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Contents

Expression of p53 protein in the endometrial polyp in postmenopausal women
Hyperplasia in endometrial polyps could be due to the increase of p53 expression.

Attitudes and practices of Korean gynecologists towards hormone replacement therapy in endometrial cancer survivors
S.J. Lee, S.G. Yeo, S.B. Kang, D.C. Park - Seoul, KOREA
Hormone replacement therapy given after treatment for endometrial cancer is considered acceptable by Korean gynecologists.

Research on sequence variations analysis of HPV-16 type in Southwestern China
Y. Wang, X. Ding, Y. Zhu - Chongqing, CHINA
Compared with other HPV-16 reference sequences, nucleotide sequencing analysis of HPV-16, E6, E7, and L1 gene from cervical biopsies in southwestern Chinese women show point mutation.

Evaluation of the outcome benefit conferred by intensive surveillance strategies in women with early-stage endometrial cancer
G. Kiran, J.P. Kesterson, K. Ozerman, M. Kanis, A. Groman, S. Lele - Buffalo, USA
Intensive follow-up in patients treated for early-stage endometrial cancer does not improve the survival rate compared to patients with symptomatic recurrence.

Response to neoadjuvant chemotherapy with paclitaxel and cisplatin in locally advanced cervical cancer
A.S. Mousavia, S. Vahidi, M. Karimi-Zarchi, M. Modarress-Gilania, F. Ghaemmaghamia - Tehran, IRAN
Neoadjuvant chemotherapy in advanced cervical cancer with paclitaxel and cisplatin improve the outcome of surgery.

When to perform palliative surgery in the treatment of ovarian cancer: a brief review
Palliative surgery in ovarian cancer patients may improve the survival and the quality of life.

Correlation of cervical intraepithelial neoplasia with expressions of p16 and Ki67 in exfoliated cervical cells in fluid-based thin-layer samples
K. You, Y.L. Guo, L. Geng, J. Qiao - Beijing, CHINA
Immunocytochemical examination of p16 and ki67 in exfoliated cervical cells could be used for the detection of CIN II or greater.

Evaluation of the importance of the serum levels of CA-125, CA15-3, CA-19-9, carcinoembryonic antigen and alpha fetoprotein for distinguishing benign and malignant adnexal masses and contribution of different test combinations to diagnostic accuracy
M. Bozkurt, A.E. Yumru, İ. Aral - Istanbul, TURKEY
CA-125 and CA 15-3 serum levels were found to be statistically significant to distinguish benign and malignant ovarian tumor.

The effect of coexisting uterine myomas on clinico-pathological variables of endometrial carcinoma
J. Menczer, E. Ben-Shem, A. Golan, T. Levy - Tel Aviv, ISRAEL
The presence of myomas does not affect clinico-pathological variables of endometrial cancer patients nor their survival.
Post-treatment human papillomavirus status and recurrence rates in patients treated with loop electrosurgical excision procedure conization for cervical intraepithelial neoplasia

Loop electrosurgical excision procedure can eliminate HPV infection.

Comparing letrozole with medroxyprogesterone acetate (MPA) as hormonal therapy for simple endometrial hyperplasia without atypia in adult and middle-aged women

A. Tabatabaie, M. Karimi Zarchi, M. Dehghani-Tafti, A. Miratashi-Yazdi, S. Teimoori, A. Dehghani - Yazd, IRAN
Letrozole seems to have less complications than medroxyprogesterone acetate in the hormonal therapy of endometrial simple hyperplasia.

Is the 2009 FIGO staging system really valuable for Stage I endometrial cancer?

F. Atalay, K. Cetinkaya, A. Bacinoglu - Ankara, TURKEY
The 2009 FIGO staging system has better predictive value than the 2008 system, for early stage endometrioid endometrial cancer.

In vitro chemosensitivity assay of ascites in epithelial ovarian cancer

X. Xu, H. Dai, Y. Zhao, Y. Wang, X. Xu, Z. Qian, X. Chen - Nanjing, CHINA
The result of the authors' experience demonstrates that chemotherapy based on chemosensitivity testing improved patient outcome, helps to avoid adverse effects, and is economical.

Characteristics of diagnosis and therapy of adolescent malignant ovarian tumors

M. Su, W. Chang, T. Xu, M. Cui, S. Wu, P. Su - Changchun City, CHINA
A review of adolescent malignant ovarian tumor, especially regarding early diagnosis and therapy, is presented.

A case of ovarian psammocarcinoma with homolateral serous cystoadenofibroma and thecoma associated with Brenner tumour and cystoadenofibroma of the contralateral ovary

G. Giordano, F. Brigati, E. Varotti - Parma, ITALY
Multiple co-existing malign and benign tumors in the same ovary, with contralateral cystoadenofibroma.

XY gonadal dysgenesis – development of a germ cell tumor: case report

J. Diessner, T. Stüber, B. Niederle, M. Pawlik, J. Dietl, A. Honig - Würzburg, GERMANY
A case of gonadoblastoma with monolateral dysgerminoma is reported.

Bilateral poorly differentiated Sertoli-Leydig ovarian tumor associated with dysgerminoma: case report

M. Zamurovic, V. Soldo, N. Cutura - Belgrade, SERBIA
A rare case of bilateral poorly differentiated Sertoli-Leydig ovarian tumor associated with a mainly cystic structure dysgerminoma is described.

An unusual clinical presentation of a pure yolk sac tumor of the ovary: case report

D. Caserta, E. Ralli, G. Bordi, M. Moscarini - Rome, ITALY
A case of pure yolk sac tumor of the ovary is referred.

Intraparenchymal metastasis to the accessory spleen from ovarian cancer: a case report

V. Mihmanli, G. Toprakci, N. Cetinkaya, A. Kilickaya, G. Kamali - Istanbul, TURKEY
A case of primary ovarian cancer metastasizing to accessory spleen is described.
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Introduction

In postmenopausal women, abnormal uterine bleeding includes vaginal bleeding 12 months or more after the cessation of menses, or unpredictable bleeding in postmenopausal women who have been receiving hormone therapy for 12 months or more [1]. Further evaluation of abnormal uterine bleeding depends on the patient’s age and the presence of risk factors for endometrial cancer, which include anovulatory cycles, obesity, nulliparity, age greater than 35 years, and tamoxifen and estrogen therapy [2-4]. Initially, medical management is recommended for premenopausal women at low risk for endometrial carcinoma and in patients who are at high risk who continue abnormal bleeding despite medical management. The increased risk of endometrial cancer developing in polyps in this iatrogenic context is estimated between 2.5% and 10% in the literature [7]. In fact, Pettersson et al suggested that the polyp might be considered high-risk factor to development of endometrial cancer. However, there is a great concern on what polyp may turn to cancer. For this reason, the aim of this study was to evaluate the p53 expression on polyps of postmenopausal women.

Materials and Methods

A total of 200 postmenopausal women from Gynecology Department of Federal University of São Paulo – Escola Paulista de Medicina (last natural menstruation at least 12 months prior to entering the study) were evaluated between December 2000 and May 2004. Of these, 118 were selected for enrollment to this study. In order to be considered for inclusion to this study, women had to have uterine bleeding and should not have been using hormone therapy in the 180 days preceding admission to the study. Exclusion criteria consisted of body mass index (BMI) ≥ 35, women who were taking herbal substances or phytoestrogens; those who practiced physical exercise (with the exception of light walks fewer than three times a week); those who had endometrial thickness < three mm according to ultrasonography carried out within the previous two months; those who had any abnormality detected by recent cervico-vaginal oncological colpocytology. Women presenting dyslipidemia, diabetes mellitus, and acute or chronic hepatopathies were also excluded, as well as those using cholesterol-reducing medication, androgens, relaxifene, tamoxifen, barbiturates, hydantoïn, carbamazepine, phenylbutazone, meprobamate and rifampicin, and those with hormone-dependent cancer. The authors also excluded the pro-

Original Articles

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Expression of p53 protein in the endometrial polyp in postmenopausal women

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Summary

Objective: To evaluate p53 protein expression in the endometrial polyp and compare with adenocarcinoma and atrophic endometrium of postmenopausal women. Materials and Methods: Ninety-eight postmenopausal women were included in this study and divided into three groups related to histopathologic diagnosis: Group A - endometrial adenocarcinoma (n = 40), Group B – endometrial polyp (n = 38), and Group C – endometrial atrophy (n = 20). The length of this study was from 1990 to 2004. The endometrial samples were collected from hysteroscopic biopsy or surgery then processed for histopathologic routine. One thousand cells of each histological section were evaluated for immunohistochemical analysis using p53 antibodies. The ANOVA test was performed for the statistical analysis. Results: The expression of p53 in adenocarcinoma samples was the highest. The expression of polyp was positive when associated to hyperplasia without atypia. All samples of atrophic endometrial were negative. Conclusions: The present data suggested that presence of hyperplasia in the endometrial polyp is factor to increase the expression of p53.

Key words: P53; Endometrial polyp; Postmenopause; Women; Adenocarcinoma.
liferative or hyperplasic endometrium. Only polyps (with and without hyperplasia) were included. All subjects voluntarily agreed to participate in the study, which was approved by the Institution’s Ethics Committee in Research, and all patients signed informed consent forms.

Prior to the initiation of study protocol, all patients were submitted to general physical and gynecological examinations, and their medical history was recorded. Blood samples of all patients were collected in the morning, following a 12-hour fasting for the measurement of serum levels of glucose, follicle stimulating hormone (FSH), and 17beta-estradiol. In addition, all participants were submitted to pelvic ultrasonographic evaluation. After that, all participants submitted to hysteroscopy using CO2. The same equipment was used in every hysteroscopy and every examination was performed by the same experienced hysteroscopist, who was unaware of the women’s background. The hysteroscope utilized had a diagnostic sheath with a diameter of 2.8 mm and lances with an inclination of 30°. The Hamou microhysteroflator with a maximum pressure of 200 mmHg and a gas flow of 25 ml/min was used for the infusion of CO2. The illumination of the endometrial cavity was achieved by a source supplying cold light xenon illumination, through a wire of optical fibres. The hysteroscopy began with a gas flow of 25 ml/min and a pressure of 40 mmHg. In many cases, this pressure was inadequate for panoramic examination of the endometrial cavity and the imaging of the tubal ostia, and therefore it was increased during the examination. The maximum pressure, which was used in order to distend the endometrial cavity, was 100 mmHg. All of the hysteroscopies were recorded on video and were safely kept in the record room of the hysteroscopy office at the Federal University of São Paulo – Escola Paulista de Medicina. After that all participants were submitted to endometrial biopsy using Pipelle instrument. When the sample was insufficient for analysis, the patient was submitted to hysteroscopic resection under anesthesia. A total of 118 patients were divided into three groups related to histopathologic diagnosis: Group A - endometrial atrophy (n = 40), Group B – endometrial polyp (n = 38), and Group C – endometrial adenocarcinoma (n = 40). Figure 1 shows the representative image of endometrial cavity in each group.

![Figure 1](image1.png)

**Figure 1.** — Representative image of endometrial cavity in each group: A – endometrial atrophy, B – endometrial polyp, and C – endometrial adenocarcinoma.

Histological analysis

Paraffin-embedded blocks were sectioned and the slides were stained with hematoxylin-eosin to be evaluated through light microscopy. Two pathologists performed the analyses and established the final histological diagnosis. They had no access to clinical information. The samples were classified and graded according to WHO recommendations.

Immunohistochemistry

The samples were prepared for the immunohistochemical procedure. The p53 was detected with antihuman p53 monoclonal antibody. Briefly, five-µm sections were deparaffinized and hydrated through graded alcohols and water. Peroxidase...
was blocked for 7.5 minutes in a peroxidase-blocking solution. Then the slides were incubated with the primary antibodies for 30 minutes and washed a buffer solution. The peroxidase-labeled polymer was then applied for 30 minutes. After washing in a buffer solution, the slides were incubated with diaminobenzidine substrate chromogen solution, washed in water, counterstained with hematoxylin, washed, dehydrated and mounted.

The measurements were performed using digitalized images (Figure 2) obtained directly from the light microscope via a video camera and stored in magnetic medium. Afterwards, measurements were performed using specific software. The percentage of cells positive for p53 immunoexpression on the epithelium was calculated on the basis of the staining presented on an average of ten microscopic fields at x400 magnification. Tissue samples were considered positive for p53 when the ratio of cells with only nuclear staining was equal to or more than ten percent. Also, we counted the number of positive cells (p53 expression) in 1000 cells. The positive control for p53 marker was a breast carcinoma sample from the laboratory archives.

Statistical analysis
The characteristic data of groups was analyzed by one-way repeated measures analysis of variance subsequently corrected by less significant difference comparison test (Fisher test). The power calculation of 90% was 30 cases per group.

Results
Characteristic (clinical and laboratorial) data are in Table 1. The statistical analyses showed not a difference among groups in all parameters.

Table 1. — Characteristics of participants (mean ± standard error) in groups: A – endometrial atrophy, B – endometrial polyp, and C – endometrial adenocarcinoma

<table>
<thead>
<tr>
<th>Groups</th>
<th>A (n = 40)</th>
<th>B (n = 38)</th>
<th>C (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at menarche (years)</td>
<td>12.9 ± 0.2</td>
<td>13.0 ± 0.3</td>
<td>12.9 ± 0.3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.2 ± 1.8</td>
<td>63.5 ± 1.9</td>
<td>66.7 ± 1.4</td>
</tr>
<tr>
<td>Time since menopause (years)</td>
<td>12.7 ± 2.5</td>
<td>8.8 ± 4.7</td>
<td>9.1 ± 4.1</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>30</td>
<td>28</td>
<td>31</td>
</tr>
<tr>
<td>Non-Caucasian</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Number of pregnancy (n)</td>
<td>3.9 ± 0.5</td>
<td>4.7 ± 0.7</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>Number of abortion (n)</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>112.5 ± 7.5</td>
<td>103.8 ± 4.1</td>
<td>105.4 ± 11.2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122.1 ± 13.9</td>
<td>123.1 ± 15.4</td>
<td>118.1 ± 15.1</td>
</tr>
<tr>
<td>FSH (mUI/ml)</td>
<td>69.1 ± 19.9</td>
<td>62.1 ± 17.4</td>
<td>67.3 ± 18.1</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>12.1 ± 9.3</td>
<td>13.1 ± 7.4</td>
<td>11.1 ± 8.5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81.7 ± 10.9</td>
<td>82.6 ± 14.3</td>
<td>83.9 ± 11.3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.4 ± 1.1</td>
<td>29.3 ± 1.7</td>
<td>27.4 ± 1.6</td>
</tr>
</tbody>
</table>

The statistical analyses showed not a difference among groups in all parameters.

Table 2. — The p53 expression in groups: A – endometrial atrophy, B – endometrial polyp, and C – endometrial adenocarcinoma

<table>
<thead>
<tr>
<th>Groups</th>
<th>A (n = 40)</th>
<th>B (n = 38)</th>
<th>C (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive of p53 in 1,000 (mean ± standard error)</td>
<td>0</td>
<td>16.5 ± 6.9</td>
<td>149.4 ± 41.5*</td>
</tr>
<tr>
<td>Number of positive case</td>
<td>0/40</td>
<td>2/38</td>
<td>21/40</td>
</tr>
</tbody>
</table>

*p < 0.001 compared to other groups.

Discussion
The endometrial polyp is a benign pedunculate or sessile excrescence of the endometrium, which contains variable amounts of glands, fibrous tissue, and blood vessels. Clinically, this lesion usually causes endometrial thickness and abnormal uterine bleeding [1-4]. In postmenopausal women, endometrial polyp is asymptomatic and frequent, and is observed in about 15% [9]. Also, carcinomatous changes in endometrial polyps have also been reported [8-10]. These polyps have been named malignant endometrial polyps. The great concern is what type of polyp may have the potential to develop the endometrial cancer. For this reason, the authors analyzed the p53 expression on the endometrial polyp.

Overexpression of the p53 oncogene appears to play an important role in the biology of endometrial cancers type II (serous), which show significantly higher expression of
p53 than do endometrioid subtypes. Unlike the endometrioid subtypes, p53 mutations occur early in tumorigenesis. The endometrial carcinoma has abnormal p53 overexpression. A p53 mutation is associated with loss of ER and PR expression, and even among cancers, confers poorer overall survival. In fact, some authors suggested that p53 might be an independent prognostic indicator among metastatic risk cases. In addition, some authors suggested that p53 is a good for identifying the endometrial cancer [11-12].

Proliferative diseases of the endometrium represent a broad continuum of morphologic and cellular changes, ranging from simple hyperplasia to invasive carcinoma. Hyperplasia is an abnormal proliferation of the endometrial glands, and in some cases, it precedes endometrial cancer as a premalignant phase. In fact, the authors only found p53 expression on the polyps with hyperplasia with atypias suggested that these cases might be the risk for developing endometrial cancer.

References


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Attitudes and practices of Korean gynecologists towards hormone replacement therapy in endometrial cancer survivors

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Summary

Purpose: To investigate the attitudes of Korean gynecologists towards prescribing hormone replacement therapy (HRT) after treatment for endometrial cancer. Materials and Methods: A questionnaire, addressing attitudes towards HRT and treatment strategies for patients previously treated for endometrial cancer, was distributed to 163 Korean gynecologists. Results: Of the 163 gynecologists that were sent this questionnaire, 98 (60.1%) responded. Among the respondents, 81 (82.7%) had previously prescribed HRT to patients with endometrial cancer. Of the latter, 75 (92.6%) had prescribed HRT to patients with Stage I, and more than half to patients with Stage II, endometrial cancer. Of the respondents who had prescribed HRT, 33 (40.7%) did so without regard for cancer-cell type and 33 (40.7%) began patients on HRT more than two years after endometrial cancer treatment. Tibolone was the most commonly prescribed drug (61.9%). The most common reason not to prescribe HRT was fear of cancer recurrence (38.1%). Conclusion: Most of the Korean gynecologists surveyed had experience prescribing HRT to endometrial cancer patients. Although HRT is not actively recommended, HRT given post-therapy to endometrial cancer patients is considered acceptable.

Key words: Endometrial cancer; Menopause; Hormone replacement therapy; Tibolone; Estrogen; Progesterone.

Introduction

Endometrial adenocarcinoma is the most common gynecological cancer in Western countries, with an incidence of 22 per 100,000 women. The disease occurs more frequently in postmenopausal women, although 25% of endometrial cancers occur in premenopausal women [1]. Recently, the incidence of endometrial cancer has increased in Korean women [2]. The treatment of choice in women with endometrial cancer consists of a total hysterectomy and bilateral salpingo-oophorectomy, which can induce an abrupt onset of menopausal symptoms in premenopausal women. Postmenopausal women can also suffer from discomfort arising from estrogen-deficiency. Therefore, hormone replacement therapy (HRT) is utilized to relieve menopausal symptoms in endometrial cancer patients [3].

In 2003, the North American Menopause Society stated that menopausal symptoms, such as vasomotor symptoms and sleep disruption, may be primary indicators of the need for systemic HRT [4]. HRT has been shown to be beneficial in the prevention of osteoporosis, coronary heart disease, and colon cancer, and in promoting patient quality of life [5, 6]. However, HRT has also been reported to increase the risk of breast cancer [7] and is absolutely contraindicated in endometrial cancer patients. Factors associated with an increased risk of recurrence in women who are administered HRT after treatment for endometrial cancer have not been identified [3]. Practically, HRT is often administered to women treated for Stages I or II endometrial cancer who complain of menopausal symptoms [3]. Although the safety of exogenous estrogen with regard to the risk of endometrial cancer recurrence has not been established, the absolute recurrence rate was low [8]. Thus, gynecologists have had difficulty deciding on whether to prescribe HRT to endometrial cancer patients.

Although many surveys of gynecologists have assessed their use of HRT in cancer patients, only two studies to date have reported a consensus among gynecologists in recommending HRT for endometrial cancer patients [9, 10]. In Korea, the indications for HRT in endometrial cancer patients have not been clearly determined. The authors therefore evaluated the attitudes of Korean gynecologists towards the use of HRT in survivors of endometrial cancer and their practices in prescribing HRT for these patients.

Materials and Methods

From September to December 2011, a questionnaire was sent by e-mail to 163 gynecologists registered as members of the Korean Gynecologic Oncology Group, part of the Korean Society of Gynecologic Oncology, and to other oncologists who did not join the group or had retired. The questionnaire addressed the attitudes and practices of gynecologists with respect to prescribing HRT for patients who had been previously treated for endometrial cancer.

Gynecologists who had expressed their wish not to participate in the study were excluded. The questionnaire asked for the following information: (1) personal data on the gynecologist,
including his or her age, sex, place of work, and areas of expertise; (2) the number of patients per year and the number of patients with endometrial cancer per year treated by the gynecologist and the rate of administration of HRT to the latter; (3) the Stage, grade, and cell type of endometrial cancer; (4) the types of HRT prescribed to patients with endometrial cancer; and (5) the side-effects of HRT. All of the questions were designed as closed questions except for those relating to personal information about the gynecologists and the side-effects of HRT (Figure 1).

Collected responses were analyzed using SAS statistical software (version 8.0). Differences in frequency were evaluated using Student’s t tests. A p value < 0.05 was considered statistically significant.
Attitudes and practices of Korean gynecologists towards hormone replacement therapy in endometrial cancer survivors

Results

Demographic data of the responding gynecologists

Of the 163 gynecologists sent questionnaires, 98 responded (60.1%). Table 1 shows the demographic characteristics of the study sample. Of the 98 respondents, 88 (90.0%) reported expertise in gynecologic oncology; 93 (94.9%) were male, and five (5.1%) were female. The authors found that 87 (88.8%) worked in academic settings and nine (9.2%) in private offices ($p < 0.05$); the other two (2.0%) had recently retired. In Korea, most patients with endometrial cancer, except for those undergoing incidental or emergency operations, are treated by a gynecologic oncologist, and most patients prefer an academic hospital. Over a one-year period, 72 of the 98 respondents (73.5%) had treated more than ten patients newly-diagnosed with endometrial cancer, and 12 (12.5%) had treated more than 50 such patients.

Prescription of HRT

Of the 98 respondents, 81 (82.7%) had previously prescribed HRT to patients with endometrial cancer, although 54 (54.8%) had prescribed HRT to fewer than 10% of their patients with endometrial cancer. When the authors divided the gynecologists into two groups according to age, < 45 and > 45 years, they found that the younger gynecologists were more willing to prescribe HRT than the older gynecologists (86.8% vs 73.3%), but this difference was not statistically significant. There was no significant difference in HRT prescription rate between male and female gynecologists, most likely due to the small number of the latter included in this study.

Indications for HRT

Stage: When the authors evaluated patient selection criteria for prescribing HRT, they found that 75 of the 81 (92.6%) respondents who prescribed HRT to endometrial cancer patients did so for patients with Stage 1, and more than half for patients with Stage II, endometrial cancer.

Cell type: Of the 81 gynecologists who had prescribed HRT to endometrial cancer patients, 44 (54.3%) did so only for patients with endometrioid-cell tumors, whereas 33 (40.7%) prescribed HRT to endometrial cancer patients without regard for cancer cell type.

Grade of tumor: The authors found that 79 of the 81 (97.5%) respondents who had prescribed HRT to endometrial cancer patients did so for patients with tumor grades I and II, with 19 (23.5%) prescribing HRT only to patients with grade I tumors.

Onset of treatment: Thirty-three of the 81 respondents who had prescribed HRT (40.7%) did so two years or more after the patient had completed treatment for endometrial cancer, whereas 19 (23.5%) started patients on HRT after the onset of climacteric symptoms, even during cancer treatment.

Duration of HRT

The authors found that 62 of the 81 (76.5%) gynecologists who had prescribed HRT preferred that patients be treated for less than five years.

Choice of medication for HRT

Of the 81 respondents who had prescribed HRT, 50 (61.7%) preferred tibolone, 27 (33.3%) preferred combined continuous estrogen/progesterone therapy, 12 (14.3%) preferred selective estrogen receptor modulators (SERMs), and 15 (18.5%) preferred estrogen only (p =
0.03). Preferences were unrelated to the location of the practice or the age or sex of the gynecologist. Figure 2 shows the preferred types of HRT.

**Side-effects of HRT**

Nine (11.1%) of the gynecologists reported minimal adverse effects of HRT, including breast tenderness, headache, and weight gain. The only serious adverse effect was thromboembolism in one patient, but a direct relationship between this thromboembolism and HRT was not identified. Furthermore, the authors could not determine whether any of the adverse effects was correlated with treatment for endometrial cancer.

**Reasons not to recommend HRT**

The three most frequent reasons cited by respondents to avoid HRT in women with endometrial cancer were (1) HRT may promote the recurrence of endometrial cancer (38.1%); (2) the patient has no complaints of menopausal symptoms (26.2%); and (3) patient refusal (21.4%).

**Discussion**

In Korea, the tendency to treat menopausal women with HRT has increased, with 25% of menopausal Korean women having a history of undergoing HRT [7]. HRT contributes to the relief of menopausal symptoms, such as hot flashes, sweating, and vaginal dryness. In the Women’s Health Initiative clinical trial, treatment with estrogen plus progesterin reduced the risks of colon cancer and bone fractures; however, it increased the risks of breast cancer and deep vein thrombosis [11]. These findings have led to changes in HRT prescription patterns, including decreasing the number of prescriptions and increasing the use of alternative medications, such as non-estrogenic drugs and phytoestrogen [12, 13].

Menopausal symptoms are a serious problem for endometrial cancer survivors [8]. Since endometrial cancers are estrogen-dependent, prescribing HRT has been generally contraindicated in endometrial cancer survivors, since HRT may stimulate cancer recurrence. Gynecologists have more confidence than other physicians regarding the role of HRT during the climacteric [14]. Of four studies assessing the use of HRT in endometrial cancer survivors, there was no increased risk of recurrence or death in the HRT group [8, 15-17]. Together, these findings suggest that HRT is safe in endometrial cancer patients.

Sixty-seven percent of Belgian physicians preferred to prescribe HRT to endometrial cancer survivors, with 49% of the latter prescribing estrogen-only therapy [9]. In contrast, 69.6% of Greek gynecologists reported not recommending HRT because of fears of endometrial cancer recurrence [10]. A review of other gynecological cancers showed that 48% of Greek gynecologists prescribed HRT to ovary cancer survivors, with 60% of the latter prescribing tibolone [18].

The present authors found that the percentage of Korean gynecologists who had prescribed HRT to endometrial cancer patients was very high (82.7%), although 54.8% prescribed HRT to fewer than 10% of their patients with endometrial cancer. These findings suggest that Korean gynecologists were generally hesitant to prescribe HRT to endometrial cancer patients. Similar to Greek gynecologists, the most common reason cited by Korean gynecologists for not offering HRT was the fear of stimulating endometrial cancer recurrence.

The present authors found that 61.9% of the Korean gynecologists who responded to this survey preferred tibolone as an alternative to HRT after endometrial cancer treatment. In comparison, 24.8% of Greek gynecologists preferred alternatives to HRT, such as selective estrogen receptor modulators (SERMs) and phytoestrogen [10]. Tibolone is useful for treating menopausal symptoms arising from estrogen deficiency, without inducing endometrial proliferation [19]. Raloxifene, a SERM, has also been reported to not have a proliferative effect on the endometrium. Unfortunately, the safety of tibolone and raloxifene as alternatives to HRT after endometrial cancer treatment has not been adequately investigated [4]. Therefore, women must be informed about the potential risks of using these alternatives [3].

