly the presence of microinvasion, and invasive implants. Expansion of knowledge about genetic nature of the tumor characteristics may more closely specify the scope of therapeutic approach for individual patients. The authors report a case report of serous borderline ovarian tumor patient with tumor cell dispersion into the gastrointestinal tract. The genes of tumor vascular markers GPM6B and DR6 were also studied and compared to a group of healthy patients.

Key words: Borderline ovarian tumor; Tumor vascular markers; DR6 gene; GPM6B gene; Ovarian cancer.

Introduction
Borderline ovarian tumors (BLOT), also known as tumors with low malignant potential, represent a type of epithelial ovarian tumors exhibiting some characteristics of malignancy [1]. Despite an accurate classification remains the borderline category of ovarian tumors, which is one of the most controversial topics in gynecologic oncology and is confusing to both clinicians and patients. The etiology of BLOT remains unclear due to a small number of cases and lack of randomized and controlled studies. Compared to malignant ovarian tumors, BLOT generally occur at younger ages, i.e. approximately ten years younger than that of women with malignant ovarian cancer [2, 3]. The symptoms, reported by several studies, include abdominal pain and an unexplainable increase in girth around the waist and abdomen, although up to 23% of patients were asymptomatic [4]. Histologically, two main subtypes are defined: serous and mucinous, with serous being more common.

Serous tumors are presumed to originate from the germinal epithelium. Mucinous tumors do not have a clearly defined origin [5]. Different results also exist respect to overexpression of various oncogenes and tumor suppressor genes comparing to malignant ovarian cancer [6]. Affecting younger women, the treatment of BLOT is often confronted with the requirement of a fertility-sparing approach.

Case Report
A 24-year-old nulligravida, student, non-smoker, with regular menstrual cycles, was admitted to the hospital in December 2011 with intermittent pain in the lower abdomen. Patient denied any other complaints. The family history was free of cancer. Ultrasoundography (USG) was performed which revealed a multicellular cystic tumor at the left adnexa measuring 15 x 13 cm and rightsided ovarian enlargement measuring 8 x 7 cm. Computed tomography (CT) was performed immediately showing minimal ascites and USG confirmed the diagnosis. There were no visceral metastases based on both USG and CT. Gastric fibroscopy and cystoscopy were also performed and the findings were normal. CA-125 was measured which was slightly elevated (42.5 IU/ml) compared to reference values (< 35.0 IU/ml). All other parameters including blood count, biochemistry, and coagulation parameters were within the reference range.

The patient was surgically treated on the eighth day after admission. Lower median laparotomy was performed and the abdominal cavity was opened. Minimal free abdominal fluid was found and sent for cytologic examination. Thereafter left adnexectomy was performed and the specimen was sent for rapid peroperative pathology [Figure 1]. The result of pathology assessment showed a serous borderline ovarian tumor without signs of invasion. Due to the location of exophytic tumor on the right ovary, and to achieve fertility-sparing approach, partial resection of the right ovary was performed. Subsequently appendectomy and omentectomy were performed. Then 120 cm from the ileocecal valve, Meckel’s diverticulum was found and resected [Figure 2], as well as intestinal polyp located 30 cm orally from the diverticulum. The harvested samples were sent for definitive pathology and the assessment included: ascites, part of the right ovary, appendix, omentum, Meckel’s diverticulum, and intestinal polyp.

Definitive results of pathologist were the following: ascites – blood cells and groups of cytologically abnormal and atypical cells suspected of tumor origin. Left adnexa - serous borderline ovarian tumor with microinvasion. Part of the right ovary - serous borderline ovarian tumor with microinvasion. Appendix – without tumoral changes. Omentum – omental cells with non-invasive desmoplastic implants and abundantly present psammomatous bodies. Meckel’s diverticulum – without tumoral changes. Intestinal polyp – rarely isolated atypical cells of tumor origin is likely.

Postoperative blood work was obtained for genetic testing. The patient was discharged from the hospital on postoperative day number eight and was referred to the oncological institute for continuation of the treatment.