The present authors found that 33.3% of Korean gynecologists gave combined continuous estrogen/progestrone treatment (CECEPT) to survivors of endometrial cancer. A German study found that CCEPT did not induce de novo hyperplasia in any woman, but restored endometrium with complex hyperplasia to normal histology [20]. CCEPT is still frequently used as a form of HRT in post-endometrial cancer patients [4].

The main factor influencing the attitudes of breast cancer patients toward HRT is the presence or absence of menopausal symptoms, with more postmenopausal than premenopausal women willing to try HRT [21]. Although the available evidence indicates no association between HRT and endometrial cancer, many Korean gynecologists are reluctant to prescribe HRT to survivors of endometrial cancer, mainly because of the fear of triggering disease recurrence or of adverse professional and legal consequences.

In conclusion, the majority of the Korean gynecologists who responded to this survey had prescribed HRT to endometrial cancer patients. Although HRT is not recommended for these patients, it is considered relatively acceptable in Korea.

**Acknowledgements**

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References


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Research on sequence variations analysis of HPV-16 type in Southwestern China

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Summary

Objective: This study was designed to analyze sequence variations in E6, E7, and L1 of human papillomavirus type 16 (HPV-16) to identify novel HPV-6 variants and correlate them with the progression of cervical cancer. Materials and Methods: Cervical biopsies from 12 HPV-16 positive cervical neoplasia cases were analyzed by polymerase chain reaction (PCR) for E6, E7, and L1 gene. The products of PCR were ligated into pGEM-T vector and used to transform e. coli DH5α. Finally, the sequence of the insert was determined by an automated DNA sequencer. Results: Compared with the European-Germany (EG) (131), 20 mutations were detected, of which eight mutations were detected from all the four biopsies: 131(G-A)(Gly-Arg), 178(T-G)(Asp-Glu), 350 (G-T)(Val-Leu), 647(A-G)(Asn-Asp), 846(T-C)(synonymous mutation), L1 966th(C-T)(synonymous mutation), L1 1302 (C-T)(synonymous mutation) and L1 1434th(A-G) (synonymous mutation). Conclusions: Compared with other HPV-16 reference sequences, nucleotide sequencing analysis of the HPV-16, E6, E7, and L1 gene from cervical carcinoma biopsies in Southwestern China consistently shows point mutations, including synonymous mutation and nonsynonymous mutation. Above all, the results may have implications for future researches of viral persistence, transmission, onco- genic potential, developing diagnostic probe, and designing vaccine or biological preparation for a particular population.

Key words: Human papillomaviruses; Cervical cancer; Sequence analysis; Phylogenetic analysis.

Introduction

Cervical cancer is the major cancer in Chinese women and a leading cause of cancer deaths. Every year, more than 130,000 new cases and about 70,000 deaths are recorded. The persistent infection by specific types of high-risk human papillomaviruses (HR-HPVs) is essential for the progression of cervical lesions, and women who are infected with HR-HPVs are likely to develop cancer. Various studies have demonstrated that more than 70% of invasive cervical cancers harbor HPV type 16 (HPV-16) and HPV-18, and the products of viral transforming E6 and E7 genes have been shown to contribute to tumorigenesis by functionally inactivating two important cellular tumor suppressor proteins, p53, and retinoblastoma. More than 100 types of HPV are known, but only about 30 types are associated with anogenital cancer. According to the Papillomavirus Nomenclature Committee, a new HPV type is defined by a nucleotide sequence variation of more than ten percent compared to that of other known HPV types in the E6, E7, and L1 open reading frames. Those differing by two to ten percent are referred to as subtypes, whereas intratype variants may vary by up to two percent in the coding region and five percent in the noncoding region compared to that of the prototype [1].

On the basis of sequence variations in E6, L1, L2, and the long control region (LCR), HPV-16 variants have been identified and grouped into six distinct phylogenetic branches: E (European), AA (Asian-American), Af1 (African 1), Af2 (African 2), As (Asian), and NA (North American). These variants have been found to show different geographic distributions, with various oncogenic potentials. A number of sequence variations have been reported for HPV-16 E6, E7, and L1 genes in cervical cancer. Studies also have shown that specific intratype variants may influence the persistence of HPV infection and the progression of precursor lesions to cancer. HPV variants may also affect virus assembly, immunologic responses, pathogenicity, p53 degradation, immortalization activity, and the regulation of transcription. These variations immediately affect the sensitivity and specificity of different PCR-based genotype diagnostic methods [2, 3].

Of the two HPV-16 oncogenes: E6 and E7, E6 has been found to show more variations than E7, which is relatively conserved. The analysis of the L1 gene, which codes for the viral major capsid protein, is of immense importance because of its high diagnostic value. The range of intratype variation observed in this region allows the distinction and assessment of known and novel HPV types. This is also an important target for the development of HPV vaccines. Very few reports are available on HPV variants from this region.

In China, as high as 98% of the cervical carcinoma cases are found to harbor HPV infection and the most prevalent (80%) type is HPV-16 [4]. Although the prevalence of HPV and cervical cancer in China is the highest in the world, there is not much information on HPV variants from different regions of China. In the present study, the authors have examined the sequence variations in E6,
E7, and L1 genes of the most prevalent HR-HPV type, HPV-16, and correlated them with the histopathologic grades of tumors, and oncogenic potential.

Materials and Methods

Specimens

Specimens were available from cervical biopsies of 12 women with CC positive for HPV-16 treated at the Cancer Hospital of Sichuan Province and the 4th People’s Hospital of Chongqing. The information of these patients was collected, including age, histological diagnosis, and the clinic stages of disease. The mean age of the patients in the study was 43 years (range 22-64). All tumors were classified as International Federation of Obstetrics and Gynecology (FIGO) Stage II, eight as Stage III.

Genomic DNA extraction

DNA was extracted using a commercially available kit. DNA extraction and the storage of extracted DNA were in a designated area free from amplification products.

Polymerase chain reaction (PCR)

PCR reagents were mixed under hoods in a designated room that was free from amplification products. The room was decontaminated nightly through scheduled ultraviolet light application. Amplification of the integrated HPV genes was performed by using the primers that designed according to HPV-16 reference sequence published in GenBank (accession number NC_001357) (Table 1).

A five-min denaturation step at 95°C was followed by 35 cycles of amplification. Each cycle included a denaturation step at 94°C for 45 s, an annealing step at 55°C for 45 s, and an elongation step at 72°C for 45 s. The final elongation step was prolonged for future ten min at 72°C.

PCR products were submitted for DNA purification and sequencing and the sequences were then analyzed and determined by NCBI Blast and DNAMAN version 5.2.2. The sites of HPV-16 DNA nucleotides were numbered according to the HPV-16 reference sequence (NC_001357).

All data were confirmed twice at least by repeat PCR amplification and sequence analysis.

Results

Results reverse membrane hybridization: in 12 specimens, 11 cases (91.7%) were HPV-positive, of which four were infected with HPV-16 alone (Table 2).

HPV-16 E6 E7 oncogenes and L1 capsid protein gene sequence variation analysis four cases of HPV-16 infection in specimens from individual HPV-16 oncogenes E6, E7 and L1 capsid protein gene by sequencing of the decision, and with the standard strains [5] to compare them with standard strains of different sites were considered variations. Compared with the EG131, a total of four specimens found 20 mutations, of which eight are shared by the four specimens (Tables 3 and 4).

For the E6 gene, a total of four specimens found four mutations: 131 (G-A) (Gly-Arg), 178 (T-G) (Asp-Glu), and 350 (G-T) (Val-Leu), of which 320 (A-G) (Ile-Val) is unique in one specimen.

For the E7 gene, four samples of three mutations were found, two of which are shared by the four samples: 647 (AG) (Asn-Asp) and 846 (TC) (the same nonsense mutations). Another 843 (T-C) was synonymous mutation.

As for the L1 gene mutations were found in 13 points, a total of three to four samples: 966 (CT) (synonymous mutation), 1,302 (CT) (synonymous mutation), and 1,434 (AG) (synonymous mutation). Seven unique to LEE: No. 503 (AG) (Asp-Gly), section 620 (AC) (Asn-Thr), section 627 (TC) (synonymous mutation), p. 648 (AG) (synonymous mutation), section 736 (GA) (Val-Ile), section 1,113 (TC) (synonymous mutation), and 1,256 (CG) (Ser-Cys).

Section 20 (A-G) (Thr-Cys) is unique CHEN. Section 112 (T-G) (Thr-Asp) is unique HUANG. Section 1,508 (A-G) (Lys-Arg) is common to ZHOU.

Discussion

Worldwide, cervical cancer incidence is each year about 100,000 new cases, 80% of which in developing countries, and in China every year about 131,500 new cases, accounting for new cases worldwide of 28.8%. Cervical cancer in recent years, occurs at a younger age: from the original 40-50 years to 35 years of age. HPV infection is the main risk factor for cervical cancer: 60% of these are HPV type 16. More than 99% of patients with cervical cancer are affected by HPV DNA [6]. The prevention of cervical cancer consists in the early individuation of HPV infection. The distribution of subtype of HPV can vary in different geographic areas. This factor should be considered when a prevention by vaccine is programmed. This paper considered cervical cancer patients in Southwestern China with the HPV-16 subtype characteristics of the main gene sequence variations.

In this study, in 12 specimens, 11 cases were HPV-positive infection rate was 91.7%, of which four were reinfected with HPV-16 alone; the other seven cases, three were single heavy infection, two cases of double infection, and two cases of triple infection. Multiple HPV infections increase or promote the occurrence of cervical lesions has been the concern of scholars. Most people believe that multiple infections did not increase the incidence of cervical cancer. In the IARC study, multiple infections patient group was 81.1%; in the control group it was 13.19%; in the control group multiple infection rate is higher. Kay et al. [7] on the South African cervical cancer and patients with CIN2-3 found that CIN2-3 has dual infection of HPV (12.4%), while 16% of cervical cancer has multiple infection related cervical lesions. Multiple infection does not increase the incidence of cervical cancer [8]. However, some authors believe that...
multiple infections and the incidence of cervical lesions and cervical cancer patients with multiple infections are less sensitive to radiotherapy [9]. Some authors have found that dual infection of HPV-16 is the most common subtype infection. HPV-16, 18, and 33 often have dual infections [10].

HPV-16 E6, E7 genes are the major transforming viral genes, which encode the E6 and E7 proteins containing “Cys-XX-Cys” zinc finger DNA binding protein, E6 protein of the N-and C-side of the formation of two zinc finger structure, were combined, the degradation of tumor suppressor gene p53. In vitro studies showed that the number of E6 mutant proteins on the degradation of p53 significantly reduced, while some other variation of the T cell receptor leads to potential HLA binding region or the amino acid changes; E7 protein C-terminal there are two zinc finger structures, N terminal 37 amino acid residues; E7 protein into the active site [11, 12]. Fujnaga et al. by PCR sequence analysis showed that the majority of invasive cervical cancer and precancerous lesions are detected HPV-16 E7 sequence variants. For the E6E7 gene, the four specimens in this study were found seven mutations, did not find any deletion or insertion mutations, mutations in the seven, five total of four specimens: 131 (GA) (Gly-Arg), 178 (TG) (Asp-Glu), 350 (GT) (Val-Leu), 647 (AG) (Asn-Asp), and 846 (TC) (synonymous mutation). Of which 350 (GT) (Val-Leu), 647 (AG) (Asn-Asp) in the country other studies has also been reported [13-15].

Ll gene structure gene, which encodes the virus capsid protein Ll protein, Ll protein molecular weight of about 54-58kDa, its form is the form pentamer shell coat of the virus particle, Ll gene sequence with a conservative, both type-specific characteristics, but also a result of geographical differences among the subtypes are also some variability [16]. Touze selected from different parts of the world’s six HPV-16 virus strains, compared with the prototypetype strain of the virus found Ll protein amino acids can be up to 15 variations, the VLP in the baculovirus expression system, the output is 1-79 times the range [17]. In this study four cases were found in 13 specimens of mutations, and insertion or deletion was not found. In the 13 mutations, a total of three to four samples, but were synonymous mutations. Seven unique to LEE: the first 627 (TC), section 648 (AG) and 1113 (TC) for the silent mutations; section 503 (AG) (Asp-Gly), section 620 (AC) (Asn-Thr), section 736 (GA) (Val-Ile), and 1256 (CG) (Ser-Cys). Section 20 (A-G) (Thr-Cys) is unique CHEN. Section 112 (T-G) (Thr-Arg) is common to ZHOU. In addition, Ll gene deletion mutants of different VLP formation also have an important impact, but this study did not find any deletion or insertion mutation.

---

**Table 1. — Primers used for PCR amplification.**

<table>
<thead>
<tr>
<th>ORF Primers</th>
<th>Site</th>
<th>Primer sequences</th>
<th>Product length</th>
</tr>
</thead>
<tbody>
<tr>
<td>E6 HPV16E6F</td>
<td>81</td>
<td>5’-TTATGCAACAAAAAGAGAACTGCA-3’</td>
<td>498</td>
</tr>
<tr>
<td>HPV16E6R</td>
<td>578</td>
<td>5’-GGTGATCTCCATGAGTACAG-3’</td>
<td></td>
</tr>
<tr>
<td>E7 HPV16E7F</td>
<td>559</td>
<td>5’-ATCATGCAATGAGAATCATCATCTG-3’</td>
<td>320</td>
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<tr>
<td>HPV16E7R</td>
<td>878</td>
<td>5’-GGAGATCATGATTGCATGTTCT-3’</td>
<td></td>
</tr>
<tr>
<td>L1+</td>
<td>HPV16L11F</td>
<td>5’-ACCAACGCTCTCTTACCTCTCT-3’</td>
<td>545</td>
</tr>
<tr>
<td>HPV16L11R</td>
<td>5955</td>
<td>5’-GGTGATGCTGTTGCTGACAGGAGCTTGC-3’</td>
<td>597</td>
</tr>
<tr>
<td>HPV16L12R</td>
<td>6529</td>
<td>5’-TCCGCTGCTGATCGCTGACAGGAGCTTGC-3’</td>
<td>660</td>
</tr>
<tr>
<td>HPV16L13R</td>
<td>7188</td>
<td>5’-ACAAACACACTGAGTCAACA-3’</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. — Prevalence of HPV types identified by reverse blot assay in the 12 biopsies collected.**

<table>
<thead>
<tr>
<th>Specimen Number</th>
<th>HPV-16</th>
<th>HPV-18</th>
<th>HPV-31</th>
<th>HPV-33</th>
<th>HPV-45</th>
<th>HPV-58</th>
<th>other b</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC-01</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>CC-02</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CC-03</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CC-04</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CC-05</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CC-06</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CC-07</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CC-08</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>CC-10</td>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>CC-11</td>
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</tr>
<tr>
<td>CC-12</td>
<td>+</td>
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**Table 3. — Nucleotide sequence variations in HPV-16 E6, E7, L1 ORFs.**

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<thead>
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<th>E6</th>
<th>E7</th>
<th>L1</th>
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<tr>
<td>520</td>
<td>1529</td>
<td>1513</td>
</tr>
<tr>
<td>AA</td>
<td>AA</td>
<td>AA</td>
</tr>
<tr>
<td>AF1</td>
<td>AF1</td>
<td>AF1</td>
</tr>
<tr>
<td>EA</td>
<td>EA</td>
<td>EA</td>
</tr>
<tr>
<td>EG131</td>
<td>EG131</td>
<td>EG131</td>
</tr>
<tr>
<td>CC-03</td>
<td>CC-03</td>
<td>CC-03</td>
</tr>
<tr>
<td>CC-04</td>
<td>CC-04</td>
<td>CC-04</td>
</tr>
<tr>
<td>CC-05</td>
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<td>CC-05</td>
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<tr>
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<td>CC-11</td>
</tr>
<tr>
<td>CC-12</td>
<td>CC-12</td>
<td>CC-12</td>
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</tbody>
</table>

**Table 4. — Nucleotide sequence variations in HPV-16 E6, E7, L1 ORFs.**

<table>
<thead>
<tr>
<th>Position</th>
<th>Type strain</th>
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<th>E7</th>
<th>L1</th>
</tr>
</thead>
<tbody>
<tr>
<td>213-250</td>
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<td>1111111111111</td>
<td>1111223344555</td>
<td>1358690502</td>
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<tr>
<td>344780235333</td>
<td>4 8 9 4 4 4</td>
<td>291305010248385160911364590013002</td>
<td>13852413110975576984073513425904687</td>
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</tr>
<tr>
<td>140-153</td>
<td>G G G G G G G G</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
</tr>
<tr>
<td>201-215</td>
<td>C C C C C C C C</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
</tr>
<tr>
<td>254-268</td>
<td>G G G G G G G G</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
</tr>
<tr>
<td>313-327</td>
<td>A T A T A T A T A</td>
<td>AA</td>
<td>AA</td>
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<tr>
<td>362-376</td>
<td>G G G G G G G G</td>
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<tr>
<td>411-425</td>
<td>A T A T A T A T A</td>
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<tr>
<td>466-480</td>
<td>G G G G G G G G</td>
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<td>AA</td>
<td>AA</td>
</tr>
<tr>
<td>521-535</td>
<td>A T A T A T A T A</td>
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<td>AA</td>
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<tr>
<td>576-590</td>
<td>G G G G G G G G</td>
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<td>AA</td>
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</tr>
<tr>
<td>631-645</td>
<td>A T A T A T A T A</td>
<td>AA</td>
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</tr>
<tr>
<td>686-700</td>
<td>G G G G G G G G</td>
<td>AA</td>
<td>AA</td>
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</tr>
</tbody>
</table>

AA: Asian-American; AF1: African-1; EA: East Asia; EG131: European-German 131.
HPV virus cannot be cultured in vitro; in vivo testing cannot be easily induced immune response. Could not be detected by serological typing its way. HPV type mainly depends on the virus nucleic acid hybridization and physical mapping of the viral genome analysis method to determine, usually 50% homology as the standard type. In recent years, the international community has put forward the virus to virus L1, E6, and E7 genes homologous to type as standard. If the nucleotide sequence between different strains of the homology of less than 90% were considered to belong to a different type. Despite the homology between HPV-16 types is very close, but based on data from different places (Europe, Asia, Africa, South America, and North America), the sequence characteristics of the local strains are divided into five branches: E (European), Aa (Asian-American), Af-1 (African-1) and Af-2 (African-2) [18]. In this paper, phylib3.6 evolutionary analysis software, respectively, E6, E7, L1, and E6-E7-L1 sequence according to a preliminary study in Southwestern China HPV-16 in cervical cancer the evolutionary relationship between subtypes and found that E6 gene alone can clearly distinguish the subtypes of Southwestern China and other branches of HPV-16.

As the great dangers of HPV, the current design of specific vaccine is imperative, and the design for the HP-V16 vaccine are generally specific gene sequences, so the major subtype of HPV-16 gene sequence analysis is necessary. The project in Southwestern China for the major genetic subtypes HPV-16 were cloned and polymorphism analysis of protein expression for the corresponding follow-up to lay a solid foundation for further application to the clinical diagnosis and prognosis of patients with HPV infection develop a preventive function as biological agents and vaccines and has broad prospects and great significance.

Reference


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Evaluation of the outcome benefit conferred by intensive surveillance strategies in women with early-stage endometrial cancer

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Introduction: The optimum follow-up regimen after treatment for early-stage endometrial cancer with curative intent is unknown. The National Comprehensive Cancer Network recommends a physical exam and vaginal cytology every three to six months for two years then at six to 12 month intervals with annual chest X-rays (CXR). However, there is debate as to whether intensive follow-up results in an improvement in outcomes for those with recurrent endometrial cancer. Objective: To determine if intensive surveillance for recurrent cancer in women with early-stage endometrial cancer improves their outcomes. Materials and Methods: The Roswell Park Cancer Institute tumor registry was used to identify patients with Stage I and II endometrial cancer initially diagnosed and treated over an 18-year period, who subsequently recurred. Clinico-pathological variables were abstracted. Patients were divided into two groups, depending on their mode of diagnosis of recurrent cancer: 1) routine screening, or 2) symptomatic. The outcomes between the two groups were compared. Results: Fifty-two patients met inclusion criteria. Twenty-three patients were diagnosed via routine screening methods and 29 were symptomatic at presentation. Groups were equally represented with respect to age, stage, grade, adjuvant therapy, site of recurrence (local, distant), and time to recurrence (p > 0.05). Median survival time was 79 months for those diagnosed during routine screening and 80 months for symptomatic patients (p > 0.05). Conclusion: Pap smear and CXR appear to be of limited utility as the present study has shown that women diagnosed as a result of intensive surveillance did not have a better outcome than those who presented when symptomatic.

Key words: Endometrial cancer; Surveillance.

Materials and Methods

After obtaining Institutional Review Board (IRB) approval, the Roswell Park Cancer Institute tumor registry was used to identify patients with Stage I and II endometrial cancer initially treated at this institution from January 1, 1990 through December 31, 2007, who subsequently recurred. Data abstracted included: patient age, stage, tumor grade, type of surgery (hysterectomy, BSO ± lymphadenectomy) (LND), lower uterine segment involvement, lymphovascular space involvement (LVSI), tumor volume, adjuvant RT, time to recurrence, site(s) of recurrence, symptoms at recurrence, modality of diagnosis of recurrence, date of last follow-up, and status at last follow-up (alive, dead of disease (DOD), dead of other causes). Patients were excluded if they had non-en-
dometrioid histology, received radiation prior to surgical staging, had a synchronous or meta-synchronous primary cancer. Stages are reported as per FIGO 2009 staging criteria [12]. Patients were divided into two groups, depending on their mode of diagnosis of recurrence: 1) routine screening, or 2) symptomatic. The outcomes between the two groups were compared.

Statistical analysis

Primary interest was in the prognosis of patients’ diagnosed with endometrial cancer recurrence, relative to diagnosis method (routine screening vs. symmetric visit). The OS endpoint was defined as time (in months) to death. Patients who had not died were censored at the date of last follow-up.

Potential confounding effects of other variables associated with diagnosis method and the outcome were investigated using both univariate and multivariate methods. Univariate associations between potential confounders and diagnosis method were assessed with the Wilcoxon Rank Sum test (for continuous covariates) and the Pearson Chi Square test (for categorical covariates). Associations between potential confounders and OS were evaluated in univariate and multivariate proportional hazards models.

Multivariate proportion hazards modeling methods were used for the primary analyses comparing OS by diagnosis method. Relative prognosis was summarized using estimates and 95% confidence limits for the hazard ratio (HR). The p value assesses the probably of observing the estimated HR by random chance, given the null hypothesis of no true survival difference between the two treatment groups. Ninety-five percent confidence limits describe the plausible range of values for the true HR that is supported by the data. In addition to the diagnosis effect, multivariate modeling controlled for effects, adjuvant treatment, grade, recurrence site, and stage.

Multivariate results were supported by univariate analyses of OS. Diagnosis method differences were assessed using the Log Rank test, with time to event distributions based on Kaplan-Meier Product Limit estimation methods. The Log Rank Test assesses the null hypothesis of no diagnosis effect on the time-to-event distribution. Product limit methods was also used to estimate median time-to-event, and the event-free proportion five years after diagnosis. All statistical analyses were done using SAS version 9.2.

Potential confounding effects of other variables associated with diagnosis method and the outcome were investigated using both univariate and multivariate methods. OS associated with routine screening diagnoses and symptomatic diagnoses were compared using multivariate proportional hazards methods controlling for age, adjuvant treatment, grade, recurrence site and stage; p values < 0.05 were deemed statistically significant.

Results

Of 850 patients with Stage I and II disease, 52 patients with recurrent endometrial cancer were identified who met inclusion criteria. Their mean age was 64.5 years. A majority of patients had Stage IA disease (58%). The remainder had Stage IB and II disease (19% and 23%, respectively). A majority of tumors were moderately or poorly differentiated (77%). More than half (54%) underwent LND at time of hysterectomy, BSO. A similar percentage of patients received post-operative radiation as those that received no further therapy. Patients were followed for a mean of 75 months. The median progression-free interval (PFI) was 43 months (range, three to 129 months). At time of analysis, 29% of recurrent patients were alive and 71% had died.

For analysis purposes, patients were divided into two groups, those that were diagnosed with recurrent disease at a routine surveillance visit and those that presented secondary to symptoms. Twenty-three (44%) patients had their recurrence diagnosed at time of routine follow-up. Their median time to recurrence was 24 months (range, six months, 129 months). Recurrence diagnosis modalities included clinical exam (22%), vaginal cytology/biopsy (39%), CXR (30%), and advanced imaging technique (9%). Upon review of symptoms, 74% of patients were asymptomatic, while others admitted to vaginal bleeding or pelvic pressure (22% and 4%, respectively). Recurrence sites were equally distributed between local and distant (52% and 48%, respectively). At time of analysis, 15 patients had died (65%), with a majority of those deaths cancer-related (12/15, 80%). Median survival time was 79 months, with 55% of patients diagnosed with recurrence at routine screening alive five years after diagnosis.

Twenty-nine (56%) patients were diagnosed with recurrence at time of interval visit, scheduled secondary to symptoms. Their median time to recurrence was 50 months (range, three to 125 months). Symptoms at time of recurrence included: pain (55%), pelvic pressure (10%), and bleeding (31%). Recurrence diagnosis modalities were as follows: advanced imaging technique (72%), vaginal smear/biopsy (21%), and clinical exam (7%). A majority of recurrences were distal (72%). At time of analysis, a majority of patients were dead (76%), with a majority of those deaths being cancer-related (19/22, 86%). Median survival time was 80 months for symptomatic patients with 59% of symptomatic patients alive five years after initial diagnosis.

The two groups were similar with regards to demographic and clinic-pathologic factors (Table 1). There was no statistically significant difference between groups with regards to patient age, stage distribution, grade distribution, LVSI, and adjuvant RT. Patterns of

<table>
<thead>
<tr>
<th>N</th>
<th>Routine f/u</th>
<th>Symptomatic</th>
<th>Total</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>850</td>
<td>23 (44%)</td>
<td>29 (56%)</td>
<td>52 (100%)</td>
<td>0.083</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>67</td>
<td>62.5</td>
<td>64.5</td>
<td>0.915</td>
</tr>
<tr>
<td>Stage IA</td>
<td>13 (57%)</td>
<td>17 (59%)</td>
<td>30 (58%)</td>
<td>0.123</td>
</tr>
<tr>
<td>IB</td>
<td>5 (22%)</td>
<td>7 (24%)</td>
<td>12 (23%)</td>
<td>0.123</td>
</tr>
<tr>
<td>II</td>
<td>5(22%)</td>
<td>7 (24%)</td>
<td>12 (23%)</td>
<td>0.123</td>
</tr>
<tr>
<td>Grade 1</td>
<td>7 (30%)</td>
<td>5 (17%)</td>
<td>12 (23%)</td>
<td>0.123</td>
</tr>
<tr>
<td>2</td>
<td>8 (35%)</td>
<td>14 (48%)</td>
<td>22 (44%)</td>
<td>0.123</td>
</tr>
<tr>
<td>Grade 3</td>
<td>8 (35%)</td>
<td>10 (35%)</td>
<td>18 (36%)</td>
<td>0.123</td>
</tr>
<tr>
<td>Surgery</td>
<td>Hyst/BSO</td>
<td>12 (52%)</td>
<td>12 (41%)</td>
<td>24 (46%)</td>
</tr>
<tr>
<td>Hyst/BSO/LND</td>
<td>11 (48%)</td>
<td>17 (59%)</td>
<td>28 (54%)</td>
<td>0.438</td>
</tr>
<tr>
<td>LVSI</td>
<td>Present</td>
<td>8 (80%)</td>
<td>13 (68%)</td>
<td>21 (72%)</td>
</tr>
<tr>
<td>Absent</td>
<td>2 (20%)</td>
<td>8 (32%)</td>
<td>10 (32%)</td>
<td>0.288</td>
</tr>
<tr>
<td>Tumor size (mean)</td>
<td>Volume (cm³)</td>
<td>17</td>
<td>81</td>
<td>59</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>67</td>
<td>62.5</td>
<td>64.5</td>
<td>0.083</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>No</td>
<td>11 (50%)</td>
<td>16 (55%)</td>
<td>27 (53%)</td>
</tr>
<tr>
<td>RT</td>
<td>Yes</td>
<td>11 (50%)</td>
<td>13 (45%)</td>
<td>24 (47%)</td>
</tr>
</tbody>
</table>

f/u = follow-up; Hyst = hysterectomy; BSO = bilateral salpingo-oophorectomy; LND = lymphadenectomy; LVSI = lymphovascular space involvement; RT = radiation therapy.
Table 2. — Patterns of recurrence.

<table>
<thead>
<tr>
<th></th>
<th>Routine Fu</th>
<th>Symptomatic</th>
<th>Total</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>23 (44%)</td>
<td>29 (56%)</td>
<td>52 (100%)</td>
<td></td>
</tr>
<tr>
<td>Time to recurrence &lt; 1 year</td>
<td>5 (22%)</td>
<td>6 (21%)</td>
<td>11 (21%)</td>
<td>0.927</td>
</tr>
<tr>
<td>Time to recurrence &gt; 1 year</td>
<td>18 (78%)</td>
<td>23 (79%)</td>
<td>41 (79%)</td>
<td></td>
</tr>
<tr>
<td>(mean)</td>
<td>33 months</td>
<td>51 months</td>
<td>43 months</td>
<td>0.077</td>
</tr>
<tr>
<td>Site of recurrence Local</td>
<td>12 (52%)</td>
<td>8 (28%)</td>
<td>20 (39%)</td>
<td></td>
</tr>
<tr>
<td>Distant</td>
<td>10 (44%)</td>
<td>18 (62%)</td>
<td>28 (54%)</td>
<td>0.179</td>
</tr>
<tr>
<td>Both</td>
<td>1 (4%)</td>
<td>3 (10%)</td>
<td>4 (8%)</td>
<td></td>
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<tr>
<td>Symptoms</td>
<td>None</td>
<td>17 (74%)</td>
<td>1 (3%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>at recurrence Pain</td>
<td>0</td>
<td>16 (55%)</td>
<td>16 (31%)</td>
<td></td>
</tr>
<tr>
<td>Bleeding</td>
<td>5 (22%)</td>
<td>9 (31%)</td>
<td>14 (27%)</td>
<td></td>
</tr>
<tr>
<td>Pelvic pressure</td>
<td>1 (4%)</td>
<td>3 (10%)</td>
<td>4 (8%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Detection</td>
<td>5 (22%)</td>
<td>2 (7%)</td>
<td>7 (14%)</td>
<td></td>
</tr>
<tr>
<td>of recurrence</td>
<td>9 (39%)</td>
<td>6 (21%)</td>
<td>15 (29%)</td>
<td></td>
</tr>
<tr>
<td>Pap smear</td>
<td>7 (30%)</td>
<td>7 (14%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adv imaging</td>
<td>2 (9%)</td>
<td>21 (72%)</td>
<td>23 (44%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Status</td>
<td>Alive</td>
<td>5 (22%)</td>
<td>6 (21%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>21 (72%)</td>
<td>31 (60%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DOD</td>
<td>12 (52%)</td>
<td>19 (66%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 (21%)</td>
<td>11 (21%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dead (NED)</td>
<td>3 (13%)</td>
<td>10 (3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 (12%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lost to Fu</td>
<td>3 (13%)</td>
<td>1 (3%)</td>
<td>4 (8%)</td>
</tr>
</tbody>
</table>

f/u = follow-up; CXR = chest X-ray; Adv = advanced imaging technique; DOD = dead of disease; NED = no evidence of disease.

Discussion

The role of routine surveillance for recurrent disease in patients treated for endometrial cancer has not been fully established. Currently, the National Comprehensive Cancer Network recommends surveillance in the form of physical exam every three to six months for two years, then every six months or annually, vaginal cytology every six months for two years, then annually, annual CXR, and patient education regarding symptoms [3]. However, recent attention has focused on the efficacy of currently implemented screening modalities and schedules [4].

The goal of the present study was to determine if intensive surveillance of women with early-stage endometrial cancer improves outcomes for those with recurrent disease. In the present study with prolonged follow-up and detailed clinic-pathologic data, the authors have demonstrated that, in patients with Stage I and II endometrial cancer, those diagnosed with recurrence as a result of currently implemented screening strategies did not fare better than those diagnosed when symptomatic. This lack of survival benefit despite rigorous and scheduled screening warrants a critical analysis of currently implemented screening tests and schedules in order to rationalize their continued use.

To justify screening in a particular population, including that the condition be an important health problem for the individual and the community, that there is a useful intervention, that there be a latent or early symptomatic stage, acceptable screening tests, and that treatment started at early-stage should be of more benefit that treatment started later [13]. Screening for recurrent endometrial cancer is done so on the premise that early detection will afford an opportunity for a therapeutic intervention with a subsequent decrease in morbidity and mortality which would not be possible for the patient who presents later, when symptomatic. This currently implemented screening approach presumes several things necessary to justify screening this particular population, specifically, that recurrence affects a significant proportion of those treated with endometrial cancer, that currently implemented screening strategies (vaginal cytology and CXR) are effective in detecting pre-symptomatic disease, and that current treatments for recurrent disease are potentially curable, with those treatments more efficacious in the pre-symptomatic or latent phase in the natural history of the disease process.

Recurrent endometrial cancer is a clinically significant problem, with those even with early-stage disease at risk. Morrow et al. defined risk factors for recurrence in early-stage disease, including deep myometrial invasion and moderate to poor cellular differentiation [14]. More recently, in GOG 99, patients with early-stage disease with high-risk features (i.e., increased age, moderate to poorly differentiated tumors, LVI, and deep myometrial invasion) were randomized to adjuvant pelvic RT vs no further treatment [2]. The addition of pelvic radiation decreased the risk of localized recurrence (1.6% vs 8.9%), without any decrease in distal recurrence or in OS. These findings were consistent with those from another large randomized study evaluating post-operative RT in early-stage endometrial cancer [15]. Considering...
Similarly, Reddoch isolated vaginal recurrence, alive without disease. Sim-
ilarly, adding that lead time to the interval from symptoms until death. The reported improvement really only being an earlier detection of the same disease process is supported in the present study by the lack of statistically significant difference in OS between symptomatic and asymptomatic patients.

In conclusion, in the present study of the role of screening for recurrent endometrial cancer, specifically vaginal vault cytology and CXR. In a systematic review of the means by which recurrent endometrial cancer was detected, vaginal vault cytology detected only 0%-4% of asymptomatic recurrences [4]. The lack of sensitivity of vaginal cytology in this setting is highlighted in a report by Morice et al. [20]. In their follow-up of analysis of women with recurrent Stage I and II endometrial cancer, none of the recurrences were detected with routine Pap smear. Furthermore, all asymptomatic patients with biopsy-confirmed vaginal recurrence had a normal Pap smear. Ng et al. reported similar findings with vault cytology being negative in all patients with recurrences, including local recurrences [21]. While the Pap smear is highly-effective at detecting dysplastic lesions of the cervix, it does not appear to be very beneficial in detection of asymptomatic endometrial cancer recurrences. The inability of vaginal cytology to detect asymptomatic recurrences may be secondary to a need for the recurrent tumor to invade through the vaginal mucosa before cells will be present for capture during cytologic evaluation, at which point the patient would most likely have symptoms and/or a pelvic exam would detect the tumor [10]. Routine CXR have a similarly low yield for detecting asymptomatic recurrence, the utility of which is called in to question further considering the historically poor outcomes for those with distal recurrences.

In conclusion, in the present study of the role of screening for recurrence for patients with a history of early-stage endometrial cancer, the authors have shown that intensive surveillance does not benefit those with recurrent disease when compared to those diagnosed secondary to symptoms. Considering, the relative low-
risk of recurrence in early-stage endometrial cancer, the primarily symptomatic nature of recurrences, the low yield of currently used screening tests (e.g., vaginal cytology and CXR), and the limited effective treatment modalities for recurrence, the use of routine surveillance in asymptomatic patients does not appear to be justifiable for the purpose of improving survival. Resources should instead be re-directed to further research on development of newer screening tests and on improved treatment options for those with distant and more extensive recurrences.

References


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Response to neoadjuvant chemotherapy with paclitaxel and cisplatin in locally advanced cervical cancer

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Summary

Purpose: The purpose of this study was to evaluate the efficacy and toxicity of paclitaxel and cisplatin as neoadjuvant chemotherapy for patients with Stage IB2 to IIB cervical cancer and determine factors accountable for response. Materials and Methods: From November 2009 to January 2011, a total of 19 patients with Stage IB2 to IIB cervical cancer were treated with three ten-day courses of paclitaxel 60 mg/m² and cisplatin 80 mg/m² followed by type III radical hysterectomy and adjuvant therapy if indicated, or chemoradiation in non-resectable patients. Results. Clinical response occurred in 79% (15/19) of patients, including 10.5% (2/19) with complete response, and 68.5% (13/19) with partial response. Four (21%) patients were nonresponders including 16% (3/19) with stable and 5.2% (1/19) with progressive disease. Resectability rate was 68.5% (13/19). Pathological optimal response rate was 46% (6/13) including, 15% (2/13) with complete and 31% (4/13) with residual disease < three mm stromal invasion response (PR1). Suboptimal response (PR2) (residual disease with > three mm stromal invasion) was 54% (7/13). It appears that both clinical and pathological response were correlated with tumor stage and size. Clinical response was seen in 87.5% of tumors sized = < eight cm vs 33.3% of tumors sized > eight cm (p = 0.166) and optimal pathological response was seen in 66.7% of tumors sized < four cm vs 28.6% of tumors sized four to eight cm, (p = 0.286), although because of small number of patients, the difference was not statistically significant. Adjuvant therapy was necessary for 38.5% (5/13) patients. Toxicities were not life-threatening and all manageable. Conclusions: The present results suggest that neoadjuvant chemotherapy (NAC) with paclitaxel and cisplatin is a highly active and well-tolerated regimen. Best candidates are patients with stages IB2/IIA bulky and IIB non-bulky than IIB bulky groups.

Key words: Cervical cancer, Neoadjuvant chemotherapy; Cisplatin; Paclitaxel; Pathological response.

Introduction

Worldwide, cervical cancer is the second most common malignancy [1] and is the most prevalent female malignancy in many developing countries. Eighty percent of the world’s new cervical cancer cases and resulting deaths occur in developing countries, where 80% of cases are diagnosed with locally advanced disease. There is no agreement on the best approach for bulky (≥ four cm in diameter of tumor) or locally advanced cervical cancer (LACC), whose prognosis remains poor in spite of the therapeutic advances achieved in recent years. For many, chemoradiation is now considered the treatment of choice for these patients, but a substantial proportion of patients treated with chemoradiation will have persistent or progressive disease after primary treatment. Thus different therapeutic approaches such as neoadjuvant chemotherapy (NAC) were introduced.

Tierney et al. [2] in a meta-analysis study based on individual participant data (IPD) compared NAC followed by surgery with radical radiotherapy and showed a highly significant benefit of NAC with an absolute improvement of 14% in five-year survival from 50% to 64%. However nowadays NAC has not been adopted as the standard of care and is still considered investigational. Several explanations for this hesitancy can be given, which probably the most important is that this treatment modality being compared to radiotherapy alone, which is inferior to today’s standard of care of chemoradiation.

There are two ongoing randomized trials, the EORTC 55994 and Tata Memorial trial, comparing chemoradiation with chemotherapy before surgery. An important topic is to assess which chemotherapy scheme is more effective. The meta-analysis results suggest that it seems to be prudent to use a short cycle length and dose intense NAC scheme [2]. In addition several trials have shown that patients achieving pathological complete response to NAC do experience a significant improvement in rates of overall survival [3, 4].

The studies using regimens based on cisplatin and drugs such as bleomycin and vincristine have shown that these agents are clearly not the most effective agents against cervical cancer [5, 6]. With these regimens the overall response rate varied between 47% and 88%. However with newer drug regimens such as cisplatin plus gemcitabine [7], taxanes [8], irinotecan [9], vinorelbine [10], oxaliplatin [11], ifosfamide [8], overall response rates were achieved in the range between 78%-95% and pathological complete response were 14%-35.7%. In the present study the authors used the combination of cisplatin and paclitaxel.

The aim of this study was to focus on the efficacy (clinical and pathological results) and tolerability of induction chemotherapy followed by radical resection in patients with Stages IB2 to IIB cervical cancer, and to determine the prognostic factors responsible for clinical and pathological response.

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Materials and Methods

From November 2009 to January 2011, 19 untreated patients with Stage IB2 through IIB cervical cancer who were hospitalized at Valiasr Hospital were eligible for the study. Further entry criteria were age < 75 years, performance status (PS) of ≤ 2 according to the WHO criteria, adequate bone marrow, renal and hepatic functions, as determined by a Hb ≥ 9 gr/dl, WBC > 3,000/mm³, and platelet count of at least 100,000/mm³, total bilirubin and transaminase and creatinine less than the 1.5 times the upper limit of normal, a normal posteroanterior CXR, signed written informed consent.

Exclusion criteria included: severe systemic or uncontrolled disease that precluded the use of chemotherapy, preexisting neuropathy of any cause, pregnancy or lactation, mental illness, previous or concomitant malignancies except non-melanoma skin cancer, previous radiotherapy or chemotherapy.

All patients were staged based on FIGO clinical staging criteria for cervical cancer. Tumor size was measured before the NAC by pelvic examination and magnetic resonance imaging (MRI).

Surgical treatment was determined after the NAC. Tumor size was determined according to the WHO toxicity criteria by grade. Response to NAC was determined ten days after the third cycle of chemotherapy by pelvic examination and MRI. Clinical response was evaluated according to the WHO criteria as follows: complete response: the disappearance of all measurable disease; partial response: a 50% or more reduction of the product of two perpendicular tumor diameters; stable disease: less than 50% reduction; progressive disease: greater than 25% increase in tumor.

After NAC, if the multidisciplinary team judged that the disease could be resected obtaining margins free of disease, patients were submitted to type III radical hysterectomy and bilateral pelvic lymphadenectomy (patients with clinical objective response). The surgery was performed within two or three weeks after completion of the third cycle. Those patients who were judged to have non-resectable disease (patients with stable or progressive disease) underwent standard chemoradiation.

For the surgically treated patients, the authors also evaluated the pathologic response. Pathologic responses were defined as follows: Optimal pathologic response (OR) included a complete response and negative nodes; Partial response (PR) included a >50% reduction of the product of two perpendicular tumor diameters; Stable disease (SD) was defined as no change in tumor size or nodes; Progressive disease (PD) was defined as >50% increase in tumor size or nodes.

Statistical methods: data was described as median (range) for continuous and frequency (percentage) for categorical variables. The Fisher’s exact test was used to assess the association between clinical or pathological response rates and baseline tumor characteristics.

Results

A total of 19 patients were enrolled in this prospective study of NAC before surgery. The tumor characteristics are shown in Table 1. The median patient age was 52 years (range 30-72).

Clinical response to NAC

Clinical response occurred in 79% (15/19) of patients, including 10.5% (2/19) with complete, and 68.5% (13/19) with partial response. Sixteen percent (3/19) of patients showed stable and 5.2% (1/19) had progressive disease. Thus 15 (79%) patients were NAC responders and four (21%) were non-responders. The correlation between response rate and clinicopathological parameters has been detailed in Table 2. Whereas 90% of patients aged more than 50 years responded clinically to NAC, this rate was 66.7% in ages = ≤ 50 years old.

This result may be affected by the difference in tumor stage and size between the age groups, because when patient age group was cross-tabulated by stage and tumor size, two age groups were significantly different according to stage of tumor (p = 0.049) and were considerable different according to size of tumor. Both patients (100%) with Stage IB2 and...
IIB bulky disease had clinical response. Whereas the clinical response decrease to 85.7% in Stage IIB non-bulky and 70% in Stage IIB bulky disease. Clinical response was considerably lower in tumors sized > eight cm (33.3%) than tumor sizes of four to eight cm (88.9%) or < four cm (85.7%), ($p = 0.166$), although because of small number of patients, the difference is not statistically significant.

Local therapy

Two of the 15 patients considered surgically resectable refused surgery and underwent chemoradiation in other centers. Thus surgery was performed on 13 patients. Resectability rate was 68.5% (13/19). The four patients with SD and PD were unresectable (21%) and were subsequently referred for chemoradiation. Type III radical hysterectomy with lymphadenectomy was performed in 12 patients and in one patient a protocol violation occurred during surgery and simple hysterectomy with lymphadenectomy was performed. In this patient tumor necrosis in the cervix was too extensive that the cervix was separated off the uterus during surgery, so radical hysterectomy was technically impossible.

Pathological responses

The analysis of the surgical specimens showed complete pathological response in 15% (2/13), and PR1 response in 31% (4/13) of patients (46% optimal response rate) and suboptimal response (PR2) in 54% (7/13) of patients. Pathological data is shown in Table 3. All patients had free surgical margins. No patient had parametrical involvement. Lymph node metastases were present in one patient (7.5%), although MRI findings before chemotherapy showed lymph node involvement in six patients.

In six patients with parametrical invasion reported by MRI, after completion of chemotherapy, no parametrical invasion was observed pathologically. Thus the findings show that there was no correlation between clinical and pathological findings in this study. Table 4 shows the pathological response according to patient’s characteristics.

Although due to the small numbers of cases, the authors cannot show any statistically significant association between pathological response and baseline characteristics, but found that optimal response rate was considerably lower in age group < 50 than = > 50 (25% vs 55.6%), Stage IIB bulky than IIB non-bulky (20% vs 66.7%) or than IB2/IIA bulky (20% vs 100%) group, and tumor size four to eight cm than < four cm (28.6% versus 66.7%). Here the cut-off point for pathological response was four cm.

According to histological findings and evaluation of prognostic factors, postoperative adjuvant therapy was recommended for (7/13) 54% of patients although it was necessary for (5/13) 38.5% of patients, because in one patient, only simple hysterectomy was performed, and in the other despite having PR1 response, adjuvant chemotherapy was used due to recommending it in some other studies [12].

Table 5 summarizes the compliance and toxicities encountered during the treatment of NAC. Hematologic toxicity was anemia of grade 1-2 which occurred in four (21%) patients, there was no grade 3 or 4 toxicity. Non hematological toxicity consisted of nausea/vomiting and alopecia. Grade 1-2 nausea/vomiting occurred in six (31.5%) patients and grade 3-4 in four (21%) patients. Grade 1-2 alopecia occurred in three (16%) patients and grade 3-4 occurred in four (21%) patients.
Discussion

NAC represents a promising alternative to surgery or radiotherapy as the initial treatment of LACC. Response to chemotherapy has been confirmed as a potent predictor of survival. In the study by Benedetti-Panici et al. on 130 patients, Stage IB2-III cervical cancer treated with NAC containing cisplatin, bleomycin ± methotrexate, followed by surgery, the correlation above is supported by the fact that the parameters significantly associated with survival were the same factors as those predicting response to chemotherapy [13].

In the present study, clinical objective response was observed in 79% (15/19) of patients. Overall response rate in the present study is comparable with response rate of 78%-95% observed in published phase II trials that used newer drug regimens as NAC [7-11].

Identifying the factors predicting chemoresponsiveness may therefore allow for a more rationale selection of patients. In the long-term follow up study by Benedetti-Panici et al. on 130 patients as mentioned above, factors predicting of response were FIGO Stage (IB2-IIIB vs III), histotype (SCC vs adenocarcinoma), cervical tumor size (four to five cm vs > five cm), the extension of parametrical involvement clinically (absent-monolateral vs complete bilateral) [13]. In the study by Sardi et al., the most powerful predictor of response was pretreatment tumor volume. The critical pretreatment tumor volume was 84 cm³ (4.85 cm) in diameter [14]. In the study by Chen et al., the two parameters responsible for response, were tumor size and histologic type. In this study, tumors larger than eight cm had significantly poorer response than those with smaller size. The response was still 63.2% in tumors with a diameter of six to eight cm and it decreased to as low as 41.7% in tumors larger than eight cm [15].

The present study was not powered enough to statistically detect the predicting factors of clinical and pathological response, due to small number of patients. Nonetheless the authors found that some of baseline measured parameters may be a considerable predicting factor. These clinically important factors were tumor stage and size. The cut-off point for the tumor size in this study was eight cm, which is in accordance to Chen et al. study [15]. The present authors noted that response rate was still 89% in tumors with a diameter of four to eight cm and it decreased to 33% in tumors larger than eight cm, which suggests that extra large tumors may be poor candidates for NAC. The studies on NAC prior to surgery, underscore the importance of achieving pathological response as a potent prognostic factor for survival. The meaning of pathological response remains unsettled.

In the SNAPO1 Italian collaborative study performed by Buda et al., achievement of optimal pathological response (OR) was a strong independent predictor of survival (HR = 5.88, \( p < 0.0001 \)). The importance of OR was as much that, it was stated that OR may be a surrogate endpoint for survival [4]. In the study by Candelaria et al., the criteria for response were further more strict than the SNAPO1 study. In this study, complete pathological response, but no near-complete or partial response was associated with longer survival [3].

The combination of cisplatin and paclitaxel in the present study induced an optimal response rate of 46% (6/13) including complete pathological response rate of 15% (2/13) and PR1 response of 31% (4/13). These results approximate the results of the studies incorporating newer drug regimens in the NAC settings. In the study by Park et al. with the same regimen as the present, on 43 patients, complete pathological response was 11.6% [16] and the long-term follow up of that study showed a five-year survival rate of 89.2% among the 37 patients followed up [17]. Thus the high rate of pathological complete response in the present study may be translated to a high survival rate as the Park study.

A primary objective of chemotherapy is to increase operability rate. In fact, undergoing surgery after chemotherapy represents one of the most important prognostic factors [18]. However response to chemotherapy is not the sole factor determining operability, the aggressiveness of the surgical team also plays an essential role and it requires a highly motivated team of surgeons to attempt surgical resection in cases with no optimal response to chemotherapy. This was shown in Dueñas-Gonzalez et al. studies in which, in their primary trials using gemcitabine + cisplatin [7] and oxaliplatin + gemcitabine [11] the operability rate was 60% and 70% respectively and in their further protocol for comparable cases using carboplatin + paclitaxel, the resection rate was 95% [19]. They stated that this high rate was due to the fact that operability was defined intraoperatively.

In the present study, the resectability rate was 68.5% (13/19) which is comparable with other studies with high rate of resectability. The status of lymph nodes is the most important prognostic factor in cervical cancer. In the ten-yr follow up study by Hwang et al. on 80 patients with Stage IB2-IIIB cervical cancer treated with VBP followed by radical hysterectomy, the only significant risk factor for recurrence was pelvic lymph node involvement (\( p = 0.0016 \)) [20]. In the present study, positive lymph nodes were seen only in one patient (7.5%).

Several studies have reported a pelvic lymph node metastases rate of seven to 25% after NAC for stages IB-IIIB patients [21, 22]. The present results are in the range of the least rates. The better results in this study may be due to the efficacy of cisplatin + paclitaxel combination, or the selection of appropriate stages in the study (IB2-IIIB). It is worthwhile to note that in this study, no patients had parametrical involvement or positive surgical margins despite seven of these impressed clinically with parametrical involvement and six of these reported by MRI post chemotherapy. This lack of correspondence between clinical and pathological parametrical infiltration has already been observed in other studies [23, 24], and it shows that the clinical suspicion of parametrical disease should not necessarily contraindicate a radical hysterectomy.

In the present study, adjuvant therapy based on pathological risk factors was necessary for 54% (7/13) of patients. The percentage of patients who received adjuvant therapy was comparable with other studies that used same guidelines for adjuvant therapy. In the study by Lee et al., comparing 31 patients with Stage IB2-IIA bulky cervical cancer in the NAC followed by radical hysterectomy and 41 patients in the radical hysterectomy group, due to decreasing risk factors, adjuvant therapy was performed on 51.6% for NAC group compared to 82.9% in the primary R.H group (\( p = 0.005 \)) [25].
Cisplatin and paclitaxel were well-tolerated. The most common hematologic toxicity was anemia which was mild. The most common non-hematologic toxicity was reversible alopecia and nausea and vomiting which never caused treatment interruption and no patient required dose reduction. The limitations of this study were the small number of patients and limited long-term follow up. However the follow up of the patients in this study will be continued and the results will be reported in the future.

These preliminary findings indicate that NAC with paclitaxel and cisplatin is highly active and well-tolerated for patients with Stage IB2 to IIB cervical cancer, making surgery possible for patients with tumors considered inoperable and improve pathologic prognostic factors, and thereby decreasing the need for adjuvant therapy. Best candidates are patients with Stages IB2/IIA bulky and IIB non-bulky groups. A randomized phase III trial is required to establish the value of NAC/surgery with or without adjuvant chemoradiation vs standard chemoradiation.

References


When to perform palliative surgery in the treatment of ovarian cancer: a brief review


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Summary
The objective of this review was to address the main indications for palliative surgery in the treatment of ovarian cancer. Design: Articles from MEDLINE/PUBMED, EMBASE, and LILACS databases up to May 05, 2012 were included with no bars on foreign languages. The key words used were taken from the Medical Subject Headings and were as follows: ovarian cancer AND palliative surgery, ovarian cancer AND complications, and ovarian cancer AND intestinal obstruction. Subsequently, the references from the original articles were also analyzed. Results: Among the complications developing in the course of malignant neoplasia, intestinal obstruction stands out as the main indication for palliative surgery, which may also be indicated for rectovaginal and enterovaginal fistulas, as well as for genital and lower gastrointestinal hemorrhage. Conclusion: Although incurable, the patients with complications due to ovarian cancer may have an extended survival and an improved quality of life with palliative surgery for the following reasons: a) improvement in the nutritional state after treatment for intestinal obstruction due to the possibility of oral nutrition; and b) improvement in clinical conditions, allowing for palliative chemotherapy.

Key words: Ovarian cancer; Ovarian neoplasia; Treatment; Palliative surgery.

Introduction
Ovarian cancer is the fourth most frequent neoplasia in women, with 22,280 newly diagnosed cases and 15,550 mortality cases are projected in the U.S.A. for 2012 according to the American Cancer Society [1]. Ovarian cancer spreads on the peritoneal surface with no regard for the anatomical boundaries of the pelvic and abdominal organs and viscera [2]. Thus, when diagnosed, most cases are advanced and peritoneal carcinomatosis is already present and patients have stage IIIC/IV (FIGO)/TNM epithelial ovarian cancer (AJCC 2010) [3,4].

Despite a surgical treatment that ideally removes all of the visible peritoneal disease leaving lesions no larger than 10mm each (optimal cytoreduction) and notwithstanding the advances in adjuvant chemotherapy, many patients will relapse and be considered incurable. Gastrointestinal tract surgeries are performed not only for cytoreduction, the so-called multivisceral resection, but also for palliative purposes.5 Thus, palliative surgery is that which is performed on incurable patients and is of great help in accomplishing the objective of reducing the patient’s suffering. Therefore, it is essential that the surgeon who plans to treat ovarian cancer have the mastery of several surgical procedures, which are often outside the range of gynecological surgery per se [5-7].