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Results

Genetic testing

In recent years there has been significant progress in the understanding of molecular mechanisms that lead to the formation of tumors and metastasis. The phenotypic variability is caused by genetic and epigenetic changes, which usually lead to destruction of the balance between cell proliferative, apoptotic, and differentiation abilities. For tumor growth, it is essential to rebuild the blood vessels, as cancer cells measuring more than one mm cannot be maintained by diffusion alone. Formation of new blood vessels in the tumor is conditioned by the presence of endothelial factors, which are called angiogenic factors or tumor vascular markers (TVM). Levels of ideal TVM for ovarian diagnostics are reported as having significantly different levels of expression during natural physiological angiogenesis compared to carcinogenesis. GPM6B encodes a membrane glycoprotein. Interaction of the membrane glycoprotein M6B with μ-opioid receptors was recently demonstrated, facilitating endocytosis and cellular metabolism of serotonin, with regards to identification of the glycoprotein membrane M6B as a binding partner of serotonin transporter (SERT) [7]. Transmembrane receptor DR6 belongs to the group of tumor necrosis factor. DR6 is functionally involved in the tasks of proliferation, differentiation, and programmed cell death. High expression of DR6 in cancer cells, which probably affects the anticancer cellular response through the differentiation and proliferative effects on monocytes, correlates with significant activity of NF-kB, due to internal stimulation of oncocytic cell proliferation [8].

The changes in levels of mRNA expression of tumor vascular gene DR6, GPM6B, and β-actin in the peripheral blood of the patient presented were detected compared to those in the control group (CG). The patients in the CG (n = 35) underwent preventive examination regarding their current health disorders. They were identified as healthy by their general practitioner, and based on the results of laboratory tests, the preventive check was negative. The family history of individuals in the CG had no mention of cancer. After isolation of total RNA from four ml of venous blood drawn from patients, applying a sampling system with anticoagulant K2EDTA, made reverse transcription of mRNA into cDNA. Changes in gene expression of Gpm6b and Dr6 were detected using polymerase chain reaction. Amplification of specific genes DR6, GPM6B, and β-actin was carried out in 30 cycles (94°C for 5 min, 94°C for 30 sec, 48°C for 30 sec, and 72°C for 45 sec), using primers from Table 1. Quantification was evaluated using a documentation system. Numerical quantification of changes in expression levels was assessed using a specifically-designed software. In order to minimize the impact of variability in biological sample of BLOT and samples in the CG, each biological sample was analyzed three times. Statistical evaluation of numerical values was done using the One-way ANOVA test. To find statistical differences between mean values, the authors used the Student-Newman-Keuls tests. Statistical analysis was processed by the GraphPad INSTAT program. By analysing expression levels of mRNA gene DR6, GPM6B and β-actin in the peripheral blood of the studied patient (BLOT) in comparison to those in the CG, the authors detected significantly increased mRNA expression levels of gene DR6 - seven-fold and GPM6B - ten-fold compared with the CG (Figure 3).

Discussion

There are still no clear recommendations regarding BLOT management. Currently, the main criteria affecting the treatment extent include histopathological features, particularly the presence of microinvasion, and invasive implants. Since BLOT represent diseases affecting mainly younger women, the fertility-sparing approach is often required. Expansion of knowledge about genetic nature of the tumor characteristics may more closely specify the scope of therapeutic approach for individual patients.