Among the complications resulting from the progression of malignant neoplasia, intestinal obstruction stands out as the main indication for palliative surgery, which may also be indicated for rectovaginal and enterovaginal fistulas as well as for genital and lower gastrointestinal hemorrhage.

Materials and Methods
Articles from the MEDLINE/PUBMED, EMBASE, and LILACS databases up to May 05, 2012 were included with no bars on foreign languages. The key words used were taken from the Medical Subject Headings and are as follows: ovarian cancer AND palliative surgery, ovarian cancer AND complications, and ovarian cancer AND intestinal obstruction. Subsequently, the references from the original articles were also analyzed.

Results
Intestinal Obstruction
Intestinal obstruction is a common feature of ovarian cancer in its advanced stages or in its recurrence. Most of the patients with an obstructed intestine have a poor physical health status and limited life expectancy [8].

The primary cause of intestinal obstruction is the frozen pelvis with involvement of the sigmoid colon and the rectum along with the loops of the small intestine. Although obstruction may occur anywhere in the digestive tract, a list of intestinal obstruction sites, according to Stephen [9], includes the small intestine (44%), the large intestine (33%), and the simultaneous occurrence of the small and large intestine (22%). The main surgeries are enterectomy (Figure 1A), ileo-transverse anastomosis (Figure 1B), transverse or terminal...
sigmoid colostomy, and omentectomy (Figure 1C). The latter is performed in order to leave the entire upper colon free so as to allow a clear view of the entire digestive tract. And thus median laparotomy is carried out aiming to make an inventory of the entire abdominal cavity so as to diagnose all possible obstruction sites and determine a safe point, beyond which it is disease-free (usually up to the transverse colon, for the pelvis is frozen more often than not).

When the disease is very advanced and surgical correction of the obstruction is no longer possible, a decompressive gastrostomy may be performed or nothing at all may be done. An ileostomy is contraindicated because of its extremely high rate of incurability and morbidity for the patients. At the time of deciding whether or not to perform palliative surgery, bad prognostic factors should be taken into account, such as palpable abdominal and pelvic masses, more than three liters of ascites, multiple obstructions, a preoperative weight loss of over nine kg, and recent chemo- or radiotherapy [10].

**Intestinal fistulas**

Digestive fistulas are rarely formed in cases of ovarian cancer; nevertheless, the most frequent are those connecting the small intestine or the rectum and the vagina [11-15].

The discharge of solid feces through the vagina is characteristic of the rectovaginal fistula, which must be treated with loop transverse colostomy. A few authors advocate a loop sigmoid colostomy, but we favor a loop transverse colostomy due to the high incidence of frozen pelvis and because the former procedure often requires great technical skill and a laparotomy to perform the colostomy on the sigmoid.

Although a loop transverse colostomy is a more frequent cause of complications such as prolapse and paracolostomy hernias, it does not necessitate a laparotomy and it may be performed with a local incision (Figure 2A).

To minimize complications, technical care must be taken not to leave free space between the colostomy and the wall and a careful choice of site on the abdominal wall must be made for maturation of the stoma.

When the discharge through the vagina is profuse and liquid and made up of enteric effluent, the enterovaginal fistula should be investigated. Diagnosis is generally carried out with computed tomography assessing the intestinal transit of an oral contrast agent (Figure 2B). An opaque enema CT
When to perform palliative surgery in the treatment of ovarian cancer: a brief review

scan should also be performed to rule out the possibility of an associated rectovaginal fistula. The treatment for the fistula of the small intestine is resection of the fistulous pathway with enterography and enterectomy. [11-15].

Genital and rectal bleeding

In lower gastrointestinal bleeding, enterorrhagia generally occurs through tumor infiltration in the sigmoid colon and the rectum. When feasible, a resection of the infiltrated site is the only treatment for stopping the often recurrent hemorrhage [11-15].

In the cases of profuse vaginal bleeding, the bilateral hypogastric artery ligation performed three cm below the source of hemorrhage to avoid the origin of the superior gluteal artery is the indicated treatment when interventional radiology is not available for embolization [11-15].

Conclusions

A large number of women with advanced ovarian cancer will require surgery at some point during the course of the disease to relieve the symptoms, such as intestinal obstructions, fistulas, and hemorrhages. Although incurable, the patients with complications due to ovarian cancer may have an extended survival and an improved quality of life with palliative surgery for the following reasons: a) improvement in the nutritional state after treatment for intestinal obstruction due to the possibility of oral nutrition; and b) improvement in clinical conditions, allowing for palliative chemotherapy.

References


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Correlation of cervical intraepithelial neoplasia with expressions of p16 and Ki67 in exfoliated cervical cells in fluid-based thin-layer samples

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Introduction

Cervical cancer is the most common malignant tumor of female reproductive system and its onset age is decreasing [1, 2]. Diagnosis at an early stage is the key for treatment, and an effective practical screening method will benefit both patients and community.

Cervical cytology is the most commonly used diagnostic mean for cervical lesion screening. Since it was introduced, the incidence of cervical cancer has dramatically decreased. It has advantages of non-invasion, easy access, and low cost. Although the false negative diagnosis rate for cervical cancer and precancerous lesion decreases in both conventional Pap smears and fluid-based Pap smears, false negative diagnosis is still the bottleneck in secondary prevention of cervical cancer [3, 4]. On the other hand, timely diagnosis of cervical cancer and precancerous changes in large numbers of cells with mild morphological changes without excessive examination is not easy to achieve. Hybrid capture II (HCII) has been recently developed to screen cervical lesions since high-risk human papillomavirus (HR-HPV) infection is considered the cause of cervical cancer and precancerous lesions [5, 6]. Although the false-negative diagnosis rate of HCII for cervical intraepithelial neoplasia (CIN) II or greater is very low, its positive predictive value is also low because most people with HPV DNA positive can clear it in a short period. Therefore, HCII alone could lead to over-diagnosis [7, 8]. In addition, high cost also limits its application. Therefore, new sensitive screening strategies other than cell morphological changes are needed to evaluate the risk for tumor appearance.

It has been shown that cervical cancer, like many other cancers, is related to the dysregulation of factors related to cell cycles. Histopathological research has confirmed that the expressions of tumor suppressor gene p16 and proliferation antigen Ki67 have a clear relationship with cervical cancer and precancerous lesions [9, 10]. p16 is a multi-tumor suppressor induced specifically by overexpression of E7 protein in patients infected with HR-HPV [11, 12]. Ki67 expression reaches its peak in mitosis phase of cell cycle and rapid declines afterward [13, 14]. Since it is not expressed in static cells, Ki67 can be used in early diagnosis of precancerous lesions and varieties of malignant tumors [15, 16].

Expression of these two factors in exfoliated cervical cells has not yet been well studied. In this study, their expression in exfoliated cervical cells obtained by fluid-based thin-layer technology from 116 patients admitted to the Cervical Lesion Center of Peking University Third Hospital from January 2010 to July 2011 for diagnosis and treatment was examined by immunocytochemistry. Their relationships to CIN II or greater were analyzed and clinical diagnostic values were evaluated.

Materials and Methods

Subjects

This study included 116 patients, admitted to Cervical Lesion Center of Peking University Third Hospital for diagnosis and treatment from January 2010 to July 2011. They had abnormal fluid-based cytology or/and HPV DNA test and then accepted colposcopy-directed histopathological examination. The patients were enrolled in the study if cytology sample were taken no more than 20 days before the authors confirmed their diagnosis. Their average age was 39.7 years, Their average deliver times were 1.7. Pregnant women and women that had surgery of cervix were excluded. All the patients were divided

Summary

Objective: To understand the correlation of cervical intraepithelial neoplasia (CIN) with the expressions of p16 and Ki67 in exfoliated cervical cells obtained by fluid-based thin-layer technology. Study design: The distributions of p16 and Ki67 positive cells in 116 patients with cytologically diagnosed atypical squamous cells of undetermined significance (ASC-US) or human papilloma virus (HPV) positive were examined by immunocytochemistry. Data were analyzed by the rank sum test, crosstab test, and receiver operating characteristic (ROC) curves. Results: When the thresholds for p16 and Ki67 positive were set as 6.5 and 4.5 positive cells per 100 exfoliated cervical cells, respectively, the expressions of Ki67 and P16 are significantly higher in the exfoliated cervical cells of patients with CIN II or greater compared with those in patients with CIN I or less. Conclusion: Immunocytochemical examination of p16 and Ki67 expressions in the exfoliated cervical cells of fluid-based thin-layer samples could be used for the detection of CIN II or greater.

Key words: p16, Ki67; CIN; HPV.
Expressions of p16 and Ki67 were examined by immunocytochemistry in squamous epithelial cells of remaining Pap smear samples preserved in tripith solutions and prepared with fluid-based/thin-layer technology. Samples that had a number of fixed squamous epithelial cells no less than 1,000 were used in the study. Immunostaining of p16 and Ki67 were performed using mouse monoclonal antibodies directed against human p16 and Ki67 at 1:40 and 1:100 dilution for p16 and Ki67, respectively, and the Dako Envision System. Number of positive cells was counted in 100 randomly selected cells.

Data were analyzed using receiver operating characteristic (ROC) curves. The diagnostic values of p16 and Ki67 for CIN II or greater in patients with cytological abnormality, including atypical squamous cells of undetermined significance (ASC-US), high grade squamous intraepithelial lesion (HSIL) or human papilloma virus (HPV) DNA positive were evaluated, respectively.

Statistical differences were analyzed by rank sum test and crosstab test using SPSS software. A p value less than 0.05 was considered as statistical significance.

Results

Figure 1A shows a typical immunohistochemical staining of p16 in exfoliated cervical cells from patients with cytologically diagnosed low grade squamous intraepithelial lesion (LSIL) and pathologically diagnosed CIN II. As shown, positive p16 staining appeared in cells with mild morphological changes.

Figure 1B shows a typical immunohistochemical staining of Ki67 in exfoliated cervical cells from patients with cytologically diagnosed ASC-US and pathologically diagnosed cervical cancer. As shown, positive Ki67 staining emerged in broken cells with naked nuclei.

Table 1 shows the distributions of p16 and Ki67 positive cells. The percentages of p16 and Ki67 positive in patients with CIN I or less were 4.8 ± 5.3% and 3.3 ± 4.3%, respectively. While those of p16 and Ki67 positive in patients with CIN II or greater were 16.4 ± 9.3% and 11.0 ± 7.9%, respectively. Statistical analyses using rank sum test indicated that the percentages of p16 and Ki67 positive cells were between 0 and 36, and from patients with CIN I or less and CIN II or greater were significantly higher than those from patients with CIN I or less.

Table 2 shows the distributions of p16 and Ki67 positive in patients with cytological ASC-US or LSIL, and HPV positive. Data were analyzed using receiver operating characteristic (ROC) curves. The diagnostic values of p16 and Ki67 for CIN II or greater were screened by combination of HPV DNA test and p16 staining, The sensitivities of p16 positive for patients with ASC-US or LSIL were 75.0% and 85.7%, respectively, and the corresponding specificities were 72.4% and 74.2%, respectively.

Table 3 shows the distributions of p16 and Ki67 positive as defined by the above threshold in patients with mild cytological changes (including ASC-US and LSIL) and HPV positive to find CIN II or greater. If CIN II or greater were screened by combination of cytology and p16 staining, The sensitivities of p16 positive for patients with ASC-US or LSIL were 75.0% and 85.7%, respectively, and their corresponding specificities were 81.3% and 63.2%, respectively. Similarly, If CIN II or greater were screened by combination of cytology and Ki67 staining, the sensitivities of Ki67 positive for patients with ASC-US or LSIL were 87.5% and 71.4% and their corresponding specificities were 76.5% and 66.7%, respectively. If CIN II or greater were screened by combination of HPV DNA test and p16 staining, the sensitivity and specificity of p16 positive for HPV positive patients were 89.4% and 67.5%, respectively. Similarly, if CIN II or greater were screened by combination of HPV DNA test and Ki67 staining, the sensitivity and specificity of Ki67 positive for HPV positive patients were 83.7% and 64.1%, respectively.

Discussion

Immunocytochemical examinations in this study showed that the expressions of p16 and Ki67 in exfoliated cervical cells obtained by fluid-based thin-layer
Correlation of cervical intraepithelial neoplasia with expressions of p16 and Ki67 in exfoliated cervical cells etc.

Molecular studies have shown that tumor suppressor p16 and proliferation antigen Ki67 are important regulators of cellular activities. p16 is encoded by CDKN2A gene located at chromosome 9p21. Its absence in melanoma was first reported in 1994 [17]. Further studies have found that p16 functions as an inhibitor of cyclin-dependent kinases (CDK) by competing with cyclin D1 to bind to CDK4 or CDK6. p16 mediated inhibition of CDKs blocks Rb phosphorylation and subsequent release of E2F, leading to arrested cell cycle in G1 phase and inhibition of cell proliferation [18]. Inactivation of p16 gene and destruction of p16 protein evoke tumor development. During the occurrence and development of HPV-related cervical cancer and precancerous lesions, the negative feedback regulation between P16 and Rb is specifically interrupted by E7 protein of high-risk HPV, leading to excessive accumulation of p16 and lose of tumor suppression function, and eventually resulting in persistent cell proliferation. This study showed that abnormal expression of p16 occurs in exfoliated cervical cells from patients with subtle morphological changes in Pap smear examination, implying that p16 may be a sensitive and specific biomarker in cervical cancer screening. Recently, p16 staining has been applied in histopathological diagnosis of CIN to identify whether immature metaplasia or...
mature metaplasia is accompanied by CIN [19]. p16 positive in upper 1/3 of the cervical epithelium strongly suggests the presence of CIN II/III [20]. p16 staining has been applied to reduce the discrepancy in diagnosis of CIN II or greater among clinicians [21], while its application in cytology and comparison with HPV test are still under investigation [22].

Ki67 gene is located in human chromosome 10. As a DNA-binding protein, it is only expressed in cell nuclei and closely related to cell cycles. Its expression is absent in G0 phase, weak in G1 phase, strong in S and G2 phases, and maximal in M phase [23]. The half-life of Ki67 is one hour. Because of its short half-life and absence in G0 phase, its expression can accurately reflect cell proliferation activity. Therefore, as an indicator of proliferation activity, Ki67 is only expressed in the basal cell layer of squamous epithelium of patients with CIN I or less, but not in the upper 2/3 layer of epithelial cells in the differentiating and maturing epithelium. By contrast, in squamous epithelium of patients with cervical cancer and precancerous lesions, its expression is significantly enhanced and used in detection of CIN II or greater [24, 25]. The study shows that in the gray area of cytological diagnosis of cervical cancer such as nacked nuclei, small cells, small keratinocytes, and severely necrotic cells, examination of Ki67 expression can help to discover cervical cancer and precancerous lesions and reduce false negative diagnosis.

In conclusion, p16 expression indicates the initiation of abnormal cervical cell proliferation induced by HPV and Ki67 expression indicates the beginning of cell active state. Positive staining of p16 and Ki67 can replenish the insufficiency of diagnosis based only on morphological changes in cytological screening. Application of these two biomarkers in exfoliated cervical cells, on one hand, can timely find abnormal proliferation and molecular alterations even when only small numbers of lesion cells have been detected, and consequently reduce false negative diagnosis in patients with subtle morphological changes in cytology such as ASC-US and LSIL; on the other hand, they can help to avoid excessive diagnosis in low-risk patients who have already been infected with HR-HPV, but have not yet had abnormal proliferation. Numbers of studies have confirmed the overexpression of p16 in cervical squamous intraepithelial lesions and glandular intraepithelial lesions. Therefore, p16 may be an effective biomarker for detection cervical cancer and precancerous lesions. Because of the small sample size of this study, subjective factors, sampling errors, experimental processes, and other factors may influence the results. Moreover, the experimental sensitivity and specificity are still not satisfactory. However, since the tests do not require further patient visiting and sampling, and are easy to operate, this method as a screening tool has practicable advantages. Further studies by expanding sample size and optimizing experimental method are needed to more accurately assess its clinical potentials in the future.

References

Correlation of cervical intraepithelial neoplasia with expressions of p16 and Ki67 in exfoliated cervical cells etc.


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Evaluation of the importance of the serum levels of CA-125, CA15-3, CA-19-9, carcinoembryonic antigen and alpha fetoprotein for distinguishing benign and malignant adnexal masses and contribution of different test combinations to diagnostic accuracy

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Summary

Purpose: The aim of this study was to investigate the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the serum levels of CA-125, CA15-3, CA19-9, carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) in the differentiation of benign and malignant ovarian tumors histopathologically diagnosed in patients and to determine the effects of the different test combinations on diagnostic accuracy. Material and Methods: One-hundred sixty-eight patients that had their preoperative CA-125, CA15-3, CA19-9, CEA, AFP levels assessed and that were subsequently surgically treated for adnexal masses, were included in the study. For each tumor markers in these patients with histopathologically-confirmed diagnosis, the sensitivity, specificity, PPV and NPV, and diagnostic accuracy, and odds ratio were calculated.

Results: The sensitivity, specificity, PPV, NPV of CA-125 with cut-off 35 U/ml, were found to be 78.9%, 86.9%, 63.8%, and 93.3%, respectively. The diagnostic odds ratio of CA-125 with cut-off of 35 U/ml, was found to be 25. With cut-off 65 U/ml, the sensitivity, specificity, PPV, NPV values were 86.8%, 95.3%, 80.6%, and 90.5%, respectively. The sensitivity, specificity, PPV, and NPV of CA15-3 were 26.3%, 98%, 80.6%, and 81.6%, respectively. The sensitivity, specificity, PPV, and NPV of CA19-9 were 26.3%, 98%, 80.6%, and 81.6%, respectively. Likelihood ratio tests: positive (LR+) = 6.83 and negative (LR-) = 0.76, with an odds ratio: 8.9. The risk of malignancy for adnexal masses with higher CA15-3 increased by approximately nine times. For CA-19-9, the sensitivity, specificity, PPV and NPV were found to be 18.4%, 93%, 43.7%, and 79.6%, respectively. CA-19-9 was not statistically significant in the differentiation of benign and malignant of adnexal masses. Even the combinations of CA125 + CEA + CA19-9 + AFP and CA125 + CA15-3 made a small contribution (one, two, and four cases, respectively), but was not statistically significant. Conclusion: The levels of CA-125 and CA15-3 were found to be significant in order to distinguish benign and malign; CA-19-9, CEA, and AFP were not found to be significant. The different test combinations did not have contribution for diagnostic accuracy.

Key words: CA125; CA15-3; Adnexal mass; Tumor markers; Benign and malign masses.

Introduction

Tumor markers are the substances which are derived from the structure of hormone, enzyme, metabolit, immunoglobulin or protein, produced by the related tumor or tissue at supra-physiological levels, and measured quantitatively in the patient’s tissue, blood or other body fluids with biochemical or immunochemical methods. An ideal tumor marker should be specific for the tumor, with low false positive and negative values and able to indicate the size of the tumor, ie, the extent of disease and response to treatment [1]. The frequently used tumor markers in gynecology CA-125, CA-19-9, CA-15-3, carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) [2]. An ideal tumor marker for the differentiation of malign and benign adnexal masses has not yet been described. CA-125 is the antigenic determinant on the high molecular weight glycoprotein, recognized by the monoclonal antibody OC-125. The expression of this antigen is seen in the tissues derived from adult mesothelium. These tissues are mesothelial epithelium of pleura, pericardium and peritoneum, fallopian tubes, and epithelial components of endometrium and endocervix. CA-125 is not seen in adults or ovaries in fetus. CEA is a protein seen in many cells, but the elevated values are associated with tumors and the developing fetus. Initially, it was of great interest for it to be a potential accurate diagnosis tool for malignant tumors of the gastrointestinal tract and it was firstly defined for colon cancers. Then, it was been shown that it could arise in stomach, lung, pancreas, and breast cancers. AFP is a polypeptide oncofetal antigen in the serum of human fetuses. In in adults, it has been found in malignant neoplasms with endodermal origin, such as liver tumors and gonadal tumors, such as ovarian endodermal sinus. CA-19-9 was found as an another monoclonal antibody to react with...
Table 1. — The values and the mean values of tumor markers in patients with benign and malign disease.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Malign</th>
<th>Benign</th>
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<tr>
<td>CA-125</td>
<td>Mean: 183.34, Min: 7.76, Max: 1032</td>
<td>Mean: 20.87, Min: 2.63, Max: 143.2</td>
</tr>
<tr>
<td>CA 15-3</td>
<td>Mean: 55.09, Min: 1.39, Max: 700.8</td>
<td>Mean: 12.67, Min: 0.50, Max: 50.4</td>
</tr>
<tr>
<td>CA 19-9</td>
<td>Mean: 19.77, Min: 1.50, Max: 68.28</td>
<td>Mean: 13.25, Min: 0.53, Max: 88</td>
</tr>
<tr>
<td>CEA</td>
<td>Mean: 4.13, Min: 0.20, Max: 26.78</td>
<td>Mean: 2.27, Min: 0.15, Max: 13</td>
</tr>
<tr>
<td>AFP</td>
<td>Mean: 3.24, Min: 0.3, Max: 28.60</td>
<td>Mean: 3.10, Min: 0.10, Max: 109.4</td>
</tr>
</tbody>
</table>

Table 2. — Specificity, sensitivity, PPV, and NPV calculated for each studied marker.

<table>
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<tr>
<th>Markers</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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<td>CA 15-3</td>
<td>90.5%</td>
<td>96.1%</td>
<td>66.6%</td>
<td>81.6%</td>
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<tr>
<td>CA 19-9</td>
<td>18.4%</td>
<td>93.0%</td>
<td>43.7%</td>
<td>79.6%</td>
</tr>
<tr>
<td>CEA</td>
<td>31.5%</td>
<td>88.4%</td>
<td>44.4%</td>
<td>81.3%</td>
</tr>
<tr>
<td>AFP</td>
<td>2.6%</td>
<td>98.0%</td>
<td>33.3%</td>
<td>77.5%</td>
</tr>
<tr>
<td>CA 125 (cut off: 35 U/ml)</td>
<td>78.9%</td>
<td>86.9%</td>
<td>66.8%</td>
<td>93.3%</td>
</tr>
<tr>
<td>CA 125 (cut off: 65 U/ml)</td>
<td>65.7%</td>
<td>95.3%</td>
<td>80.6%</td>
<td>90.3%</td>
</tr>
</tbody>
</table>

The mean age of the patients with malignant ovarian tumors was 60.7 ± 12.9 years (min: 23, max: 76); those with benign ovarian tumors it was 38 ± 13.9 years (min 16, max: 83). There was a significant difference between benign and malignant ovarian tumors in terms of age, gravity, and parity.

Patients were classified according to benign or malignant disease and the minimum, maximum, and mean values tumor markers were detected (Tables 1–4).

Discussion

It has been reported in several studies that, CA-125 measurement was useful in terms of the diagnosis of ovarian masses, distinction of benign- malign, follow-up for response of chemotherapy, and tumor recurrence [3, 4]. CA-125 is the most reliable serum marker, but the role in screening is controversial [5, 6]. In the present study, the authors examined the usefulness of 30 CA-125 for identification of benign and malignant masses in 30 malign and 138 benign adnexal masses. For CA-125 with cut-off 35 U/ml, the sensitivity, specificity, PPV, NPV, were 78.9%, 86.9%, 63.8%, and 93.3%, respectively. With cut-off 65 U/ml, the sensitivity, specificity, PPV, and NPV values were 65.78%, 95.3%, 80.6%, and 90.5% were found, respectively. When the cut-off increased from 35 U/ml to 65 U/ml, sensitivity decreased from 78.9% to 65.7% and specificity increased from 86.9% to 95.3%. In addition, PPV increased from 63.8% to 80.6%. CA-125 with a cut-off 35 U/ml, was detected in 30 of the 38 malign cases. CA-125 with a cut-off 65 U/ml was detected in 25 cases. The increase of specificity showed that the number of benign cases increased from 113 to 124, (the actual negativity was detected for 11 more cases). CA-125 with cut-off 35 U/ml, LR+ = 6.03, LR- = 0.24 were detected, namely high CA-125 gave one false positive result for each six positive results. In the present study, according to these values, diagnostic odds ratio was found as 25 for CA-125 with cut-off 35 U/ml. The sensitivity and specificity of CA-125 for
The sensitivity value is not a tumor marker that could be used alone, but especially in mucinous type of ovarian cancers with a low expression of CA-125, could provide an advantage.

In the present study, four out of 38 cases were mucinous type ovarian carcinoma, and CA19-9 was elevated in two patients. In both of these cases, due to high CA-125, CA19-9 could not provide an additional contribution to the pre-diagnosis. In the study performed by Fioretti et al., in women diagnosed with ovarian mass with an age of 50 years or more, according to use of CA-125 alone for differentiation of benign and malignant masses, higher sensitivity (93.2% vs. 81.1%, \( p = 0.03, p < 0.05 \)) and non-significant specificity (78.9% vs. 86% \( p < 0.05 \)) were detected that for the combination of CA-125 (cut-off 65 U/ml) and CA19-9, respectively [13].

In the present study, in ten of 38 malignant adnexal mass, five of 130 benign mass, an increased value of CA15-3 was found. For the differentiation of malignant and benign adnexal masses, Scambia et al. measured CA15-3 levels in benign and malignant gynecological pathologies. In 74 malignant cases (41%), the values passing 30 U/ml and abnormal results were positively correlated. This ratio was 71% for malignant ovarian tumors and 6% for benign cases [14]. Similar to this present study, CA15-3 was significant in the differentiation of malignant or benign adnexal mass. Sensitivity, specificity, PPV, and NPV were 26.3%, 96.1%, 66.6%, 81.6%, respectively. (LR+ = 6.83; LR- = 0.76, and odds ratio: 8.98). Therefore the adnexal masses with high CA15-3 level had an approximately nine-fold higher risk for malignancy. The sensitivity, specificity, PPV and NPV for AFP were found to be 2.63%, 98%, 33.33%, and 77.57%, respectively. It is not meaningful in the differentiation between benign and malignant adnexal masses. Although it was a close to an ideal tumor marker for germ cell tumors, in the literature, it has been reported that the sensitivity is low for epithelial ovarian tumors [15]. The elevation of AFP was detected for only one out of 38 malignant adnexal masses and it was a serous-type epithelial ovarian carcinoma.

In a study performed by Eljito et al., it was shown that when three tumor markers (CA-125, CA19-9, CA15-3) were used together as panel to distinguish between benign and malignant adnexal masses; in all cases the three markers were high and all cases were malignant but the number of malignant cases that had higher three tumor markers, was lower frequency. In this report, CA15-3 was the most important contributor in determining malignancy in the whole group [16].

The use of tumor markers in panels was not more effective according to other test models of (ultrasonography and/or pelvic examination and/or menopausal status). Also in the present study, based on ROC curve, it has been detected that the tumor marker which had the highest value was CA-125 (Figure 1). Adding CA15-3 to CA-125 provided a very small contribution but it did not reach statisti-
In addition, in this study, it has been concluded that, the combinations of CA125 + CEA + CA19-9 and CA125 + CEA + CA19-9 + AFP were not useful for differentiation of benign and malignant adnexal mass.

**Conclusion**

CA-125 and CA15-3 serum levels were statistically significant to distinguish between benign and malignant ovarian tumors, CA 19-9, CEA, and AFP were not significant. The different test combinations did not contribute to the diagnostic accuracy.

**References**


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The effect of coexisting uterine myomas on clinico-pathological variables of endometrial carcinoma

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Summary

Purpose: To assess the prevalence of leiomyomas in patients with endometrial carcinoma (EC) and the association of their presence with clinico-pathological variables and with survival. Materials and Methods: A retrospective chart review was conducted of all endometrial carcinoma (EC) patients diagnosed and treated in the present institution between 2002 and 2008. Selected clinical data were abstracted from medical records. Pathological data such as the presence of myomas (any size), tumor grade, depth of myometrial invasion, presence of lymphovascular space involvement (LVSI), and the presence of metastases, are based on the original pathology report. Results: Coexisting myomas were found in 74 (56.9%) of 130 EC patients diagnosed during the study period. No significant difference with regard to age, histological type, stage, grade, depth of myometrial invasion, LVSI, lymph node involvement, and presence of metastases (other than lymph node involvement) was found between patients without and with myomas. There was also no significant difference in survival of EC patients without and with coexistent myomas. Conclusion: The present data seem to indicate that the presence of myomas does not affect clinico-pathological variables of EC patients nor their survival.

Key words: Uterine myoma; Endometrial carcinoma; Clinico-pathological variables; Survival.

Introduction

Endometrial carcinoma (EC) is the most common type of gynecological cancer in Western countries [1,2]. The majority of them are of type 1 i.e. – endometrioid EC. They are associated with prolonged unopposed estrogenic stimulation, are well to moderately differentiated, arise in a background of endometrial hyperplasia and have a favorable prognosis. About 10% of endometrial cancers are of type 2. These tumors are either poorly differentiated endometrioid or non-endometrioid carcinomas [3]. They are not estrogen associated, arise in a background of endometrial atrophy and most commonly are of the serous and clear cell types [4,5].

Uterine myomas are the most common benign solid pelvic tumors in women [6]. The source of these lesions is mainly the smooth muscles of the uterus and possibly also the smooth muscle of uterine blood vessels; they are also estrogen associated. Thus, they are diagnosed only after menarche, they decrease in size after menopause and under other hypoestrogenic conditions, and may increase markedly in size during pregnancy and in the premenopausal years secondary to anovulatory cycles. The estrogen receptors in myomas bind 20% more estradiol per milligram of cytoplasmic protein than the normal adjoining myometrium [7,8].

Studies with regard to the coexistence of endometrial carcinoma and uterine myomas are extremely scarce [9].

The purpose of the present study was to assess the prevalence of myomas in patients with endometrial carcinoma and the association of their presence with clinico-pathological variables and with survival.

Materials and Methods

A retrospective chart review was conducted of all EC patients diagnosed and treated in the present institution between 2002 and 2008. Selected clinical-pathological variables were abstracted from medical records. Pathological data such as the presence of myomas (any size), tumor grade, depth of myometrial invasion, presence of lymphovascular space involvement (LVSI), and the presence of metastases, are based on the original pathology report. Comparisons of the variables between EC patients without and with myomas were made using the chi-square test for categorical variables and Fisher’s exact test, for small cells. Survival was assessed using the Kaplan-Meier method and comparison of survival between women without and with myomas was made by the log-rank test.

Results

During the study period, 130 EC patients were diagnosed. Coexistent myomas were found in 74 (56.9%) patients. The presenting symptom in 95 (73.1 %) of them was postmenopausal bleeding and the diagnosis was made by Pipelle endometrial biopsy in 89 (68.5%) of them. A comparison between EC patients without and with myomas was made using the chi-square test for categorical variables and Fisher’s exact test, for small cells. Survival was assessed using the Kaplan-Meier method and comparison of survival between women without and with myomas was made by the log-rank test.

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lymph node involvement was available for 101 patients. Of 47 patients without myomas and 54 patients with myomas, six (12.8%) and five (9.3%) patients respectively had lymph node metastases. This difference was not significant ($p = 0.57$).

The overall five-year survival of the EC patients was 83.1%. There was no significant five-year survival difference between EC patients without and with myomas (84.5% vs. 82.0% respectively; $p = 0.5$).

**Discussion**

The authors found that 74 (56.9%) of 130 EC patients had concomitant uterine myomas. However no association between the presence of myomas and clinico-pathological variables and with survival was observed. A PubMed search located only one previous similar study in the English language. In that study, Koshiyama et al. [9] assessed the relationship between EC and coexistent myomas, as well as with adenomyosis and endometriosis. Only 51 (28%) of their 179 EC patients had myomas. This rate is lower than in the present study and may possibly be due to racial differences. Similar to the present findings they observed no difference between EC patients without and with myomas with regard to age, Stage, grade, and tumor type.

In addition to the variables evaluated by Koshiyama et al. [9], the present authors assessed the association between EC and coexistent myomas with regards to depth of uterine invasion, LVSI, lymph node, and other metastases. Again no association was found.

As in the study by Koshiyama et al. [9], the present authors also observed no survival difference between EC patients with and without coexistent myomas.

The present findings may be biased by the fact that the majority of the patients were post-menopausal and some of the myomas may have shrunk and disappeared. In addition, the pathological examination was not performed with special attention to the presence of myomas. Nevertheless, the present data seem indicate that the presence of myomas do not affect clinico-pathological variables of EC patients nor their survival.

**References**


The effect of coexisting uterine myomas on clinico-pathological variables of endometrial carcinoma


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Post-treatment human papillomavirus status and recurrence rates in patients treated with loop electrosurgical excision procedure conization for cervical intraepithelial neoplasia

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Summary
Purpose of investigation: The aim of the study was to evaluate the rate of human papillomavirus (HPV) infection clearance after loop electrosurgical excision (LEEP) procedure conization for cervical intraepithelial neoplasia (CIN) and the factors related to such clearance and to assess the relation between HPV and recurrence. Materials and Methods: A total 141 patients who underwent LEEP owing to high-risk human papillomavirus (HR-HPV) associated with CIN were involved in this study. All patients with negative margins on LEEP specimens were followed up with HPV testing and cervical smear after three, six, nine, and 12 months post-treatment. If necessary, cervical biopsy under colposcopy was performed. Results: LEEP can effectively eliminate HPV infection. Most patients cleared HPV infection within six months. The persistent HPV infection rates were 44.6%, 10.6%, 5.7%, and 2.1% after three, six, nine, and 12 months, respectively. The clearance rates were significantly slower in patients with HPV 16 infection, and not differ significantly by age, parity, and pathologic degree. Patients with persistent HR-HPV infection after treatment had a significantly higher risk for recurrence/residual after LEEP compared to patients with negative HPV infection. Conclusion: The authors concluded that patients who were positive for HPV infection, especially for HPV 16, should be followed up closely after treatment.

Key words: Cervical intraepithelial neoplasia; Persistent HPV infection; HPV genotype; Negative margins; Residual/recurrence.

Introduction
Cervical cancer is one of the most serious health problems in women, which is the second most common female cancer worldwide, with 190,000 deaths each year [1]. It is largely preventable in view of its well-known nature with detectable and treatable precancerous lesion: cervical intraepithelial neoplasia (CIN). CIN bears a significant risk of developing invasive cancer if not treated [2]. Persistent infection with high-risk human papillomavirus (HR-HPV) is a necessary factor of the vast majority of CIN and cervical cancer cases, and such HPV can be detected in almost all tissues of cervical cancer [3, 4]. Women diagnosed with CIN require treatment to prevent the progression to cancer. Effective treatments include CIN removal and eradication of HPV.

The loop electrosurgical excision procedure (LEEP) is being widely used for the management of CIN, which has been accepted as the gold standard for CIN therapy, as well as for diagnosis. Several studies have suggested that effective conization also eradicated HPV infections in most patients with CIN [5, 6]. However the residual/recurrent rate after LEEP varies between 5%-30%, even women with negative margins, required follow-up [7, 8]. HR-HPV detection techniques have been proposed for follow-up after treatment for CIN. Several studies have reported that age, cytological grade, menopausal status, resection margin involvement, cytological grading, and HPV viral load are risk factors of residual or recurrent disease [9, 10]. However, the status of HPV infection after LEEP for CIN and factors affecting persistence of HPV has not been well-defined, and if post-treatment HPV persistent infection could be regarded as a predictor for residual/recurrent disease of CIN after LEEP, it has not been adequately determined.

The aims of this study were to assess the status of HR-HPV after successful treatment for CIN with negative margins and to assess the risk factors associated with such status, and to determine HPV persistent infection as a predictor for residual/recurrent disease of CIN with negative margins after LEEP.

Materials and Methods
Study patients
There were 254 patients who had received LEEP in the Clinic between October 2008 and August 2009 at the Department of Gynecology of the First Affiliated Hospital of Xin Jiang Medical University. Inclusion criteria consisted of histologically-verified CIN I, CIN II-III based on LEEP specimen, and negative margins without additional hysterectomy. Other inclusion criteria were an HPV-positive result and tumor-free end-cervical excervical resection margins of LEEP specimens. Exclusive criteria: patients who had previous histories of diagnosis or treatment for CIN or cervical cancer, who had any malignant disease, who had any immunosuppressive disease or under-treatment, who were pregnant, or who had undergone complete hysterectomy for carcinoma in situ of uterine cervix. The patients underwent postoperative follow-up visits at regular intervals. Postoperative follow-ups were done every three months during first year. Each visit included pelvic examination, cervical inspection, HPV testing, liquid-based cytology test, and colposcopic examination of the...
cervix. Colposcopically-directed punch biopsy of the cervix was performed for abnormal cytology or HR-HPV detection by HPV tests. Abnormal cytology was defined as equal to atypical squamous cells of undetermined significance (ASCUS) or higher according to the 2001 Bethesda system [11] criteria for the presence of residual/recurrent disease, based on positive histology of colposcopy-directed biopsy; histologically confirmed presence of CIN of any grade was considered as residual/recurrent disease.

A total of 177 patients between 23 to 65 years with a median age of 41.14 ± 8.81 years met the aforementioned requirements. Among them, 36 patients were excluded because of inadequate follow-up; the remaining 141 patients were satisfactory for study inclusion criteria.

**HPV sample and genotyping**

The collection of cervical cells scrapings for each patient were performed using a plastic cervical swab from ecto- and endocervix of uterus. Each plastic swab was well-mixed with one ml of specimen transport medium and stored immediately at 4°C. All specimens were finally sent to clinical laboratory for HPV analysis. Using gene amplification and flow-through hybridization technology, which conclude three processes of extraction of total DNA of cervical cells, gene amplification (PCR), and flow-through hybridization. HPV testing were performed strictly according to the manufacturer’s instructions. The final results were detected by colourimetric change on the chip under direct visualization. There were a total of 21 HPV types (13 High-risk HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 types, and five low-risk HPVs 6, 11, 42, 43, and 44; popular HPV types in Chinese population 53, 66, and CP8304).

**LEEP**

LEEP was performed under local anesthesia using wire loop electrodes. The electrosurgical procedure was performed with a high-frequency electrical generator, with diathermy apparatus set to 50W for cutting and 40W for coagulation. All specimens were marked with a suture at 12-o’clock, fixed with 10% buffered formalin before submitted for pathologic examination. Histological diagnosis of LEEP specimens were made according to the World Health Organization (WHO) classification. All LEEP specimens were finally sent to clinical laboratory for HPV test. Abnormal cytology was defined as equal to atypical squamous intraepithelial lesion (LSIL) (36.9%), and 38 high-grade squamous intraepithelial lesion (HSIL) (26.9%). Type HSIL (26.9%). Type

**Results**

**Patients’ characteristics**

One hundred and forty-one patients who underwent LEEP with negative margins were enrolled in this study. The median age was 41.14 ± 8.81 years old (range from 23 to 65). Before treatment the thin-layer cytological test (TCT) results showed that 141 patients had 30 ASCUS (21.3%), 21 ASC-H (14.9%), 52 low-grade squamous intraepithelial lesion (LSIL) (36.9%), and 38 high-grade squamous intraepithelial lesion (HSIL) (26.9%). Type

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n = 141)</th>
<th>Occurrence (%)</th>
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<td>Parity</td>
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<tr>
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<td>69</td>
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<tr>
<td>≥ 2</td>
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<td>51.1</td>
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<td>Initial cytology</td>
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<tr>
<td>ASCUS</td>
<td>30</td>
<td>21.3</td>
</tr>
<tr>
<td>ASC-H</td>
<td>21</td>
<td>14.9</td>
</tr>
<tr>
<td>LSIL</td>
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<td>36.9</td>
</tr>
<tr>
<td>HSIL</td>
<td>38</td>
<td>26.9</td>
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<td>Pre-LEEP subtype</td>
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<td>CINII</td>
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<tr>
<td>CINIII</td>
<td>33</td>
<td>23.4</td>
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</table>

*Include single and multiple infections.

**Table 1. — Patients’ characteristics.**

**Table 2. — Association between age parity and persistent HPV infection after treatment.**

<table>
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<tr>
<th>Factors</th>
<th>n</th>
<th>Three months*</th>
<th>Six months*</th>
<th>Nine months*</th>
<th>Twelve months*</th>
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<tr>
<td>30-39</td>
<td>52</td>
<td>20 (38.4)</td>
<td>3 (6.1)</td>
<td>2 (4.2)</td>
<td>1 (2.1)</td>
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<tr>
<td>40-49</td>
<td>56</td>
<td>27 (48.2)</td>
<td>8 (15.4)</td>
<td>4 (8.2)</td>
<td>2 (4.1)</td>
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<td>≥ 50</td>
<td>18</td>
<td>8 (44.4)</td>
<td>2 (12.5)</td>
<td>1 (6.7)</td>
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<tr>
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<tr>
<td>&lt; 2</td>
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<td>33 (41.8)</td>
<td>5 (6.8)</td>
<td>2 (2.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>≥ 2</td>
<td>62</td>
<td>30 (48.4)</td>
<td>10 (16.9)</td>
<td>6 (10.3)</td>
<td>3 (5.2)</td>
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<tr>
<td>p value</td>
<td>0.383</td>
<td>0.784</td>
<td>0.278</td>
<td>0.246</td>
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</tbody>
</table>

*Include single and multiple infections.

**HPV16 was identified most frequently (62.4%), and the other high-risk subtype account for 37.6%. CIN was confirmed by histology; 54 patients (38.3%) had CIN I, 54 patients (38.3%) had CIN II, and 33 patients had CIN III (23.4%). Patients characteristics are summarized in Table 1.**

**The association between persistence of high-risk HPV infection and related factors after LEEP**

**Age and parity factors**

The persistent HPV infection was detected 63 (44.6%), 15 (10.6%), eight (5.7%), and three patients (2.1%), respectively, at three, six, nine, and 12 months after treatment. The rates of persistent HPV infection at each follow-up times had no significant difference among different age or parity groups (p > 0.05) (Table 2).

**Pathologic results and HPV subtypes factors**

Next, the authors studied the relationship between pathologic results, HPV subtypes, and the persistent HPV infection. They found that there was no significant difference among pathologic groups, but the rates of persistent HPV infection were significantly higher in patients with HPV 16 type infections compared with those of patients with other subtypes infections, especially at three and six months (p = 0.039; p = 0.037). However the differences were not statistically significant at nine and 12 months (p = 0.120; p = 0.234) (Table 3).
The authors analyzed the clearance of HPV infection among the different groups by survival analysis with log-rank test, and then found that there were no significant differences among age \( p = 0.397 \), parity \( (p = 0.876) \) and pathology \( p = 0.934 \) groups, but the clearance were significantly slower in patients with HPV 16 type infections compared with those of patients with other subtype infections \( (p = 0.004) \).

**Relationship between recurrent/residual disease and HPV infection after LEEP at different follow-up times**

All patients had completed follow-up. Eighteen (12.7%) patients were diagnosed with recurrent/residual diseases, seven patients presented with CINI, four patients presented with CINIII, five patients presented with CINII, one patient presented with CIS, and one patient with histologically proven invasive carcinoma. Sixteen patients had persistent HPV infection with the same subtype before LEEP and at the end of this study, and two patients with recurrent disease tested negative for HPV. A comparison of the two groups divided according to positive HPV or negative HPV after the LEEP at different follow-up times is detailed in Table 4. The recurrent rates showed significant differences between two groups at three, six, and 12 months follow-up \( (p < 0.05) \), although at nine months follow-up the differences were not statistically significant \( (p = 0.063) \).

**Discussion**

Organized cervical cancer screening systems with cytological or HPV tests have resulted in a sustained decline in incidence and mortality due to cervical cancer [12]. The main role of screening test is to detect precancerous lesion CIN to prevent them from developing cervical cancer. In recent years, LEEP has been widely used in clinical operations due to simple operation and less complication, but it is known that patients treated for CIN have a remarkable risk for cervical cancer compared to the general population. Another important concern after LEEP is treatment failure. This indicates that it is very important for early detection and treatment.

Almost all recurrent disease after conization for CIN occurs in patients with persistent HPV infection. The persistence of HR-HPV after conization treatment shows two possibilities: the presence of residual/recurrence, or cells harboring HPVs that could result in the development of new cervical lesion. In the present study within three months after treatment, 44.6% of the patients had persistent initial HPV infections, but at the sixth month, only 10.6% of the patients presented with persistent initial HPV infection. HPV infection cleared gradually, and only a few cleared at 12 months after successful treatment. A majority of the HPV infection had been eradicated within six months. Several studies had reported the clearance rates of HPV infection, but the results have been somewhat inconsistent. Elfgren et al. reported that only nine percent of patients with CINI-II after treatment remained with persistent HPV infection at three months [6]. The figure was lower than the present results. However Song et al. reported the clearance of HPV infection was 92.6% at six months, which was similar to the present (89.4%) [10]. All of these confirm that LEEP is an effective method in clearing HPV infection. On the other hand, most patients had cleared HPV infection at the sixth month, which might explain the first visit time within six months.

In many literatures, the recurrence/residual rates have been inconsistent. In the present study, the results was 12.7%, which is much higher than that reported by Ghaem-Maghami (three percent), but similar to that reported by Alonso et al. (17.7%) and Leguevaque et al. (12%) [9, 13, 14]. Early identification of risk factors that could predict patients at high risk for recurrence/residual after treatment would be of great important. In the present study group, patients with persistent HR-HPV infection after treatment had a significantly higher risk for recurrence/residual after LEEP compared to patients with negative HPV infection at different follow-up times. A systematic review of the literature shows that the sensitivity of HPV DNA testing in detecting treatment failures was quite good in most studies and that a positive HPV test, even in the presence of normal cytology, may pick up early and accurately a treatment failure [15]. Cecchini et al. reported that most CIN 2 > recurrences after LEEP occur in subjects with persistent HPV infection [16]. Bae et al. reported that the only significant risk factor for redevelopment of CIN after conization was persistence of the same HPV subtype [17]. In this study, the authors assessed the preconization HPV genotype as risk factor for predicting the recurrence/residual, and concluded that persistent abnormal HPV tests can more quickly detect the treatment failure, further diagnostic evaluation for secondary treatment, and close follow-up are needed for

---

### Table 3.

**Association between pathologic results, HPV subtypes, and persistent HPV infection.**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Pathologic degree</th>
<th>HPV subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CIN I</td>
<td>CIN II</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>59.2</td>
</tr>
<tr>
<td></td>
<td>(11.1)</td>
<td>(18.6)</td>
</tr>
<tr>
<td></td>
<td>(2.8)</td>
<td>(10.6)</td>
</tr>
<tr>
<td></td>
<td>0.063</td>
<td>0.001</td>
</tr>
<tr>
<td>p value</td>
<td>0.599</td>
<td>0.546</td>
</tr>
</tbody>
</table>

---

### Table 4.

**Association between recurrence/residual and HPV infection at different follow-up times.**

<table>
<thead>
<tr>
<th>HPV</th>
<th>Months</th>
<th>Total cases</th>
<th>Recurrence/residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (+)</td>
<td>3</td>
<td>63</td>
<td>8</td>
</tr>
<tr>
<td>Negative (−)</td>
<td>3</td>
<td>78</td>
<td>1</td>
</tr>
<tr>
<td>Positive (+)</td>
<td>6</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Negative (−)</td>
<td>6</td>
<td>117</td>
<td>1</td>
</tr>
<tr>
<td>Positive (+)</td>
<td>9</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Negative (−)</td>
<td>9</td>
<td>119</td>
<td>0</td>
</tr>
<tr>
<td>Positive (+)</td>
<td>12</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Negative (−)</td>
<td>12</td>
<td>124</td>
<td>0</td>
</tr>
</tbody>
</table>

---

The result of HPV infection after LEEP at different follow-up times is detailed in Table 4. The recurrent rates showed significant differences between two groups at three, six, and 12 months follow-up \( (p < 0.05) \), although at nine months follow-up the differences were not statistically significant \( (p = 0.063) \).
women with persistent HPV infection after treatment. Therefore assessment persistent HPV infection and the factors association with them are important to clinical detection.

Several studies had reported on the clearance of HPV infection after CIN treatment, but the results have been somewhat inconsistent. The well-known risk factors for persistence HPV infection after treatment are advanced age, parity, the use of contraceptives, cytology grade, high histological grade of the cervical lesion, glandular involvement, conization specimens, specimens margin status, and HR-HPV load before treatment. Sarian et al. reported that women whose age above 35 years (irrespective of margin status) were strongly associated with positive HPV during follow-up [18]. While Song et al. reported that age and parity, conization grade, and histology of colposcopic biopsy were not significant predictors of HPV clearance after conization, and then they showed that high viral load (RLU/PC > 500) was the only significant independent predictor of HPV persistence [10]. Consistent with these results, the present authors found that the rates of persistent HPV infection at each follow-up visit did not differ significantly among different age, parity or pathologic degree groups. However, the clearance was significantly slower in patients with HPV 16 type infections compared with those of patients with other subtype infections. The reason for this difference still remains unclear. Kreimer et al. reported that the two-year risk associated with HPV16 positivity was 37.0%, significantly higher than for other carcinogenic HPV types (10.8%, p < 0.001), non-carcinogenic types (1.5%, p < 0.001), or testing HPV negative (0%). They concluded that women who tested positive after LEEP for carcinogenic HPV types, especially HPV 16, had high risk of subsequent CIN II + HPV 16 was more difficult to be cleared than other HR-HPV infections. It may be the risk predictor of HPV persistence [19]. The present authors also obtained the same results. In addition, it was worth paying attention to one point that two patients with recurrence tested negative for HPV at third and sixth month, respectively. Two remained negative during the whole follow-up period, and both were diagnosed with CIN2. Most likely, it could be explained by a diagnostic limitation or a previously missed early CIN.

Conclusion

In conclusion, the effective conization could eradicate HPV infection in most people within six months. After this initial period, clearance continues. The clearance rates were significantly slower in patients with HPV 16 infection, and did not differ significantly according to age, parity, and pathologic degree. Patients who are positive for HPV infection, especially for HPV-16 infection after LEEP, should be closely followed-up, even with negative margin.

Acknowledgments

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References


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Comparing letrozole with medroxyprogesterone acetate (MPA) as hormonal therapy for simple endometrial hyperplasia without atypia in adult and middle-aged women

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²Gynecology Oncology Department, Reproductive Research Center, Shahid Sadoughi University of Medical Science, Yazd
³Internal Medicine, Islamic Azad University, Yazd; ⁴Yazd Branch, Islamic Azad University, Yazd (Iran)

Summary

Background: The aim of this survey was to compare the effect of letrozole with medroxyprogesterone acetate (MPA) in treatment of simple endometrial hyperplasia to preserve fertility in young women. Materials and Methods: Forty-five patients referred to Shahid Sadoughi gynecology clinics from 2009 until 2011 who suffered from abnormal vaginal bleeding or endometrial thickness, that underwent curettage with diagnosis of simple endometrial hyperplasia without atypia were enrolled. The patients were divided randomly into two groups. First group including 22 women receive ten mg MPA, for ten days during a month for three months. All cases were followed by interview, endometrial curetage, and vaginal sonography. Serum level of estradiol was checked before and after treatment. At the end of the study, biopsy was retaken in 41 patients. All the patients were under observation by two gynecologists. Results: Age range of patients was 20 to 42 years. Mean body mass index (BMI) in the MPA and letrozole groups was 29.13 ± 4.8 and 25.42 ± 4.2, respectively. Fifty and 34.8 percent of cases had history of obesity or polycystic ovarian syndrome (PCOS) in MPA and letrozole groups, respectively. Forty-one selected cases (20 of the MPA and 21 of the letrozole groups) continued the treatment for three months. The endometrial thicknesses decreased in both groups. Serum estradiol level also decreased in both groups. The most common complication in the MPA and letrozole groups was headache (27.3%) and flashing and dizziness, respectively. The side-effects were reported less in the letrozole group and the most common ones in this group were dizziness and flashing. Discussion: In women suffering from simple endometrial hyperplasia without atypia, letrozole can lead to decrease of serum estradiol level and endometrial thickness like MPA. In both groups, there was no simple hyperplasia report in curettage report following treatment. It should be noted that there was an incomplete response to treat case with pathology of disordered proliferative type. Conclusion: Letrozole is a good therapeutic option in simple endometrial hyperplasia without atypia: cases candidate for medical treatment. To confirm the effect and safety of letrozole, more studies with larger samples are recommended.

Key words: Endometrial hyperplasia; Management; Hormone therapy; Letrozol; Medroxyprogesterone.

Introduction

Hyperplasia is the result of permanent exposure of endometrium to estrogen. The risk of endometrial cancer depends on presence and severity of atypia. Lesions without spontaneous atypia regress following curettage or progestin treatment and are associated with low risk of adenocarcinoma [1]. Lesions with cytological atypia do not often regress and may be resistant to repeated curettage or long-term treatment with high-dose progestin. As lesions without treatment may progress to adenocarcinoma (10% - 30%), these lesions are considered as precancerous ones [2-5]. The best therapeutic option for hyperplasia with atypia is surgery. In women with a fertility-preservation desire, stronger and more long-term treatment of progesterin 40-80 mg of megestrol acetate daily for three to six months and repeated biopsied to monitor and confirm the therapeutic response are recommended. Seventy-five to 90 percent of these patients respond to medical treatment [5].

Aromatase inhibitor decrease serum level of estrogen to less detectable level by inhibiting androgen conversion to estrogen [1]. Letrozole and anastrozole are two aromatase inhibitors which are prescribed as an adjuvant hormonal therapy in menopause women suffering from breast cancer [2-6, 7]. Letrozole decrease local synthesis of estrogen in endometrium. With regards to intramural synthetic role of estrogen in progression of the lesion into endometrial cancer, this agent could be of assistance in treating different types of hyperplasia and even endometrial cancer. On the other hand; continuous use of letrozole in premenopausal women leads to ovulation induction which inhibits hyperplasia following progesterone secretion [4]. The aim of this survey was to study and compare the effect of letrozole on simple endometrial hyperplasia.

Materials and Methods

This randomized clinical trial was conducted on 45 women referred to Shahid Sadoughi Hospital from 2010 to 2011 with endometrial curettage diagnosis of simple hyperplasia without atypia. The cases were randomly divided into two groups. Serum
estriol level was checked in all of them before dividing the patients. First group (n = 23) received 2.5 mg letrozole daily for three months and the second group (n = 22) received ten mg medroxyprogesterone acetate (MPA) for ten days a month for three months. At end of each month, the patients were asked about their menstrual status and drug complications via telephone and then questionnaires were completed.