As previously stated, women of reproductive age are affected predominantly, with a median age of 34.5 years [9]. The patient in this case was a 24-year-old nulligravida, thus fertility-sparing was the crucial point in the range of surgical approaches. The use of imaging studies (MRI and CT) helps to detect suspected tumors, although the key imaging characteristics that would distinguish BLOT from other ovarian tumors are absent. Bent et al. in a study of MRI usage only reported that serious BLOT was significantly smaller than mucinous BLOT [10]. Comparing with the latest results of Shih et al. study, significant differences were found if the tumor size was studied. Tumor size greater than eight cm was associated with a 22.4% incidence of invasive cancer on final pathology compared to 3.2% in tumors eight cm or less [11]. The CT findings of the present patient showed that the tumor size of 15 x 13 cm, also confirmed peroperatively, nevertheless showed no invasive tumor in the final pathology. There were no clear correlations found where CA-125 in BLOT for diagnosis or follow-up was studied. However, preoperatively, it can be useful in counseling the patient as to what to expect in the operating room [12]. The CA-125 level in the presented patient was slightly elevated (42.5 IU/ml) compared to reference values (< 35.0 IU/ml). This result showed that the value was not typically high for invasive carcinoma, although higher than in non-cancer patients confirming previously made conclusions regarding CA-125.
The histopathological features of BLOT were clearly defined and represent one of the main elements for choice of therapeutic approach. As major histopathological characteristics, confirmed by previous studies, microinvasion and peritoneal implants (PI) were determined. The study of Anfinan et al. with 138 BLOT patients has shown that presence of microinvasion and invasive implants are significant risk factors for disease recurrence [13]. While the presence of non-invasive PI has almost no negative influence on ten-year survival rates, the invasive form is associated with poor prognosis, i.e. more than 50% had recurrences, and the ten-year survival rate was only about 35%. Therefore the morphology of the PI is the main prognostic factor for patients with stage II-III BLOT. The omentum is the most likely site for invasive implants. Therefore, surgeons must take a sufficient amount of omental tissue to enable the pathologist to distinguish non-invasive from invasive implants [9]. This has also been confirmed by the study of Gershenson et al., where the patients with serous BLOT and non-invasive PI had 20% recurrence rate, while in patients with serous BLOT and invasive PI, the recurrence rate was 31%-45%. In this mentioned study, there was no difference in survival between patients with serous and mucinous BLOT [14]. Thus, the high-risk patients, regarding histopathological criteria, are the patients with BLOT with microinvasion and presence of invasive implants. These types of BLOT have been designated also as “micropapillary serous carcinoma” due to their characteristic micropapillary architecture [15]. The final pathology of the present patient showed serous BLOT with microinvasion and non-invasive omental implants. The absence of invasive implants made good prognostic prospects, but the presence of atypical cells in intestinal polyp suggested more extensive surgery.

Given the excellent prognosis of patients with Stage I disease, fertility-sparing surgery is of great interest. This type of surgery is an acceptable option in confirmed Stage I serous and mucinous tumors. Advanced disease requires more extensive surgery; however the exact recommendations are still absent [16]. In order to meet the patient’s demand, fertility-sparing surgery was an option...
in this patient and was performed with only partial resection of the right ovary. Because of microinvasion presence from rapid peroperative pathology, omentectomy and appendectomy were performed. The presence of atypical cells, with suspected tumoral origin, necessitates gastrointestinal tract checking with resection of polyp and diverticulum if BLOT is diagnosed. Postoperative treatment for any stage is controversial. Up to 95% of BLOT have diploid DNA mostly associated with great prognosis. Minority of BLOT have aneuploid DNA (micropapillary carcinoma) leading to poor prognosis with higher recurrence rate. There are proposals to treat these tumors as low-grade invasive carcinoma, although no prospective and randomized clinical trials have been done due to small number of patients [16].

The progress in genetic testing of gynecological malignancies has been seen in previous years, however little was done on BLOT. The pattern of genetic alterations described in BLOT differs from that of invasive carcinomas, e.g. TP53 mutation are often absent in BLOT but present in up to 88% of invasive carcinoma cases. On the other hand, loss of heterozygosity on the long arm of the inactivated X chromosome is characteristic for BLOT and rare in carcinomas [9]. The influence of TP53 and HER2 mutation on prognosis showed different results as well. While the positivity of TP53 and HER2 in invasive carcinoma is associated with worse prognosis in case of BLOT, the positivity confer a survival advantage. Generally, 28 markers were identified for various gynecological malignancies, and for ovarian cancers six-fold higher expression was detected than in healthy subjects. Thirteen genes with reduced expression were also detected [6]. There are few studies presenting the detection of gynecological and other cancers using TVM [17]. As the authors have mentioned above, ideal levels of TVM for detection of ovary cancer must report significantly different levels of expression during natural physiological angiogenesis compared to carcinogenesis. These conditions are fulfilled for example by adican, COL11A1, GMP6B, and DR6, which show from ten-fold up to 350-fold increase in expression in gynecological cancers [6]. The present patient showed overexpression of GMP6B and DR6 when compared to healthy population. This suggests for higher risk of invasive carcinoma development and was also confirmed by histopathological findings – microinvasion and presence of atypical cells suspected of tumour origin in the intestinal polyp. The conclusions from genetic testing probably predict the need of postoperative treatment. The present results correspond with the results described by Buckanovich et al., which also showed increased expression levels of DR6 RNA gene in ovarian cancer compared to healthy subjects, but BLOT were not included in the study [17].