At end of third month, the endometrial thickness was measured by sonography and serum level of estradiol was measured. Outpatient endometrial biopsy was taken and if not possible, endometrial curettage was undertaken under general anesthesia. The data was analyzed by SPSS software version 11 and p value < 0.05 was considered significant statistically. T test (to compare body mass index (BMI) means), Wilcoxon test (to compare estradiol level and endometrial thickness before and after treatment) and Mann-Whitney test (to compare estradiol level and endometrium thickness before two groups) were used.

Results

Mean age of cases was 28-52 years. BMI, history of obesity, diabetes, hypertension, and polycystic ovarian syndrome (PCOS) were also checked. MPA side-effects such as headache, weight gain or edema, hypertension, nausea, vomiting, and abnormal uterine bleeding (AUB) are listed in Table 1. Letrozol side-effects such as dizziness, constipation, fatigue, flashing, AUB, and palpitation are listed in Table 2.

Therapeutic status (complete or incomplete) in MPA group was follow: 20 complete and two incomplete cases. The first incomplete treated case discontinued the treatment at the end of second month due to AUB whose final pathology demonstrated menstrual phase. The second one discontinued the therapy at the end of the second month due to amenorrhea whose final pathology demonstrated atrophic endometrium (Table 1).

In letrozole group, 20 and three cases underwent complete and incomplete treatment, respectively. The first incomplete treated case discontinued the therapy due to AUB and side-effects such as flashing, palpitation, and fatigue in the first month and underwent hysterectomy. The second untreated case with history of bilateral ovarian cysts and hypermenorrhea underwent hysterectomy due to ovarian cyst and AUB following two months and her pathology indicated proliferative phase. The third case with history of PCOS became pregnant following two months of letrozole therapy.

According to Table 1, the patients were divided into three BMI groups. The most prevalent complication in MPA group was headache and 90.9% of the patients received complete treatment.

According to Table 1, 56.5% of the cases had no history of any diseases. Approximately 17.4%, 8.7%, and 8.7% of the letrozole group had history of PCOS, obesity, and PCOS concurrent with obesity, respectively. There was no letrozole side-effect in 56.5% of them and 87% of the cases received complete treatment. There was no significant difference between two groups following three months of treatment (p = 0.721, Fisher Exact test).

Mean of estradiol was 43.34 ± 45.40 in 22 of cases on MPA, following three months of treatment. The data analyzed by Wilcoxon test showed that there was a significant difference before and after therapy (p < 0.001).

The mean of endometrial thickness was 11.09 ± 5.83 mm. It should be noted that the data analyzed by Wilcoxon test also indicated a significant difference before and after therapy.

According to Table 2, mean of serum estradiol and endometrial thickness analyzed by Wilcoxon test were significantly different (p < 0.001).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Medroxyprogesterone</th>
<th>Letrozole</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>Frequency Percentage</td>
<td>Frequency Percentage</td>
</tr>
<tr>
<td>&lt; 25</td>
<td>6 27.3 11 47.8</td>
<td></td>
</tr>
<tr>
<td>&gt; 25-29.9</td>
<td>7 31.8 7 30.4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22 100 23 100</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Past medical history</th>
<th>Frequency Percentage</th>
</tr>
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<tbody>
<tr>
<td>Obesity</td>
<td>5 22.7 2 8.7</td>
</tr>
<tr>
<td>PCOS</td>
<td>5 22.7 4 17.4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2 9.1 1 4.3</td>
</tr>
<tr>
<td>DM</td>
<td>0 0 1 4.3</td>
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<tr>
<td>PCOS and obesity</td>
<td>1 4.5 2 8.7</td>
</tr>
<tr>
<td>None</td>
<td>9 40.9 13 56.5</td>
</tr>
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<tr>
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<th>Frequency Percentage</th>
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</thead>
<tbody>
<tr>
<td>Complete</td>
<td>20 90.9 20 87</td>
</tr>
<tr>
<td>Incomplete</td>
<td>2 9.1 3 13</td>
</tr>
<tr>
<td>Total</td>
<td>22 100 23 100</td>
</tr>
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<table>
<thead>
<tr>
<th>Drug</th>
<th>Variable</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medroxyprogesterone</td>
<td>Estradiol level</td>
<td>69.18 ± 45.34</td>
<td>45.34 ± 45.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Endometrial thickness</td>
<td>60.95 45.4</td>
<td>5.07 5.83</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Letrozole</td>
<td>Estradiol level</td>
<td>80.37 ± 53.33</td>
<td>57.7 92.13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Endometrial thickness</td>
<td>15.23 ± 9.97</td>
<td>3.91 4.03</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Frequency Percentage</th>
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</thead>
<tbody>
<tr>
<td>Proliferative</td>
<td>6 27.3 12 57.12</td>
</tr>
<tr>
<td>Secretory</td>
<td>13 59.1 6 28.56</td>
</tr>
<tr>
<td>Menstrual</td>
<td>1 4.5 0 0</td>
</tr>
<tr>
<td>Atrophic</td>
<td>2 9.1 2 9.56</td>
</tr>
<tr>
<td>Disordered prolif.</td>
<td>0 0 1 4.76</td>
</tr>
<tr>
<td>Total</td>
<td>22 100 20 100</td>
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<table>
<thead>
<tr>
<th>Side-effects</th>
<th>Frequency Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without</td>
<td>7 30.8 13 56.5</td>
</tr>
<tr>
<td>With</td>
<td>15 69.2 10 43.5</td>
</tr>
<tr>
<td>Total</td>
<td>22 100 23 100</td>
</tr>
</tbody>
</table>
In MPA group, final pathologies were divided into proliferative, secretory, menstrual, and atrophic subgroups. In letrozole group, only 20 women underwent curettage and the pathology reports were divided into proliferative, secretory, atrophic, and disordered proliferative subgroups.

There was no simple hyperplasia in any of the groups. According to Table 3 which was analyzed by Fisher exact test, with regards to presence of complications, there was no significant difference between two groups ($p = 0.13$).

According to Table 4 and Mann Whitney test, estradiol level was not significantly different between letrozole and MPA groups following treatment ($p = 0.133$). According to the same table and Mann Whitney test, endometrial thickness was not significantly different between two groups following treatment.

**Discussion**

The present findings showed that AUB recovery following three months of treatment was 72.8% and 81.8% in MPA and letrozole groups, respectively (Table 1). In 2008 in Li et al. study on five women suffering from endometrial hyperplasia with and without atypia received letrozole for three months and AUB was not reported [4]. The present findings showed that serum estradiol level in MPA group decreased in all cases at the end of treatment. In letrozole group, the serum estradiol level decreased in all cases except for two (Tables 2 and 4).

In the Li et al. study, serum level of estradiol was checked monthly in letrozole group which showed a decrease during treatment. The endometrial thickness decreased at the end of treatment as well [4].

Barker et al. studied the effect of anastrozole and letrozole on endometrial hyperplasia with or without atypia and endometrial carcinoma. Their findings showed decrease of endometrial thickness in eight and four patients suffering from endometrial hyperplasia and endometrial adenocarcinoma, respectively, following 36 months of treatment [8].

In 2005, Agorastos et al. found that endometrium thickness decreased following anastrozole therapy for 12 months in postmenopausal and obese women suffering from endometrial hyperplasia with or without atypia [9].

Disordered proliferative phase is a borderline phase between proliferative and simple hyperplasia that compared to normal proliferative phase, has no uniform glands growth. It should be noted that compared to hyperplasia, the proportion of glands to stroma is maintained in this phase [3].

Li et al. findings in 2008 showed three secretary, two proliferative, and no disordered proliferative pattern in final pathology [4]; however, this might be due to limited number of young cases (27-38 years of age) who were not obese (BMI = 22-28).

Duration of therapy was three months in the present study and was three to six months in other studies [10-16]. It should be noted that duration of therapy is at least six to nine months in cases with atypia and the biopsy taking is repeated every six months [15].

In 2007, a 57-year-old woman with history of skin cancer and contraindication of progestin, who suffered from AUB due to simple endometrial hyperplasia, received anastrozole which led to recovery from hyperplasia and AUB [16].

Another study in 2006 was conducted on 376 women with endometrial hyperplasia on cyclic MPA (ten days a month) for three to six months. The pathology of 98% of these cases recovered which increased to 100% following increasing duration of therapy to 13 days a month [10]. The recovery of pathology was 100% in the present study (Table 3).

Serum estradiol level and endometrium thickness decrease was not significantly different at the end of treatment (Table 4). The complications reported in similar studies were vascular complications, increased level of lipid profile and lipoprotein, weight gain, and mood change [4].

The most common complication in letrozole group was dizziness and flashing (87%) and other complications such as constipation, fatigue, and palpitation and 56.5% of the cases in this group did not have any complications which were not significantly different with MPA group. However, with regards to OR = 2.78 and CI = 0.82-9.42, in a larger sample study, letrozole can be less complicated than MPA.

**References**


<table>
<thead>
<tr>
<th>Variable</th>
<th>Medroxy-progesterone acetate</th>
<th>Letrozole</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>After treatment estradiol level</td>
<td>45.34 ± 45.4</td>
<td>53.33 ± 92.13</td>
<td>&lt; 0.133</td>
</tr>
<tr>
<td>After treatment endometrial thickness</td>
<td>11.09 ± 5.83</td>
<td>9.97 ± 4.03</td>
<td>&lt; 0.74</td>
</tr>
</tbody>
</table>
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Is the 2009 FIGO staging system really valuable for Stage I endometrial cancer?

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Summary

Purpose: The aim of this study was to compare the survival predictive values of the 1988 and 2009 FIGO staging systems for the staging of patients with early-stage endometrioid type endometrial cancer. Materials and Methods: Two hundred twenty-four patients treated for endometrial cancer from 1996 to 2006 at Ankara Oncology Education and Research Hospital were staged according to the two staging systems. Early-stage patients with a histological diagnosis of endometrioid adenocarcinoma were included in the study. The Kaplan-Meier method was used for survival analysis. Results: The charts of 224 patients treated for endometrial cancer were retrospectively evaluated. The five-year overall survival (OS) for Stage IA and IB cases was 100% (n = 19) and 96.2% (n = 79), respectively, with no significant difference between the OS values (p = 0.126) with the FIGO 1988 system and 96.4% (n = 112) and 87.5% (n = 48), respectively with a statistically significant difference (p = 0.05) with the FIGO 2009 system. Conclusion: The authors found that the survival prognostic value of the 2009 FIGO staging system was more effective than the 1988 FIGO staging system for cases with early-stage endometrioid type endometrial cancer.

Key words: Endometrial cancer; Staging system; Early-stage; Survival.

Introduction

Endometrial cancer staging was performed clinically until 1971 when a new classification that again used clinical staging but contained new prognostic factors that added tumor grade came into use [1]. A series of surgical-pathological studies by the Gynecologic Oncology Group (GOG) lead to the International Gynecology and Obstetrics Federation (FIGO) Gynecologic Oncology Committee to decide that endometrial cancer should be surgically staged in 1988. The 1988 FIGO system consisted of three subgroups of stages according to the myometrial invasion depth [2, 3].

FIGO revised the staging system for endometrial cancer in 2009. The revised 2009 FIGO staging system for endometrial cancer has several major changes. The lack of myometrial invasion or a rate of less than 50% is defined as Stage IA in 2009 system. The 2009 FIGO system Stage IA was expanded compared to the 1988 FIGO system by including Stage IA, IB, and IIA cases with endocervical glandular involvement where myometrial invasion is less than 50% and Stage IIIA cases where the peritoneal fluid is positive and myometrial invasion is less than 50%. Stage II cases are no longer divided into two subgroups as A and B and the endocervical glandular involvement of the cervix is accepted as Stage I in the new system. Stage II now only contains patients with cervical stromal involvement [1, 4]. Positive peritoneal cytology has been excluded in the staging so a positive peritoneal fluid no longer influences the Stage [1, 3, 5, 6, 7]. These changes represent a significant change in classifying early-stage patients. The new system has merged patients who were previously classified as advanced-stage with the early-stage.

The aim of this study was to compare the survival results of the 2009 FIGO staging system with the 1988 FIGO staging system for our early-stage endometrial cancer cases and to discover whether the predictive ability of the revised system is better or worse than the 1988 system in the early stages of the disease.

Materials and Methods

This study was approved by the ethics committee of Ankara Oncology Education and Research Hospital. Study data were obtained by retrospective evaluation of the charts of patients treated for endometrial cancer between 1996 and 2006. The patients were staged again according to the 1988 and 2009 FIGO staging systems. Early-stage patients with a histological diagnosis of Stage I endometrioid adenocarcinoma were included in the study. Cases without follow-up were excluded. The survival status of the cases was recorded. The overall survival (OS) was accepted as the number of months from the date the cancer diagnosis was received to the date of death. The Kaplan-Meier method was used for survival analysis. The log rank test was used to obtain the p values for univariate survival analysis. All analyses were performed using SPSS 15.0 software.

Results

The authors evaluated the charts of a total of 224 cases treated between 1996 and 2006 for endometrial cancer. Stage I endometrial cancers made up 164 and 189 cases, respectively, with no significant difference between the OS values (p = 0.126) with the FIGO 1988 system and 96.4% (n = 112) and 87.5% (n = 48), respectively with a statistically significant difference (p = 0.05) with the FIGO 2009 system.
58.63 ± 8.80 (27 - 80), respectively, according to the FIGO 1988 and 2009 systems. Based on the 1988 system, 139 Stage I patients — including IA (19 / 13.7%), IB (79 / 56.9%), and IC (41 / 29.4%) — were identified (Table 1). The five-year OS for 1988 FIGO system for Stage IA, IB, and IC were 100%, 96.2% and 87.8%, respectively. There was no significant difference for OS values (p = 0.126).

When the cases were restaged according to the FIGO 2009 system (n = 160), there were 112 (70.0%) and 48 (30.0%) in Stage IA and IB, respectively, and the five-year OS was 96.4% and 87.5%, respectively (Table 2). The difference was statistically significant (p = 0.05). OS curves obtained for FIGO 1988 and 2009 staging systems with Kaplan-Meier analysis are presented in Figures 1 and 2. Table 3 presents the five-year OS rates of Stage I endometrioid type endometrial cancer patients for both staging systems.

With the 1988 FIGO system, there were 17 Stage IIA and eight Stage IIB cases, 100% and 75% five-year OS, respectively. According to the 2009 FIGO system, there were ten Stage II cases with 80% five-year OS.

**Discussion**

The aim of the classification and staging of any cancer is to determine the approximate prognosis of the patients and ensure a consistent terminology among healthcare professionals [1]. A good staging system should therefore be valid, reliable, and practical [5, 8]. The prognostic significance of tumor grade and myometrial invasion for endometrial cancer limited to the uterus has been shown years ago [9].

The present authors especially focused on early-stage changes in the current study. An analysis of similar conditions in the FIGO system reported similar prognosis for Stage IA and IB cases in the 1988 FIGO system and therefore found the inclusion of these two subgroups in Stage IA in the 2009 FIGO system to be reasonable [2]. Similarly, the authors did not find a significant difference for the five-year OS between Stage IA and Stage IB according to the FIGO 1988 system. This finding supports the merging of the IA and IB groups in the 1988 system into Stage IA in the 2009 system. When the present authors staged their cases again with the FIGO 2009 system, the five-year OS difference between Stage I and Stage IB was statistically significant (p = 0.05). OS differences in the current study. An analysis of similar conditions in the FIGO system reported similar prognosis for Stage IA and IB cases in the 1988 FIGO system and therefore found the inclusion of these two subgroups in Stage IA in the 2009 FIGO system to be reasonable [2]. Similarly, the authors did not find a significant difference for the five-year OS between Stage IA and Stage IB according to the FIGO 1988 system. This finding supports the merging of the IA and IB groups in the 1988 system into Stage IA in the 2009 system. When the present authors staged their cases again with the FIGO 2009 system, the five-year OS difference between Stage I and Stage IB was statistically significant (p = 0.05). OS curves obtained for FIGO 1988 and 2009 staging systems with Kaplan-Meier analysis are presented in Figures 1 and 2. Table 3 presents the five-year OS rates of Stage I endometrioid type endometrial cancer patients for both staging systems. With the 1988 FIGO system, there were 17 Stage IIA and eight Stage IIB cases, 100% and 75% five-year OS, respectively. According to the 2009 FIGO system, there were ten Stage II cases with 80% five-year OS.

**Table 1.** Endometrioid type uterine carcinoma staged according to FIGO 1988.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Total</th>
<th>Exitus</th>
<th>Alive</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>I A</td>
<td>19</td>
<td>0</td>
<td>19</td>
<td>100.0%</td>
</tr>
<tr>
<td>I B</td>
<td>79</td>
<td>3</td>
<td>76</td>
<td>96.2%</td>
</tr>
<tr>
<td>I C</td>
<td>41</td>
<td>5</td>
<td>36</td>
<td>87.8%</td>
</tr>
</tbody>
</table>

**Table 2.** Endometrioid type uterine carcinoma staged according to FIGO 2009.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Total</th>
<th>Exitus</th>
<th>Alive</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>I A</td>
<td>112</td>
<td>4</td>
<td>108</td>
<td>96.4%</td>
</tr>
<tr>
<td>I B</td>
<td>48</td>
<td>6</td>
<td>42</td>
<td>87.5%</td>
</tr>
</tbody>
</table>

**Table 3.** Overall five-year survival rates of Stage I according to the 1988 and 2009 FIGO systems.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total</th>
<th>Exitus</th>
<th>Alive</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>139</td>
<td>8</td>
<td>131</td>
<td>94.2%</td>
</tr>
<tr>
<td>2009</td>
<td>160</td>
<td>10</td>
<td>150</td>
<td>93.8%</td>
</tr>
</tbody>
</table>

**Conclusion**

The authors did not find a statistically significant difference between the five-year OS values of 1988 FIGO Stage IA and IB cases. However, there was a statistically
significant difference between the five-year OS values of Stage IA and IB cases according to the 2009 FIGO system. In conclusion, these data indicate that the 2009 FIGO staging system has better prognostic predictive ability than the 1988 system for early-stage disease in endometrioid type endometrial cancers.

References

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e-mail: kacetinkaya@gmail.com
In vitro chemosensitivity assay of ascites in epithelial ovarian cancer

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Summary

Objective: This study aimed to investigate the predictive value of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for chemosensitivity test in ascites. Materials and Methods: The relationship of the in vitro sensitivity results and the clinicopathological characteristics, objective response rates (ORRs) of chemotherapy, and time to progression (TTP) were retrospectively analyzed in 120 epithelial ovarian cancer (EOC) patients. The clinical response criterion was based on the Response Evaluation Criteria in Solid Tumors (RECIST) standard. The log-rank test and Kaplan-Meier curve were used to estimate TTP. Results: MTT assays revealed that tumor cells from ascites of primary and type II EOC were more sensitive to paclitaxel (PTX) and carboplatin (CBDCA) than relapse 

Introduction

The five-year survival rate of epithelial ovarian cancer (EOC) patients increased from 36% in 1977 to 44% in 2007 [1]. Nevertheless, more than 50% of EOC patients still relapse after their initial remission. Among them, 40%-60% acquire resistance to chemotherapeutic drugs and molecular target agents, which cause treatment failures [2]. The majorities of patients with EOC require chemotherapy in the course of their disease. Whether these patient’s tumors are sensitive to a certain drug prior to chemotherapy initiation is uncertain. Chemotherapeutic regimens for ovarian cancer are usually based on clinical trials, which depend on the histological type of tumor rather than on the sensitivity of an individual’s cancer cells to specific anti-cancer drugs [3]. Randomized trials comparing chemotherapeutic regimens or agents have contributed numerous pieces of evidence that cytotoxic treatment benefits ovarian cancer. However, given their heterogeneity, ovarian cancers respond differently to the same chemotherapeutic agent. A diagnostic assay that can predict the response of a given agent may help improve the clinical outcome of ovarian cancer patients. The premise of sensitivity-guided chemotherapy should be the consistency of in vitro sensitivity and in vivo response.

About 20%-40% objective response rates (ORRs) to single agents such as paclitaxel (PTX), carboplatin (CBDCA), epirubicin (EPI), cyclophosphamide (CTX), and cisplatin (CDDP) in ovarian cancer have been reported. The ORRs may be more than 70% for combinations of these agents [4, 5]. To date, apart from CBDCA + PTX regimens in primary therapy [6], no particular regimen has been shown to be superior to others in terms of prolonging overall survival in primary or platinum-sensitive ovarian cancer. The sensitivities of individual patients to anti-cancer agents such as PTX and CBDCA in relapse or platinum-resistant ovarian cancer must be assessed.

Recent studies have grouped EOC into two broad categories: type I and type II. These types are based on distinct clinicopathological and molecular genetic features. Type I tumors commonly include low-grade, well-differentiated serous, endometrioid, mucinous, and clear cell carcinomas. Type II tumors include high-grade serous, high-grade endometrioid, and undifferentiated carcinomas, as well as malignant mixed mesodermal tumors (carcinosarcomas). Type I ovarian cancers slowly grow and infrequently respond to platinum-based therapy. Type II ovarian cancers, constitute approximately 75% of EOCs, and aggressively grow but commonly respond to platinum-based therapy.
Tumor cells resistant to a single drug are difficult to identify in vivo, and the administration of a multidrug regimen is common. A cell culture drug-resistance assay can facilitate the isolation of a single drug-resistant cancer cell [7]. Numerous studies on chemosensitivity in vitro assays have been performed using established cell lines [4-6]. Clonogenic assays have successfully been used to predict the initial response of EOC patients to chemotherapy, but technical problems and long culture time have limited the clinical use of these assays [8]. Most of these assays are short-term total cell killing tests, where the cell isolation and culture procedures are essentially the same but the methods of determining viable cells are different. Typically, in the 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the surviving cells can convert MTT into formazan, which can be directly quantified by spectrophotometry [9]. Compared with other in vitro chemosensitivity assays, the MTT test is short term (two to four days) and requires a very low amount of cells in suspension [10-12]. A significant correlation between in vitro results and in vivo outcomes for ovarian cancer (p < 0.0001), with an assay sensitivity of 81%, has been found in ovarian cancer [13, 14]. However, the MTT assay in tumor tissue has two major drawbacks; namely, sensitivity investigation results may 1) lag behind the requirement, and 2) be interfered with by the amounts of non-tumor cells.

To evaluate the chemosensitivity testing results, ORRs, and overall survival, chemosensitivity assay results must be verified in a large number of ovarian cancer patients. In the present study, the authors evaluated the results of chemosensitivity testing using highly purified tumor cells from ascites through an MTT assay in 120 cases of ovarian cancer. The results were assessed in terms of the correlation with clinicopathological findings, clinical response, and time to progression (TTP) by comparing with those of patients treated by experienced clinicians. The tumor cells from ascites excluded the major interference of non-tumor cells in the test. The MTT assay was consistent with the objective therapy response in some agents, which indicated the possibility of further optimized protocol on the chemosensitivity assay results in EOC patients.

Materials and Methods

Clinicopathological characteristics

This retrospective study was approved by the Institutional Review Board of Jiangsu Cancer Hospital. A total of 120 EOC patients confined in this hospital between January 1, 2005 and January 1, 2010 were recruited. Those who did not receive the standard primary treatment, mostly including chemotherapy and cytoreduction surgery, in the present hospital were excluded. Patients were monitored according to the routine follow-up protocol for EOC recommended by the MD Anderson Cancer Center, which included visits every three months for the first two years, every four months at year three, and every six months at years four and five after primary treatment.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)/median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.7 years (29.2-4)</td>
</tr>
<tr>
<td>Baseline CA-125 level</td>
<td>801 U/ml (733439)</td>
</tr>
<tr>
<td>Grade, n = 120</td>
<td></td>
</tr>
<tr>
<td>Low grade</td>
<td>28 (23.3%)</td>
</tr>
<tr>
<td>High grade</td>
<td>92 (76.7%)</td>
</tr>
<tr>
<td>Histology, n = 120</td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>71 (59.2%)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>18 (15.0%)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>9 (7.5%)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>5 (4.2%)</td>
</tr>
<tr>
<td>Transitional</td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>7 (5.8%)</td>
</tr>
<tr>
<td>MMMT</td>
<td>8 (6.7%)</td>
</tr>
<tr>
<td>FIGO Stage, n = 120</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>12 (10.0%)</td>
</tr>
<tr>
<td>II</td>
<td>7 (5.8%)</td>
</tr>
<tr>
<td>III</td>
<td>63 (52.5%)</td>
</tr>
<tr>
<td>IV</td>
<td>36 (20.0%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Adjuvant chemotherapy, n = 112</td>
<td></td>
</tr>
<tr>
<td>Carboplatin/paclitaxel</td>
<td>103 (92.0%)</td>
</tr>
<tr>
<td>Other regimes</td>
<td>9 (8.0%)</td>
</tr>
<tr>
<td>n (%)</td>
<td>133 (26.8%)</td>
</tr>
</tbody>
</table>

The authors evaluated the relationship between drug sensitivity and patients’ clinicopathological data, including the histological type and grade of tumors, stage of disease as defined by the International Federation of Gynecology and Obstetrics, type of primary treatment, ascites volume, time of disease relapse, management of relapse disease, immunohistochemistry (IHC) staining. The primary antibodies were as follows: p53 (1:100, DO-7), Ki67 (1:100, clone MIB-1), ER (1:100, Dako ID5), and PR (1:100, Novocastra PGR-312). Negative and positive control slides were included in the present assay. For p53 immunoassay, nuclear staining in more than 10% of the neoplastic cells was deemed as a positive cutoff. Considering that Ki67 expression is commonly homogenous, Ki67 protein was scored by the percentage of positively stained cells with a 10% cutoff. Chemotherapy was given every 21 or 28 days. A total of 82 patients received neo-adjuvant chemotherapy, including 56 patients with CBDCA (area under the curve (AUC) = 5) and PTX (185 mg/m²); 22 with CDDP (75 mg/m²) and PTX (185 mg/m²); and four with CBDCA (350 mg/m²) and CTX (600 mg/m²). After primary surgery, 102 patients were treated with CBDCA (AUC = 5) and PTX (185 mg/m²); 14 with CBDCA (350 mg/m²) and CTX (600 mg/m²); two with CDDP (75 mg/m²) and PTX (185 mg/m²); and two with CBDCA (AUC = 5) and DOC (100 mg/m²). Clinical response to chemotherapy was assessed by clinical examination every three months. Computerized tomography (CT) scans were performed when necessary. The Response Evaluation Criteria in Solid Tumors (RECIST) and World Health Organization (WHO) criteria were used to assess tumor therapy response and clinical relapse [15-17]. The follow-up period ranged from 0.7 months to 67 months.
Drugs and ovarian cancer cell lines

Eight commonly used agents in EOC were used for in vitro chemosensitivity assay, including PTX, CBDCA, 5-fluorouracil (5-FU), TPT, etoposide (VP-16), PLD, and GEM. CBDCA and PTX are now preferentially prescribed in EOC as first-line agents, and the others are commonly administered to relapse patients in accordance with current clinical practice. In the present study, the drug concentrations for sensitivity test were chosen to mimic those in vivo [18, 19]. The SKOV3 cell line was used in the MTT assay and as blank controls. CBDCA was dissolved in distilled water, and other drugs including PTX were dissolved in saline. All drugs were further diluted with RPMI-1640. The SKOV3 cell line was obtained from the American Type Culture Collection.

Separation of cells and culture suspension
Ascites for sensitivity test were obtained from abdominocentesis or surgical procedures. Ascites were centrifuged at 3,000 rpm for 30 min before immersion in a complete medium containing collagenase (2 mg/ml, Type V-S) and DNase I (0.4 mg/ml). The cells were harvested after incubation for 40 min at 37°C, washed, and suspended in a complete medium. The single-cell suspension was then centrifuged at 400 × g for 30 min. The collected interface was suspended in a complete medium at 1 × 10⁶/ml density. Discontinuous gradients consisting of 10 ml of 100% and 15 ml of 75% Ficoll–Hypaque were applied for onto the cell layer. A tumor cell-rich fraction was then obtained from the 75% interface after centrifugation at 400 × g for 30 min. Discontinuous gradients comprising four ml each of 25%, 15%, and 10% Percoll were then applied onto the tumor cell-rich suspension layer. Tumor cells depleted of lymphoid cells were obtained from the bottom and at the 25% interface, and then suspended in a complete medium at a density of 1 × 10⁶/ml after centrifugation was performed at 25 × g for 7 min.

In vitro chemosensitivity assay
With different drug concentrations in RPMI, the cell suspension was incubated in a 96-well round-bottomed microculture plate. Blank and control tumor cells (SKOV3) were cultured in RPMI without any other reagent. Wrapped in cling film, the plates were incubated for 48 h at 37°C in humidified air containing 5% CO₂. After 48 h of incubation, 50 g of MTT in five mg/ml concentration was added to each well. The plates were then incubated for another five hours at 37°C and 5% CO₂. During exposure, yellow MTT was transformed by viable cells into purple formazan.

After dissolving in 100 µl of dimethylsulfoxide, the formazan crystals were quantified by a microplate spectrophotometer at 540 nm. The following equation was used to calculate the ovarian cancer cell viability: (OD value of drug exposed well/mean OD value of control wells) × 100%. The OD of blank wells was used to adjust the control and test wells.

Criterion of chemosensitivity assay results
For each single drug, the result of MTT chemosensitivity assay was determined to be sensitive (i.e., greater than the mean inhibition rate). Based on the chemotherapeutic regimen, patients were classified into three categories in accordance with the combination of two sensitivities. The sensitive (S) category was defined as being sensitive to both drugs, the intermediate (I) was sensitive to only one of them, and resistant (R) was sensitive to none. The primary chemotherapeutic regimen was mostly CBDCA/PTX in 87 patients receiving neo-adjuvant chemotherapy and 43 patients who did not obtain satisfactory cytoreduction surgery. Regimens other than CBDCA/PTX were evaluated in 87 platinum-resistant relapses patients.

Statistical analyses
The relationship between the MTT assay results and clinical characteristics was tested for statistical significance by the t-test, whereas the clinical responses were assessed using the chi-squared test. The Kaplan-Meier method was used to estimate the TTP distribution, and a log-rank test was used to analyze differences between groups. A p value less than 0.05 was considered significant. All analyses were performed using the SPSS 11.5 software package.

Results
Correlation of in vitro chemosensitivity results with clinical characteristics
During the study period, 188 pieces of ascites specimens with ovarian cancer from 120 patients were tested by MTT assay. A total of 182 specimens were considered to be suitable for evaluation (success rate = 96.8%). Table 2 shows the overall results of chemosensitivity for each drug. The inhibition rates of tumor cells for PTX and CBDCA were significantly higher than 5-FU, VP-16, and EPI (p < 0.01, respectively). The inhibition rate for PLD did not different from those for GEM (p = 0.39) and TPT (p = 0.72).

The correlation of the clinicopathological characteristics with in vitro chemosensitivity test results was investigated. The inhibition rates for PTX and CBDCA in the relapse (p = 0.01 and p < 0.01, respectively) and type I (p = 0.03 and p = 0.02, respectively) tumors were significantly lower than those in the primary and type II tumors, respectively. The authors also found that the inhibition rates for GEM and 5-FU in type I tumors (p = 0.01 and p = 0.01, respectively) were significantly higher than those in type II tumors. No statistical difference between the inhibition rates in FIGO stages was observed for all tested drugs (Table 2). The inhibition rates for VP-16 in serous tumor cases were higher than those in endometrioid and mucous tumor cases (p < 0.01 and p < 0.01, respectively). No statistical difference existed between the inhibition rates for other drugs, except in p53 and Ki67 expression (Table 3).

Correlation of in vitro chemosensitivity results with in vivo clinical response
The authors analyzed the in vitro chemosensitivity test results and patients’ ORRs using these tested drugs in EOCs. Most patients who received CBDCA/PTX had a high ORR in the S and I categories than those in the S category both in neo-adjuvant and adjuvant chemotherapy (Table 4; p = 0.03 and p = 0.02, respectively). For platinum-resistant relapse patients, chemotherapeutic regimens in S and I categories were associated with a higher ORR (Table 5; p = 0.04).
Correlation of MTT sensitivity results with patient outcomes

The MTT sensitivity assay results of adjuvant chemotherapy were insignificantly correlated with the overall survival ($p = 0.33$). For platinum-resistant relapse EOC, the Kaplan–Meier survival analysis demonstrated that the patients in the S and I categories (95% confidence interval, median = 7.0 months, range = 5.0-9.0 months) with longer TTP than those in the R category (95% confidence interval, median = 7.0 months, range = 2.4-7.2 months) (Figure 1; $p = 0.043$).

Discussion

To the best of the authors’ knowledge, the current study on in vitro chemosensitivity by MTT assay in 120 ovarian cancer patients is the largest retrospective study conducted so far [13, 14, 20-23]. Based on the overall results, CBDCA and PTX showed higher sensitivities than PLD, TPT, EPI, and GEM in primary ovarian cancer, in agreement with clinical reports. Both CBDCA and PTX are presently first-line regimens for primary

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>5-Fu</th>
<th>PTX</th>
<th>CBDCA</th>
<th>EPI</th>
<th>VP-16</th>
<th>GEM</th>
<th>PLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>41.6 ± 9.2</td>
<td>50.5 ± 10.2</td>
<td>51.5 ± 7.2</td>
<td>48.7 ± 9.7</td>
<td>42.3 ± 9.1</td>
<td>40.7 ± 5.1</td>
<td>45.6 ± 9.5</td>
</tr>
<tr>
<td>Relapse</td>
<td>28.7 ± 5.1</td>
<td>35.1 ± 6.8</td>
<td>34.1 ± 4.7</td>
<td>32.2 ± 5.2</td>
<td>30.9 ± 6.7</td>
<td>33.0 ± 3.7</td>
<td>30.8 ± 7.9</td>
</tr>
<tr>
<td>Type I</td>
<td>36.9 ± 7.8</td>
<td>38.8 ± 7.4</td>
<td>37.8 ± 5.0</td>
<td>37.6 ± 6.8</td>
<td>32.8 ± 6.3</td>
<td>33.1 ± 3.9</td>
<td>42.1 ± 8.1</td>
</tr>
<tr>
<td>Type II</td>
<td>31.3 ± 7.0</td>
<td>46.7 ± 9.6</td>
<td>47.7 ± 5.8</td>
<td>44.7 ± 7.4</td>
<td>39.5 ± 8.6</td>
<td>38.4 ± 4.8</td>
<td>34.0 ± 8.4</td>
</tr>
<tr>
<td>Stage I</td>
<td>35.2 ± 8.2</td>
<td>44.3 ± 8.9</td>
<td>45.3 ± 5.9</td>
<td>43.2 ± 7.4</td>
<td>36.2 ± 7.9</td>
<td>36.3 ± 4.3</td>
<td>39.1 ± 8.8</td>
</tr>
<tr>
<td>Stage II-IV</td>
<td>33.8 ± 7.4</td>
<td>40.4 ± 8.1</td>
<td>39.4 ± 5.1</td>
<td>38.9 ± 6.9</td>
<td>35.1 ± 7.5</td>
<td>35.0 ± 3.5</td>
<td>37.2 ± 8.4</td>
</tr>
</tbody>
</table>

**Table 2.** Comparison of chemosensitivity between clinicopathologic characteristics in ovarian cancer.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>5-Fu</th>
<th>PTX</th>
<th>CBDCA</th>
<th>EPI</th>
<th>VP-16</th>
<th>GEM</th>
<th>PLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53+</td>
<td>36.7 ± 7.7</td>
<td>45.3 ± 9.4</td>
<td>46.1 ± 5.5</td>
<td>44.9 ± 7.5</td>
<td>40.4 ± 8.8</td>
<td>38.9 ± 4.8</td>
<td>45.0 ± 9.4</td>
</tr>
<tr>
<td>P53-</td>
<td>31.8 ± 7.2</td>
<td>39.2 ± 7.6</td>
<td>39.9 ± 5.2</td>
<td>37.0 ± 6.7</td>
<td>31.8 ± 6.2</td>
<td>32.8 ± 3.8</td>
<td>32.8 ± 8.1</td>
</tr>
<tr>
<td>Ki67 strong</td>
<td>41.0 ± 9.1</td>
<td>52.5 ± 10.3</td>
<td>52.5 ± 7.3</td>
<td>49.8 ± 9.9</td>
<td>43.2 ± 9.1</td>
<td>41.7 ± 5.2</td>
<td>43.3 ± 9.2</td>
</tr>
<tr>
<td>Ki67 weak</td>
<td>29.6 ± 5.3</td>
<td>34.1 ± 6.7</td>
<td>33.8 ± 4.7</td>
<td>31.4 ± 5.1</td>
<td>30.1 ± 6.6</td>
<td>32.1 ± 3.5</td>
<td>32.1 ± 8.0</td>
</tr>
<tr>
<td>ER+</td>
<td>32.5 ± 7.2</td>
<td>40.0 ± 8.1</td>
<td>38.4 ± 5.0</td>
<td>39.3 ± 6.9</td>
<td>35.2 ± 7.5</td>
<td>35.2 ± 3.7</td>
<td>37.6 ± 8.4</td>
</tr>
<tr>
<td>ER-</td>
<td>35.4 ± 8.5</td>
<td>44.6 ± 8.9</td>
<td>47.6 ± 6.1</td>
<td>42.7 ± 7.3</td>
<td>36.0 ± 7.9</td>
<td>36.1 ± 4.3</td>
<td>38.7 ± 8.8</td>
</tr>
<tr>
<td>PR+</td>
<td>34.2 ± 7.5</td>
<td>42.0 ± 8.3</td>
<td>41.7 ± 5.3</td>
<td>39.8 ± 6.9</td>
<td>34.6 ± 7.2</td>
<td>35.4 ± 3.9</td>
<td>37.1 ± 8.6</td>
</tr>
<tr>
<td>PR-</td>
<td>36.1 ± 7.8</td>
<td>43.1 ± 8.8</td>
<td>43.7 ± 5.6</td>
<td>42.2 ± 7.5</td>
<td>36.9 ± 7.8</td>
<td>36.2 ± 4.3</td>
<td>39.2 ± 8.9</td>
</tr>
</tbody>
</table>

**Table 3.** Comparison of chemosensitivity between IHC characteristics of ovarian cancer.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>5-Fu</th>
<th>PTX</th>
<th>CBDCA</th>
<th>EPI</th>
<th>VP-16</th>
<th>GEM</th>
<th>PLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53+</td>
<td>36.7 ± 7.7</td>
<td>45.3 ± 9.4</td>
<td>46.1 ± 5.5</td>
<td>44.9 ± 7.5</td>
<td>40.4 ± 8.8</td>
<td>38.9 ± 4.8</td>
<td>45.0 ± 9.4</td>
</tr>
<tr>
<td>P53-</td>
<td>31.8 ± 7.2</td>
<td>39.2 ± 7.6</td>
<td>39.9 ± 5.2</td>
<td>37.0 ± 6.7</td>
<td>31.8 ± 6.2</td>
<td>32.8 ± 3.8</td>
<td>32.8 ± 8.1</td>
</tr>
<tr>
<td>Ki67 strong</td>
<td>41.0 ± 9.1</td>
<td>52.5 ± 10.3</td>
<td>52.5 ± 7.3</td>
<td>49.8 ± 9.9</td>
<td>43.2 ± 9.1</td>
<td>41.7 ± 5.2</td>
<td>43.3 ± 9.2</td>
</tr>
<tr>
<td>Ki67 weak</td>
<td>29.6 ± 5.3</td>
<td>34.1 ± 6.7</td>
<td>33.8 ± 4.7</td>
<td>31.4 ± 5.1</td>
<td>30.1 ± 6.6</td>
<td>32.1 ± 3.5</td>
<td>32.1 ± 8.0</td>
</tr>
<tr>
<td>ER+</td>
<td>32.5 ± 7.2</td>
<td>40.0 ± 8.1</td>
<td>38.4 ± 5.0</td>
<td>39.3 ± 6.9</td>
<td>35.2 ± 7.5</td>
<td>35.2 ± 3.7</td>
<td>37.6 ± 8.4</td>
</tr>
<tr>
<td>ER-</td>
<td>35.4 ± 8.5</td>
<td>44.6 ± 8.9</td>
<td>47.6 ± 6.1</td>
<td>42.7 ± 7.3</td>
<td>36.0 ± 7.9</td>
<td>36.1 ± 4.3</td>
<td>38.7 ± 8.8</td>
</tr>
<tr>
<td>PR+</td>
<td>34.2 ± 7.5</td>
<td>42.0 ± 8.3</td>
<td>41.7 ± 5.3</td>
<td>39.8 ± 6.9</td>
<td>34.6 ± 7.2</td>
<td>35.4 ± 3.9</td>
<td>37.1 ± 8.6</td>
</tr>
<tr>
<td>PR-</td>
<td>36.1 ± 7.8</td>
<td>43.1 ± 8.8</td>
<td>43.7 ± 5.6</td>
<td>42.2 ± 7.5</td>
<td>36.9 ± 7.8</td>
<td>36.2 ± 4.3</td>
<td>39.2 ± 8.9</td>
</tr>
</tbody>
</table>

**Table 4.** Chemosensitivity of carboplatin/paclitaxel regime and the objective response rate in ovarian cancer.

<table>
<thead>
<tr>
<th>Inhibition rates</th>
<th>NACT (n = 72)</th>
<th>CR + PR</th>
<th>SD + PD</th>
<th>CT (n = 43)</th>
<th>CR + PR</th>
<th>SD + PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP (S)</td>
<td>22</td>
<td>7</td>
<td>12</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP (I)</td>
<td>18</td>
<td>5</td>
<td>8</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP (R)</td>
<td>9</td>
<td>11</td>
<td>5</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5.** Comparison of chemosensitivity and objective response rate in platinum-resistant relapse ovarian cancer.

<table>
<thead>
<tr>
<th>Inhibition rates</th>
<th>CR + PR</th>
<th>SD + PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP (S)</td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>CP (I)</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>CP (R)</td>
<td>9</td>
<td>15</td>
</tr>
</tbody>
</table>

$p = 0.03$, $p = 0.02$ compared with the inhibition rate and objective response rate on carboplatin/paclitaxel regime.
and platinum-sensitive EOC [6, 24, 25]. On the other hand, 5-FU and VP-16 showed lower inhibition rates than the other drugs, suggesting that 5-FU and VP-16 alone were ineffective for most ovarian cancers. The authors found that the chemosensitivity of CBDCA and PTX were higher in primary, type II, and p53 immunostaining positive tissues than those in relapse, type I, and p53-negative ovarian cancer. This result suggested that the chemosensitivity testing results of primary tumor should not be referenced for second-line chemotherapy, and that the subtype of ovarian cancer should be a major agent selection factor. In relapse cases, tumor cells can acquire a tolerance for anti-cancer drugs that have been previously administered. Chemosensitivity test may play an important role in selecting a regimen of second-line chemotherapy and should be assessed as frequently as possible while tumors relapse. Ovarian cancer is not a single-disease entity but rather comprises many different subtypes with distinct clinicopathological characteristics [26, 27]. Type I ovarian cancer is apt to be less malignant without p53 mutation, and more resistant to CBDCA and PTX than type II. The present study revealed that Ki67, as a typical cell-proliferation and cell-cycle time marker, is associated with agents such as TPT, GEM, and so on. Thus, Ki67 may be an indicator of the effect of these agents on tumor metabolism and mitosis. Clinicopathological findings such as FIGO stage and ER/PR immunostaining were not correlated with chemosensitivity in all test agents.

Generally, chemotherapy benefits patients with platinum-sensitive relapse ovarian cancer, but the extent of this benefit is limited and no standard, universally accepted regimen exists for platinum-resistant patients. Anti-cancer drugs such as trabectedin [28], PLD, TPT, and GEM are already available and reportedly effective for ovarian cancer. However, the efficacy rates of these drugs are commonly < 30% and their adverse effects cannot be ignored. In the current work, the authors found by MTT assay that a higher inhibition rate in platinum-resistant ovarian cancer is associated with higher ORRs and prolonged TTP. To improve prognosis and avoid adverse effects in platinum-resistant relapse ovarian cancer patients, the chemotherapeutic regimen should be established in accordance with in vitro testing-based individualized chemosensitivity, which may enable personalized ovarian cancer treatment in the future.

The in vitro MTT tumor sensitivity assay had some inherent drawbacks. First, the tumor tissues for this assay were mostly obtained through biopsy, which is an invasive, surgical procedure. The drug sensitivity results were unavailable and may have been influenced by neo-adjuvant chemotherapy. Second, the false positive and negative rates of MTT assay for clinical response were about 30%-50% and 5%-15% [29, 30]. Besides, the difference between the internal and external environments of tumor cells, non-tumor cells (such as lymphocytes and fiber cells), and interference also affect the accuracy of the results and drug sensitivity test in vitro. The authors recruited ascites by abdominocentesis for MTT assay, and the results were available within two to four days, which enabled quick application to patients in vivo. More importantly, MTT assay on ascites may partly avoid these interference factors.

In conclusion, patients have different sensitivities to anti-cancer drugs. Moreover, predicting the appropriate anti-cancer drug in accordance with the clinicopathological findings of tumors, including the difference between primary and relapse lesions, is difficult. Chemosensitivity testing is one of the most effective strategies for establishing an appropriate anti-cancer regimen, especially for platinum-resistant ovarian cancer, to improve patient outcomes. Chemotherapy based on the results of chemosensitivity testing can also help avoid potential adverse effects and be economical.

Acknowledgements

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References

Characteristics of diagnosis and therapy of adolescent malignant ovarian tumors

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Summary

Background: To improve the early diagnosis rate of adolescent malignant ovarian tumors, avoid misdiagnosis, select proper therapy, avoid excessive therapy, and render therapy more tolerable. Materials and Methods: A comprehensive review of adolescent malignant ovarian tumors, such as types, difficulties of early diagnosis, therapeutic principles, prognosis, fertility preserving through the authors' clinical experience, with reference to Chinese and international literatures. Results and Conclusion: The majority of adolescent malignant tumors are malignant germ cell, the malignancy is high and it is difficult to diagnose in the early stage, likely to be ignored. Their diagnosis is therefore fundamental, while selecting the most appropriate therapeutic approach that also considers fertility-sparing without compromising prognosis and avoiding over-treatment.

Key words: Adolescent; Ovarian cancer; Diagnosis; Therapy.

Introduction

Although the incidence rate of malignant ovarian tumors holds the third place of malignant tumors of the female genital tract, their associated death rate is highest. The difficulties in the current diagnosis and therapy of ovarian cancer include lack of specific early-stage diagnosis methods, resistance of chemotherapy, higher relapse rate, etc. Regarding adolescent ovarian tumors, there are some particular characteristics in the types, selection of diagnosis methods and therapeutic principle, prognosis, issues faced, etc. This paper discusses the aforementioned questions, aimed to improve the early diagnosis rate of adolescent malignant ovarian tumors, avoid misdiagnosis, select proper therapy; moreover, enhancing survival rate as well while fully considering the characteristics of adolescent marriage and child-bearing, avoid excessive therapy, and render therapy more tolerable.

Literature Reports

Types of adolescent malignant ovarian tumors

Epithelial ovarian cancer is the most common malignant ovarian tumor, accounting for 85%–90% of malignant ovarian tumors, mostly among the middle- and old-aged women [1], and relatively seldom among adolescents. The conventional therapy is surgery plus adjuvant therapy, mainly chemotherapy, and the five-year survival rate is low; aside from very few early-stage patients, full-staging surgery should be carried out generally, and it is difficult to maintain the child-bearing and endocrine functions.

Ovarian germ cell tumors are a set of tumors with different histological characteristics, originated from original germ cells of embryonic gonad and only second to the epithelial tumors; the young women and girls before puberty account for 60%–90% patients, commonly before menophania, and one-third are malignant or have the tendency to become so [1,2], including the immature teratoma, dysgerminoma, endodermal sinus tumor, embryonic cancer, etc. The malignancy and death rates of ovarian germ cell tumors are high, and by the application of effective chemotherapy in recent years, the five-year survival rate has increased from no more than 20% up to 75%–90%, making the fertility preserving therapy possible; the sex cord-stromal tumors with endocrine function is rare among the adolescent, 5%–10% of granulosa cell tumors occur before puberty [3]. The rare gonadoblastoma often results from gonadal dysgenesis, mostly before 20 years of age, and seldom before ten years of age [4]. Therefore, germ cell tumors are common ovarian types among the adolescents.

Difficulties of early diagnosis

The main diagnostic means of malignant ovarian tumors includes pelvic inspection, radiographic inspection (ultrasonic, CT, MRI, PET), tumor label, proteomic examination, laparoscopy, etc.; however, all these methods suffer certain limits. Due to lack of specific early diagnosis method, lack of ideal tumor specific label, ovarian anatomic particularity (deep in pelvic cavity), no early-stage symptom available, etc., the early-stage diagnosis of malignant ovarian tumors is very difficult [5]. Different from women in the child-bearing period, another difficulty of early diagnosis of adolescent malignant tumors is delay in seeing a doctor; some patients are young, and can not clearly de-
scribe their disease history; moreover, the majority of them are unmarried and have no sex life; the regular general gynecological inspections cannot be performed; while free of symptoms, the gynecological inspections are almost unacceptable; even if some discomfort emerges, the department of gynecology is not preferred; even they come to the department, since the pelvic examination cannot be performed and the accuracy of rectal examination is poor, the misdiagnosis happens. The main inspection means for unmarried adolescent patients coming to the department of gynecology is abdominal ultrasound scan [6]; besides, the fullness degree of urinary bladder also influences the accuracy of examination results; when the abdominal mass is obvious, even the pressing symptoms such as difficult defecation and micturition, or abdominal bloating or ascites occur, the early stage has been passed [7]. The misdiagnosed disease for adolescent malignant ovarian tumors is pelvic tuberculosis; both may show the abdominal mass, ascites, becoming thin, CA125, HE4 increase, and the radiographic results are similar; the tuberculin test is one of identification method; however, the false negative is possible. α-fetoprotein (AFP) is a very specific tumor label of malignant ovarian germ cell tumor, especially endodermal sinus tumor and embryonic cancer, and can be used as one differential diagnostic method [8,9].

Characteristics of selection of therapeutic principle
Aside from malignant trophoblastic tumors, the conventional therapy for malignant ovarian tumors is surgery plus adjuvant therapy, mainly platinum-based chemotherapy. The selection of surgery shall be based on the tumor stage, patient’s age, histological type of tumor, married and pregnancies or not, desire for child-bearing, etc. For women with children or menopause, both early full-staging surgery and terminal cytoreductive surgery [10], the conventional choice is to remove the uterus and both adnexa. However, fertility-preserving is a special question to be considered while choosing the proper surgical range for adolescent malignant ovarian tumors [11].

Epithelial ovarian cancer
For patients of early-stage epithelial ovarian cancer, who have given birth, the full staging surgery is mainly carried out, removal of the entire uterus and adnexa, inspection of the peritoneal surface and complete and careful biopsy, maintaining the ascites or abdominal lavage to carry out the abdominal cytological examinations, removal of colonic greater omentum, removal (or selective removal) of the pelvic and para-aortic lymph nodes, etc.; however, the patients with early-stage epithelial ovarian cancer [12] and expecting children, carry out the conservative fertility-sparing surgery is carried out, provided that it is Stage IA, IB, smooth activities of tumor, well-differentiated cells (G1, G2), normal appearance of ovary at opposite side (or negative biopsy), negative ascetic cytological examination, negative biopsy at various locations of peritoneum and greater omentum, negative selective lymph nodal biopsy, and able to follow up the patient. Surgical approach is: unilateral uterine adnexectomy and colonic omentectomy and pelvic or abdominal aorta lymphadenectomy (or selectively), and after giving birth, according to follow-up, secondary surgery to remove the uterus and adnexa at opposite side [13,14]. However, since the prognosis of some special types of epithelial cancers, such as clear cell cancer, undifferentiated cancer, etc., is poor, and the relapse rate is higher, the conservative surgery for early-stage epithelial ovarian cancer is only applicable to the serous cancer, mucinous cancer or endometrioid carcinoma [15]. Whether the ovary at opposite side has metastasis or not is one of important factors influencing the choice of conservative surgery and postoperative safety. Because the prognosis of terminal epithelial cancers is poor and the death rate is high, while carrying out the cytoreductive surgery, the uterus and adnexa at both sides are generally removed, without considering the age and child-bearing requests [16,17].

Malignant ovarian germ cell tumors
While performing surgery on patients with malignant genital tract tumors and having given birth, the surgical range and method are same with the aforementioned epithelial ovarian cancer [18]. For young patients expecting children, differing from epithelial ovarian cancer [19], the clinical staging cannot indicate whether the pelvic organs should be removed or not; with regards to the uterus and ovary, if the ovary on the opposite side has not been invaded, regardless of the tumor stage, fertility can be preserved. The adnexa of the affected side is removed together with the greater omentum, retro-peritoneal lymph nodes, and pelvic focus, while preserving the uterus and ovary on the healthy side. If there no normal ovarian tissues, preserving the uterus should be considered; postoperative, hormone replacement therapy and IVF can be carried out [20]. The main criteria are: this type of malignant tumor is sensitive to chemotherapy; good prognosis can be expected in many cases; the sensitive tumor labels can provide the long-term follow-up monitoring [21]; most tumors are located at one side of ovary; pelvic relapse is rare; removal of adnexa at one side does not affect the tumor prognosis; and the survival rate does not decrease.

Dysgerminoma
Young patients should undergo fertility-sparing surgery as much as possible. By removing the adnexa on the affected side, maintaining the uterus and the ovary on the opposite side, and carrying out postoperative chemotherapy, can lead to good results. The dysgerminoma is sensitive to radiotherapy, but which will harm ovarian function should therefore be assessed, while carefully evaluating the advantages and disadvantages [22,23].
Ovarian borderline tumor

For patients without child-bearing requests, carry out the hysterectomy and bilateral adnexectomy are carried out; for mucous borderline tumors, the greater omentum and vermiform appendix should also be removed for patients in Stage III or IV; the surgical range should be the same as epithelial ovarian cancer, and the cytoreductive surgery should be performed; for the adolescent patients desiring children, in Stage I and long-term follow-ups, fertility should be preserved [24]; the adnexa at the affected side should be removed, and biopsy of the ovary at the opposite side should be taken; for the bilateral ovarian borderline tumor, as long as normal tumor tissue exists, it is advisable to carry out the focal excision and maintain normal ovarian tissue; however, it is reported that the incidence rate of bilateral ovarian borderline tumor is 38%–43% [25], since the relapse rate of adnexectomy and focal excision is 2%–3% and 20%, respectively. Therefore, surgery which excises the focus and maintains the ovarian tissue is only limited to patients with bilateral ovarian borderline tumors or to those having only one ovary excised [26]. Nowadays, some researchers hold that the patients with late-staged ovarian borderline tumor and peritoneal implantation could consider fertility-preserving surgery. Because its five-year survival rate can reach up to 77%–96% [27], and the relapse rate of fertility-preserving surgery is only 17%; furthermore, the relapsed tumor is still borderline type, and can be treated with the second surgery. However, for patients with exogenous papillary structure and peritoneal infiltration and implantation, the fertility preserving surgery must be carefully weighed, its advantages and disadvantages should be fully informed, and the choice of surgical method should be accepted by the patient or by her relatives.