References


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An undescribed coexistence of benign metastasizing leiomyoma in the lung with serous borderline tumor of the ovary

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Summary
Pulmonary benign metastasizing leiomyoma (BML) is a rare disease occurring predominantly in women of reproductive age and usually develops several years after the resection of a uterine leiomyoma. Serous borderline tumor (SBT) occurs most frequently in the ovary originated from sex hormone dependence. This report describes such a co-existing case. A 46-year-old woman developed a uterine leiomyoma co-existing SBT of the right ovary ten years ago and then underwent abdominal total hysterectomy and right side oophorectomy. In 2008, she developed a co-existing pulmonary BML and SBT of the left ovary. Left side oophorectomy was performed and no further therapeutic actions were taken. The patient is currently alive and well. To the authors’ knowledge, this is the first case of a co-existing BML and SBT. Herein, they describe the clinicopathological features of BML and the possible existence of a close causative association between BML and SBT.

Key words: Benign metastasizing leiomyoma; Serous borderline tumor; Uterine leiomyoma; Sex hormone.

Introduction
Benign metastasizing leiomyoma (BML) is a rare disease which was initially described by Steiner in 1939 and there are now about 150 cases reported [1, 2]. Such lesions have commonly been reported in young premenopausal women with seldom symptoms and therefore, are often discovered incidentally by routine chest roentgenogram. The pathogenesis of BMLs is still the subject of controversy as hormone-level changes may have an effect on the general course of the disease.

Case Report
In February 2008, a 46-year-old woman was admitted to the respiratory department of this hospital because of cough, expectoration for one month, and fever for three days. Computed tomography (CT) of the chest showed multiple nodular shadows in both lungs (Figure 1). Abdominal ultrasound showed a cystic solid mass in the left region of the adnexa. The liver, spleen, gall bladder, and both kidneys were normal. Tumor markers, including CA72-4, CA19-9, CA12-5, and neuron-specific enolase (NSE) were within the normal ranges. Routine blood chemistry and inflammatory reaction test results were normal. Physical examination and other laboratory investigations did not reveal any significant abnormalities.

Her medical history included a uterine myoma and ovarian tumor of the right side (Figure 3A) eight years prior for which she underwent abdominal total hysterectomy and right side oophorectomy at another institution. Fortunately, the authors were able to review the uterine and ovarian tumors, which were both pathologically confirmed to be benign leiomyoma (Figure 2A) and serous papillary cystadenoma of borderline malignancy of ovary (Figure 3A).

Figure 1. — (A,B). Chest CT scan showing multiple pulmonary nodules in bilateral lung.
Figure 2. — Uterine benign leiomyoma showing nodular proliferation of smooth muscle cells (A). Pulmonary benign metastasizing leiomyoma composed of spindle cells with no atypia under respiratory epithelium (B). Immunohistochemical stainings for desmin (C) and estrogen receptor (D), showing diffuse and strong positivity (A and B: H&E stain × 100; C, D: immunostain × 100).

Figure 3. — The tumor is a serous borderline ovarian tumor with a micropapillary and cribriform pattern. A marked epithelial proliferation can be seen. (A) is the right side in 2000 and (B) is the left side in 2008. (A and B: H&E stain × 100).
The patient underwent a CT-guided percutaneous needle biopsy of the lung tumor on August 11, 2008. Histologically, biopptic specimens showed a completely benign appearance consisting of proliferating smooth muscle fibers with a mixture of respiratory epithelia (Figure 2B). In particular, no mitoses, necrosis, or inflammatory responses of the host tissues were detected. Neoplastic cells expressed desmin (Figure 2C), smooth muscle actin, estrogen (Figure 2D), and progesterone receptors, but neither CD10 nor HMB-45. The epithelial cells were positive for cytokeratin and thyroid transcription factor-1. Therefore, the lung tumor was diagnosed to be a BML. Left side oophorectomy was performed on September 2, 2008. The pathologic diagnosis of left side ovarian tumor was serous papillary cystadenoma of borderline malignancy of the ovary (Figure 3B).

No further therapeutic actions were taken. The patient is alive and well during the last two years.

Discussion

BML describes a different pattern of spread, whereby a histologically-benign leiomyoma exhibits metastatic qualities and is found at sites distant to the uterus [3]. It is a unique condition occurring in women with BML and are pathologically described as extraterine proliferations of cytologically bland smooth muscle cells that are mitotically inactive, show no evidence of nuclear pleomorphism or necrosis, and have similar histologic appearances to the patient’s known primary benign uterine tumor. Most BMLs have been found to express estrogen and progesterone receptors [4].

BML mainly affects sexually-mature women when the hormonal effects are at their maximal level, and usually regresses after menopause or parturition or with the use of selective estrogen modulators or gonadotropin-releasing hormone agonists (GnRHa). The pathogenesis of BMLs has been the subject of controversy and several hypotheses have been proposed. Skepticism has been expressed regarding the existence of BML, suggesting that the primary lesion in BML could actually be low-grade sarcomas with metastatic potential [5]. Metaplastic transformation of the coelomic epithelium may explain BML in virtually any location where mesothelial mesenchyme exists [6]. Most of the views regarding this disease are similar to the mechanisms used to explain the etiology of endometriosis, in which the hormone-level changes that occur during pregnancy and menopause may have an affect on the general course of the disease [2-7].

Serous papillary cystadenoma of borderline malignancy is classified as a serous borderline tumor (SBT) and is found most frequently in the female ovary. Over 90% of serous borderline ovarian tumors are estrogen-receptor positive [8]. To the authors’ knowledge, there is no reported case of BML co-existing with SBT. In the present case, the co-existing tumors, as well as uterine leiomyoma co-existing SBT of the right ovary eight years ago, indicated that the patient had a high sex hormone level which played a key role in their management. Based on the literature, removal of estrogen stimulation by oophorectomy or hormonal therapy using GnRHa, selective estrogen receptor modulator, and aromatase inhibitor have been suggested as the best options for both BML and SBT [9]. Due to the oophorectomy performed in 2008, the pulmonary BML had no further development during the past two years.

In summary, this is an interesting case of pulmonary BML co-existing with SBT which seems to have originated from the sex hormone dependence. The authors believe this report will help to promote the understanding of BML. Meanwhile, the current report perhaps gives additional information regarding the pathogenesis of BML.

References


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Vulvar melanoma and endometrial polyp following breast carcinoma: a case report

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Summary

The authors describe the occurrence of a 55-year-old female patient presenting with a vulvar melanoma, endometrial polyp, and a prior history of breast carcinoma excised from the left chest wall, radiotherapy, chemotherapy, and tamoxifen maintenance for two years. This case exemplified secondary primary vulvar melanoma following breast cancer and supported that radiotherapy might play a role in the onset of secondary cancer. This case report also emphasizes the onset of endometrial polyp induced by tamoxifen.

Key words: Vulvar melanoma; Endometrial polyp; Breast carcinoma.

Introduction

The incidence rate of malignant secondary cancer significantly increases among female breast cancer patients, and includes secondary cancers of the thyroid, uterine corpus, and skin melanoma increasing the incidence to 80% [1]. However, secondary primary cancer is vulvar melanoma which is infrequent, because it is a rare disease with malignant tumor affecting the vulva and vagina with an incidence of three to seven percent in all melanomas. For breast cancer treatment, radiotherapy slightly increases the risks of secondary leukemia, endometrial, and breast cancers [2]. Recently, many studies focus on the side-effects of tamoxifen, which has been used for more than 30 years to treat breast cancer [3]. This case report presents a combination of first primary breast cancer and secondary primary vulvar melanoma, and endometrial polyp with tamoxifen maintenance.

Case Report

A 55-year-old female presented with vulvar melanoma. Her main complaint was the presence of a mass on her vulva that had been increasing in size over a six-month period, in addition to vulvar itching and swelling. The mass presented superficial ulceration, bleeding, and was located in the upper half of the right labia minora. It was radically excised with vulvectomy and the diagnosis of melanoma was confirmed using HMB-45, Melan-A, and anti-S-100 (Figure 1). Melanoma cells were positive for vimentin, HMB45, MelanA, S100, and EMA and negative for SMA, cytokeratin, myogenin, and MyoD1. The histopathological tumor, node, metastasis (TNM) stage was pT4bN0M0, Stage IIC, concordant with the clinical stage, in both American Joint Committee on Cancer (AJCC) and Union for International Cancer Control (UICC) classification systems. The patient has no family history of melanoma or dysplastic nevi.

Before radical excision with vulvectomy, the gynecologic examination, revealed a mass measuring 3 x 4 x 4 cm protruding from the cervical os. The mass was extirpated under general anesthesia. The mass originated from the endometrial cavity. The endometrial polyp measured 10 x 6 x 3 cm macroscopically and was confirmed to be benign under microscopic examination (Figure 2).

Two years prior she underwent left radical mastectomy and axillary lymphadenectomy. The patient stated that she found a lump in her breast one year prior before accepting a physician examination, but she chose to not undergo treatment which allowed the mass to enlarge. She had an excision of a left chest wall breast carcinoma with a 3.5 cm lump in the upper outer quadrant. The final pathology of the right radical mastectomy revealed that the lump was an infiltrating duct carcinoma diagnosis, pT2 pN1 bipMx, Grade 2 Stage IIB (Figure 3). Left axillary nodal clearance showed that four out of twelve lymph nodes contained metastatic carcinoma. Then, the patient underwent six cycles of adjuvant chemotherapy and radiotherapy on the left side of the chest and in axillary and supraclavicular lymph nodes area, and tamoxifen maintenance for about two years.

Discussion

Breast cancer patients subsequently developing a melanoma

Many previous studies have noted associations between the two malignancies: breast cancer and melanoma. The BRCA2 gene has been associated not only with an increased risk for breast cancer, but also with increased risk for melanoma. There were families that breast cancer and melanoma within either the same pedigree or the same patient [4]. This report supported these findings suggesting an association and that common genes influenced the onset of breast cancer, melanoma, and especially vulvar melanoma, although its incidence is rare.

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of melanoma in breast cancer patients following radiation [5]. As nearly one in ten cancer diagnoses were second (or higher) malignancies [6], it is important to understand the contribution of radiotherapy to second cancer induction.

**Tamoxifen citrate and melanoma**

Tamoxifen, an anti-estrogenic synthetic drug, is widely used in the treatment of breast cancer. Due to its partial agonistic effect, long-term tamoxifen usage may give rise to both pre-malignant and malignant uterine corpus lesions and functioning ovarian cysts. Physicians should be aware of the confounding effects of tamoxifen on the histological and ultrasonographic appearance of the endometrium, and suggest patients to accept the exam at regular intervals.

**Radiation treatment**

Radiation is known to induce malignant transformation, and radiotherapy was suggested to induce secondary malignancies. Specifically there is a 42% increased risk of melanoma in breast cancer patients following radiation [5]. As nearly one in ten cancer diagnoses were second (or higher) malignancies [6], it is important to understand the contribution of radiotherapy to second cancer induction.

**References**

Vulvar melanoma and endometrial polyp following breast carcinoma: a case report


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Figure 3. — The primary breast carcinoma (hematoxylin-eosin stain, × 200), and metastasis carcinoma in a lymph node (hematoxylin-eosin stain, × 100).
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