Prognosis

Aside from clinical staging, whether the cytoreductive surgery is ideal, pathologic type, differentiation degree, resistance of chemotherapy, the patient’s health, age, immunity, also influence the prognosis; the negative factors influencing the prognosis include poor health, relatively older, and poor immunity and resistance; generally, the younger patients are healthy and immune; in comparison with older patients and those with incorporated diseases, their prognosis is optimal. Transplantation of ovary, cryopreservation of embryo, cryopreservation of oocyte, slicing and cryopreservation of cortex of ovary, cryopreservation of ovarian tissues [28,29], are methods available before surgery for patients demanding preservation of fertility, however, these methods are still being evaluated. Among patients with gynecological tumors, who have gained the complete response, some cannot get pregnancy in the natural way, and assisted pregnancy is necessary[30]. When to use the assisted reproductive technologies? How to use the assisted reproductive technologies? These are questions common to both gynecological tumor scientists and reproductive scientists. The patient’s fertility should be a priority, since the most important target in young patients is pregnancy.

Conclusion

The majority of adolescent malignant tumors are malignant germ cell tumors: the malignancy is high and it is difficult to diagnose in the early stage, it is likely to be ignored, so care must be taken to identify them; while selecting the therapeutic program, the fertility question should be considered as much as possible, without compromising the prognosis, and excess therapy should be avoided. Pregnancy is the ideal target of fertility-preserving surgery for malignant ovarian tumors; for the epithelial ovarian cancer, after giving birth, the second exploratory operation should be considered; while performing the adjuvant chemotherapy, the ovarian functions shall be carefully protected. It is reported that conservative surgery for malignant ovarian tumors is only suitable for young patients demanding fertility; the questions such as whether the conservative surgery influences the patients’ survival time and survival rate or not, whether the relapse rate will increase or not, etc., still require long-term and larger sample-sized studies to be confirmed. The critical factors affecting the prognosis of adolescent malignant ovarian tumors include: early diagnosis and reasonable therapy.

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A case of ovarian psammocarcinoma with homolateral serous cystoadenofibroma and thecoma associated with Brenner tumour and cystoadenofibroma of the contralateral ovary

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Department of Biomedical, Biotechnological, and Translational Sciences, Section of Pathology, Parma University, Parma (Italy)

Summary

Psammocarcinoma of the ovary is a rare serous neoplasm, with only 32 cases reported in the international literature. Characteristically, this tumour shows extensive formation of psammoma bodies, low-grade cytological features, and invasion of the ovarian stroma, peritoneum or intraperitoneal viscera. The behaviour of this entity is unpredictable, with benign, low malignant and metastatic potential. Herein the authors report a case of psammocarcinoma of the ovary with homolateral serous cystoadenofibroma and thecoma, which were associated with Brenner tumour and adenofibroma of the contralateral ovary, in a 78-year-old woman. Thus, this example shows an unpredictable tumour associated with multiple benign epithelial neoplasms and a benign stromal tumour. Moreover, this example of psammocarcinoma is very interesting because it measures only 1.5 x 0.5 x 1.5 cm and, to the best of the author’s knowledge, represents the smallest case of psammocarcinoma described so far in the literature.

Key words: Serous psammocarcinoma; Serous cystoadenofibroma; Thecoma and Brenner tumour.

Introduction

Serous psammocarcinoma is a rare form of ovarian carcinoma with only 32 cases reported in the literature [1]. This neoplasm was first described by Gilks et al. as a lesion with massive psammoma body formation. The other histological criteria suggested by Gilks et al. for diagnosis of psammocarcinoma were as follows: (a) destructive invasion of ovarian stroma, vascular invasion, or invasion of intraperitoneal visceral; (b) moderate nuclear atypia; (c) presence of nests of solid epithelial proliferations no greater than 15 cells in diameter; (d) psammoma bodies that replace at least 75% of the papillae [2].

Here the authors report a further case of psammocarcinoma of the right ovary with homolateral serous cystoadenofibroma and thecoma which was associated with Brenner tumour and cystoadenofibroma of the contralateral ovary.

Case Report

A 78-year-old nulliparous woman, with a previous history of hypertension, and a subtotal hysterectomy as a result of severe uterine bleeding and leiomyomas at the age of 29 years, was admitted to the present institution to have her uterine cervix removed. Seric levels of Ca 125, Ca 19.9, and CEA were normal. A preoperative pelvic magnetic resonance image (MRI) revealed the presence of a right hand mass measuring 3.5 x 3 cm. This lesion was solid with a cystic area. A small cystic lesion was present in the left ovary: the patient underwent bilateral salpingo-oophorectomy.

The surgical specimens were fixed in ten percent neutral-buffered formalin for a routine light microscopic examination.

Sections of neoplasms were submitted to histological examination and the samples were embedded in paraffin and stained with haematoxylin-eosin.

Macroscopically, the right ovary disclosed the presence of three lesions. Two of these were solid, while the third was cystic. One of the solid lesions was a small, heavily calcified, grey sub-capsular nodule measuring 1.5 x 0.5 x 1.5 cm (Figure 1). Histologically, this small nodule corresponded to a psammocarcinoma and was characterized by numerous psammoma bodies (Figure 2A) which were occasionally surrounded by papillary and tubular structures, lined by cytological bland cuboidal or low columnar epithelium (Figure 2B).

The cystic neoplasm macroscopically contained serous fluid and papillary projections. Histologically, this neoplasm corresponded to a serous cystoadenofibroma which, characteristically, had formed broad papillae which projected into the lumen of a cyst. Both these papillae and the wall of the cyst showed cellular fibrous stroma and were lined by a single layer of columnar epithelium without nuclear atypia (Figure 3A). The other solid lesion appeared as a mass measuring 3 x 1.5 cm, with a yellow sectioned surface (Figure 1A). Microscopically, this mass revealed the features of thecoma showing a fibromatous background with spindle cells and masses of cells which had abundant pale cytoplasm and oval/round nuclei (Figure 3B).

Macroscopically, the left ovary revealed the presence of another epithelial neoplasm which corresponded to a benign Brenner tumour, showing the presence of epithelial nests with cystic structures containing eosinophilic debris (Figure 4A). The epithelial elements were characterized by eosinophilic cytoplasm and oval nuclei with small nucleoli and longitudinal grooves (coffee bean nuclei) (Figure 4B). In addition, in other areas of the same ovary, there was a small cyst measuring one by one cm into which projected some papillary excrescences which microscopically revealed the histological features of cystoadenofibroma (Figure 4C). After 48 months from the diagnosis, the patient is alive without evidence of disease.
Discussion

Thecomas or theca cell tumours are benign ovarian neo-
plasms composed only of theca cells. Histogenetically,
they are classified as sex cord stromal tumours. They are
typically estrogen-producing and occur in older women
(mean age 59 years; 84% after menopause). They can,
however, appear before menopause [3].

Sixty percent of patients present with abnormal uterine
bleeding, and 20% have an endometrial carcinoma.

Grossly, the tumour is solid and yellow. Microscopi-
cally, the tumour cells have pale, abundant, and lipid-
filled cytoplasms.

Ovarian cystoadenofibroma are infrequent superficial
epithelial tumours. They can appear at all ages. Macro-
scopically, it has been seen that in younger patients, they
are cystic with small papillae, while in older patients they
form fibrotic nodules.

Brenner tumours are uncommon surface-epithelial
stromal cell tumours They are most frequently found as
incidental findings on pelvic examination or at laparo-
tomy [4]. These tumours may be very small to very large,
and may be solid or cystic.

Its epithelial cell (which defines these tumours) is a
transitional cell and similar in appearance to bladder

Figure 1. — Right salpingo-oophorectomy specimen containing a
sub-capsular, small calcified nodule (arrow heads). A: a solid
yellow mass and a cystic lesion with papillary structures (arrows).

Figure 2. — On histological examination, the sub-capsular cal-
cified lesion corresponds to a psammocarcinoma with extensive
psammoma bodies (A: haematoxylin-eosin x 40) which are sur-
rounded by single layer of cytological bland cuboidal or low
columnar epithelium (B: haematoxylin-eosin x 400).

Figure 3. — The cystic lesion shows features of serous cy-
stoadenofibroma revealing the presence of broad papillae with
cellular fibrous stroma and a single layer of epithelium project-
ing into the lumen of a cyst (haematoxylin-eosin x 100). The
solid yellow mass corresponds to a thecoma showing a fibroma-
tous background with spindle cells and masses of cells which
have abundant pale cytoplasm and oval/round nuclei (e: haema-
toxylin-eosin x 200) haematoxylin-eosin x 40).
epithelium. The nests of transitional cells are surrounded by tissue that resembles normal ovary and their nuclei present longitudinal grooves (coffee bean nuclei).

As a rule, the neoplasms which can be associated with Brenner tumour have already been described and they can be epithelial, germinal or sometimes ovarian stromal tumours [5-7].

A review of the literature reveals that psammocarcinoma of the ovary can be associated with cystoadenofibroma [8], but was never observed with thecoma or other stromal ovarian tumors.

In the present case, the authors observed the coexistence of different types of epithelial neoplasms and a stromal tumour associated with a psammocarcinoma, which can be considered a malignant neoplasm.

Moreover, this example of psammocarcinoma is very interesting because it measures only 1.5 x 0.5 x 1.5 cm and, to the best of the author’s knowledge, represents the smallest example of psammocarcinoma described so far in the literature [1].

In the authors’ opinion, because psammocarcinoma is characterized by unpredictable behaviour with both low malignant, aggressive [9], and metastatic potential [10], accurate follow-up is always mandatory.

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References


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Introduction

Gonadal dysgenesis (GD) is a genetically caused heterogeneous disorder of the reproductive system that affects about one in 3,000 births [1-3]. A complete form can be differentiated from a partial form of XY-GD. The complete loss of primordial germ cells in developing gonads of an embryo leads to extremely hypoplastic (underdeveloped) and dysfunctional gonads, mainly composed of fibrous tissue [4]. Complete GD is therefore characterized by hypergonadotropic hypogonadism, female external genitalia, and streak gonads consisting only of fibrous stroma without hormonally active tissue, such as Sertoli cells. The development of Müllerian derivatives like fallopian tubes, uterus, and vagina are not impaired, because of the absence of the anti-Müllerian hormone (AMH). Clinically the complete form is characterized by primary amenorrhea and absence of breast development [5, 6].

In contrast to the complete form, endocrine active tissue can be present in partial XY-GD. The androgen production from Leydig cells induces the differentiation of the Wolffian ducts into seminal vesicles, epididymis and vasa deferentia. Most frequently the AMH production by Sertoli cells is partially impaired causing a coexistence of Wolffian and Müllerian ducts [5, 7].

Case Report

Medical history

The authors present a case of a 17-year-old girl with primary amenorrhea and poor breast development. Inconspicuous female phenotype, normal growth, and psycho-social development corresponded to her age. There was no reported history of hormonal intake, cyclical pain, exposure to radiation, cytostatic therapy or any nervous system symptoms. Furthermore there was no history of relevant trauma or surgical procedures. She is the first child of a non-consanguineous marriage and her mother’s age at time of delivery was 28 years. Her family medical history was inconspicuous.

Diagnostics

Physical examination: 165 cm tall female, weighing approximately 59 kg. No evidence of acne, hirsutism, acanthosis nigricans, goiter or cushingoid stigmata. Breasts were small and poorly-developed with hypopigmented areola (Tanner’s Stage II). External genitalia showed a female phenotype, with no evidence of clitoromegaly. Further examination revealed intact hymen. Vagina and cervix were small and hypoplastically developed (Figure 1).

Laboratory tests: hypergonadotropic hypogonadism, AMH 0.5 ng/ml (–), follicle-stimulating hormone (FSH) 57.4 mU/ml (+), luteinizing hormone (LH) 32.4 mU/ml (+), 17β-estradiol < 5 pg/ml (–), dehydroepiandrosterone sulfate (DHEAS) 260 µg/ml (–), prolactin 309 mIU/l (=), testosterone 0.13 ng/ml (=), androstenedione 0.9 ng/ml (–).

Transvaginal ultrasonography of the pelvis showed a small and hypoplastic uterus; ovaries were not detectable (Figure 2).

Genetic investigation: The karyotype showed an inconspicuous male 46 XY genome, SR-Y and SF-1 locus without pathological findings.

Therapy

The patient was admitted to this present hospital and the authors conducted an operative laparoscopic procedure under general anesthesia. The laparoscopy revealed a hypoplastic uterus, inconspicuous fallopian tubes, and streak gonads on both sides. They performed a bilateral gonadectomy. The histopathology of the excised gonads revealed bilateral streak gonads with gonadoblastomas and a right-sided dysgerminoma.

The authors conducted two cycles of cytostatic therapy with cisplatin/etoposid according to the MAKEI 96 protocol. The cytostatic treatment was well-tolerated by the patient. Subsequently a hormone replacement therapy was initiated with cyclo-progynova. After the first cycle of hormonal treatment, menstruation initiated and breast growth increased.

Summary

Gonadal dysgenesis (GD) is a rare congenital malformation that affects about one in 3,000 births. The authors present a case of a 17-year-old woman with primary amenorrhea and poor breast development. They conducted a laparoscopic surgery and bilaterally removed hypoplastic streak gonads. Histopathology of the ovaries revealed bilateral streak gonads with gonadoblastomas and a right-sided dysgerminoma.

Key words: 46XY karyotype; Gonadal dysgenesis; Gonadoblastoma.
Figure 1. — Infantile external genial, vagina, and cervix existent but hypoplastically developed.

Figure 2. — Vaginal sonography of the hypoplastic uterus (upper left). Images from the endoscopy reveal a very small uterus and bilateral streak gonads (upper right, bottom left and right).
Discussion

The patient suffers from a male pseudohermaphroditism: female phenotype and male genotype. Wieacker suggested in 2003 the following diagnostic algorithm: the existence of Müllerian structures like the upper 2/3 of the vagina, uterus, and fallopian tubes indicates the lack of AMH production during embryonic development. This is the case in the complete XY-GD. The lack of these structures however leads to the existence of AMH producing Sertoli-cells. The differential diagnosis includes different disturbances of the steroid hormonal synthesis, androgen insensitivity or a defect in the LH receptor. The diagnosis of an agonadism is difficult (e.g. vanishing testis). In this case variable forms of the external and internal genital variations are possible according to the time point of testicular regression during embryonic development [8].

The development of the testis is a strictly regulated process. Variable genes are involved: SRY-gene (sex-determining-region Y, induces Sertoli-cells (AMH)), SF-1 (steroid genic factor 1, early development of the gonads, AMH-expression), DHH (desert hedgehog, Leydig-cell-differentiation (testosterone), spermatogenesis) [1]. Different mutations in this signaling cascade cause variable forms of the external and internal genital.

The incidence of malignant gonadoblastoma in patients with dysgenetic gonads is about 30% [5]. Therefore a prophylactic, bilateral gonadectomy is recommended before the onset of puberty. The response rate to chemotherapy is excellent. Five-year survival rate: 100% / 85% / 79% / 71% (Stage I-IV). Seven-year survival rate: 96% at FIGO Stage I (MAKEI-96).

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Bilateral poorly differentiated Sertoli-Leydig ovarian tumor associated with dysgerminoma: case report

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Summary
Sertoli-Leydig cell tumors are rare stromal tumors of the ovary. They account for less than 0.5% of ovarian neoplasms. From a histological point of view, they show large diversity, making their clinical symptoms diverse as well. They are mostly unilateral, with an average diameter 13.5 cm at the moment of diagnosis. Histologically, poorly-differentiated Sertoli-Leydig tumors pose a diagnostic problem, often being clinically asymptomatic which makes their detection relatively late, preventing efficient treatment, and resulting in worse prognosis. This article presents a rare case of bilateral poorly-differentiated Sertoli-Leydig ovarian tumor, characterized by heterologous histological structure, without hormonal unbalance, and without signs of defeminization and/or virilization, its diagnostics, and treatment.

Key words: Sertoli-Leydig tumor; Rare ovarian malignancy.

Introduction
Sertoli-Leydig cell tumors are very rare. They account for approximately 0.5% (even less then 0.2% according to some authors) of all ovarian tumors [1]. In 75% of all cases, they are diagnosed in patients of 20 to 30 years of age with an average of around 25 years. According to data reported in literature, these tumors appear in all age categories; cases showing a clinical picture of premature puberty have been described in premenarchal period; they appear, although rarely, in postmenopausal women [2].

From a histological aspect, these rare tumors contain both Sertoli and Leydig cell elements of varying degrees of differentiation. Sertoli-Leydig cell tumors are classified into four histologic subtypes depending on the degree of differentiation. According to data reported in literature, 11% are well-differentiated tumors, 54% are moderately-differentiated tumors, 3% are poorly-differentiated tumors, while 22% are histological types of this tumor having heterologous elements. Clinical characteristics associated with this tumor vary widely and are related to the presence of a retiform pattern and/or heterologous elements. The degree of tumor differentiation appears to be age-associated. Well-differentiated tumors are more common in elderly women, while moderately- and poorly-differentiated tumors are more common in younger women.

In about one-third to one-half of all Sertoli-Leydig tumor cases, elevated values of androgen hormones are present. Such a hypersecretion of androgens causes menstrual cycle disorders, amenorrhea, hirsutism, i.e., clinical signs of masculinization, which may direct diagnostics towards detection of ovarian tumors.

Sertoli-Leydig tumors which are small in size, do not secrete androgens, do not cause cycle disorders or signs of masculinization, represent a diagnostic problem, and are often asymptomatic before detection of secondary deposits occurs, followed by detection of primary tumor. Tumor cell aggressive behavior, rapid metastasizing, and late detection of the primary tumor all contribute to bad prognosis [3].

Case Report
A case of Sertoli-Leydig ovarian tumor in a 40-year-old patient, virgo intacta, is presented. The patient reported for a checkup because of lower back pains and amenorrhea. Previously, her cycles were orderly, lasting 28 days with usual four-day long menstruation. Color Doppler ultrasonographic examination verified slightly enlarged ovaries of dimensions 48 x 42 x 38 mm with atypical vascularization in the stroma of both ovaries, without any distinguished tumor lesions. Ultrasonographic characteristics of the uterus were normal, no free fluid inside the abdomen, and tumor marker Ca 125 was inside reference values. Patient hormonal status was normal. Considering the suspect pathological vascularization of the ovaries and increasing lower back pains, the patient was referred to nuclear magnetic resonance (NMR) of the abdomen and small pelvis. NMR examination of abdominal organs verified a normal finding. NMR examination of small pelvis verified bilateral ovarian tumors, a smaller one, 20 x 22 mm in size in the left ovary, while the tumor in the right ovary was somewhat bigger in size, partly-cystic in structure, and about 50 mm in diameter. An X-ray imaging of lumbosacral spine was performed which created doubt in existence of secondary localization in vertebral bodies L2-L5. X-ray imaging finding of the lungs was normal as were all lab analyses, including those which could indicate pathological osteolysis caused by secondary vertebral localization. After two weeks the patient underwent surgery. Left ovary was verified by intraoperative means to be cystic, approximately 4 cm in size, while right ovary was significantly increased and changed by tumor which was partly-cystic, partly-solid in structure, and approximately 24 cm in diameter. Cystic zones were filled with serous content, while the solid component was made up of compact whitish foreign tissue containing patches of hemor-
rhage and necrosis. There was no free fluid inside the abdomen. Classical hysterectomy with bilateral adnexectomy and omentectomy was performed. Histopathological findings verified a bilateral contemporary ovarian tumor, one dysergminoma, and one poorly-differentiated Sertoli-Leydig tumor. No malignant cells were found in the omentum. Surgery was complication-free and postoperative course was normal. The patient recovered quickly after surgery, although she was diagnosed with secondary deposits in spinal bodies i.e., already advanced malignant disease. A month later, bone scintigraphy was performed and it showed that the disease was spreading to thoracic vertebrae as well, i.e., secondary deposits in vertebral bodies of both thoracic and lumbar vertebrae Th4 - L5 were verified. Palliative radiation therapy was begun but it did not help as the disease kept spreading, and pathological fractures appeared on vertebral bodies leading to paraplegia and sphincter dysfunction, i.e., symptoms related to spinal cord compression. The patient died 4.5 months after diagnosis was set and treatment attempted.

Discussion

Bilateral Sertoli-Leydig tumors are extremely rare. According to literature data, bilateral tumors are described in less than two percent of all Sertoli-Leydig ovarian tumor cases. Their size varies. The biggest such tumor described in the references was 50 cm in diameter; their average size was approximately 13.5 cm in diameter. Macroscopic description of these tumors usually corresponds to partly-solid, partly-cystic tumor in approximately 58% of cases. Purely solid tumors were described in 35% of cases, while cystic Sertoli-Leydig tumors are extremely rare.

This article describes a bilateral poorly-differentiated Sertoli-Leydig ovarian tumor with component of dysergminoma in one ovary, small in size, and purely cystic in structure, which is a rarity, and another partly-solid, partly-cystic in structure in the other ovary, twice the average size. References cite different cases of Sertoli-Leydig tumor with heterologous elements but also associated with other tumors. For example, a case of dominantly mucinous ovarian tumor was described containing in its stroma a theca cell tumor and granulosa cell tumor [4], as well as a moderately-differentiated Sertoli-Leydig tumor associated with mucinous adenocarcinoma of the ovary [5]. Association of Sertoli-Leydig tumor with papillary tumor of the thyroid gland has also been described [6]. An unexpectedly high incidence of Sertoli-Leydig ovarian tumor and thyroid disease, including benign thyroid nodules, carcinoma, and multinodular goiter, has also been reported.

Concerning the use of tumor markers as tools for diagnostics of Sertoli-Leydig tumors, references cite different data which corresponds to histological diversity of these tumors i.e., to the clinical symptomatology associated with them. However, the value of serum tumor markers to assist in the diagnosis of ovarian stromal malignancies has not yet been established. Serum inhibin and alpha-fetoprotein (AFP) have been evaluated as potential tumor markers in patients with Sertoli-Leydig cell tumors; anti-Müllerian hormone as well. Research performed by Rey in 2000 [7] was based on the fact that only granulosa and Sertoli cells produce anti-Müllarian hormone, thus the assumption was made that this hormone could be used as the tumor marker. However, the same research showed that this assumption was not exactly correct and that measurement of the quantity of this hormone in the serum can be used in certain cases only. Even widely-used tumor marker Ca 125 is not significant in diagnostics of Sertoli-Leydig ovarian tumors. Hormonal status of patients accompanied by a separate analysis of androgen hormones status (possibly during gonadotropin stimulation) can be useful in cases characterized by clinical picture of defeminization i.e., masculinization where, together with ultrasonographic findings of ovarian tumors, can have a certain diagnostic value. Thus it can be said that the usefulness of these tumor markers to assist in the diagnosis of this clinical entity remains questionable [8].

Early diagnostics of small asymptomatic yet aggressive gonadal stromal tumors, such as Sertoli-Leydig tumor, still represents a large diagnostic and therapeutic problem. Fortunately, Sertoli-Leydig tumors are rare so studies and descriptions of each individual case increase our experience in researching this rare malignancy.

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An unusual clinical presentation of a pure yolk sac tumor of the ovary: case report

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Summary

Introduction: Yolk sac tumor (YST) of the ovary is a rare neoplasm, which belongs to the group of ovarian germ cell tumors. It most commonly occurs in children and young women and it is characterized by high malignancy given its premature metastasis. An early diagnosis is important but not easy. Case: An 18-year-old girl came to the authors’ observation for amenorrhea lasting approximately 16 weeks. Abdominal examination revealed a painless palpable mass in the right lower abdomen. At admission ultrasonography (US) revealed a complex mass of the right adnexa with a diameter of about 15 cm. The alpha-fetoprotein (AFP) serum level was elevated to 960 UI/ml. Fertility-sparing surgery was undertaken and the histopathology revealed a Stage IA pure YST. Chemotherapy was avoided and an intensive 36 months follow-up was performed without clinical and radiological evidence of recurrence. Conclusion: This is the first case report of a pure YST of the ovary presented with amenorrhea. It is also a very interesting case for its Stage IA despite prolonged duration of symptoms and AFP high levels.

Key words: Yolk sac tumor; Chemotherapy; Ovary; Alpha-fetoprotein; Follow-up.

Introduction

Yolk sac tumor of the ovary (YST), also called endodermal sinus tumor (EST) is a rare neoplasm, which belongs to the group of malignant ovarian germ cell tumors (MOGCT). It is the second most common MOGCT after dysgerminoma, representing about one percent of all ovarian malignancies [1]. Among all subtypes of MOGCT, YST is highly malignant and has the worse prognosis [2]. Its relative frequency is higher in Japan (up to 19% of ovarian malignancies) than in North America and Western European countries [3]. Because YST principally affects women of child-bearing age, preservation of fertility is the most important objective in its management. Therefore it is imperative that these tumors are managed with accurate diagnosis, staging and treatment. In this paper the authors report a case of YST in an 18-year-old woman with an unusual presentation. It is important to consider these tumors when a pelvic or abdominal mass is detected in children or young women, although when symptoms are not typical.

Case Report

An 18-year-old Caucasian woman came to the authors’ observation for amenorrhea lasting approximately 16 weeks. She denied any family history of malignancy and any previous surgical therapy. She reached menarche at the age of 12 years and always had regular menstrual cycles until then. A transabdominal ultrasonography (US) had been performed before presenting to the authors’ observation and it showed right ovary largely enlarged and entirely occupied by a heterogeneous mass of 95 x 65 x 89 mm, which included mixed echogenic and hypoechoic components with arterial blood flow at color Doppler.

At admission to the present Obstetrics and Gynaecology Unit, abdominal examination was performed and a painless palpable mass in the right lower abdomen was detected. Vaginal examination was not performed because the patient was virgin. Then, a transabdominal US revealed a complex multiseptated mass, predominantly anechoic of the right adnexa measuring 148.7 x 78.1 mm with no free fluid in the pelvis (Figure 1). In two weeks the mass had greatly grown to about ten cm.

Pelvic magnetic resonance imaging (MRI) showed a complex mass of 150 x 93 x 83 mm, which dislocated the uterus to the left and caused compression on the pelvic vessels. It was an encapsulated and multiloculated mass with several areas of cystic degeneration, showing low signal intensity at T1-weighted images and high signal intensity at T2-weighted images (Figure 2). There was no free fluid in the pelvis. The size and the morphology of left adnexa were normal.

Serum tumor markers levels were obtained: alpha-fetoprotein (AFP) was 968 UI/ml (normal values: <10 UI/ml); serum β-human chorionic gonadotropin (β-hCG) and CA 125 were within normal range. The patient was presumptively diagnosed with a malignant germ cell tumor of the ovary, however for a definitive diagnosis, surgery was necessary. After informed consent the patient underwent surgical treatment, which consisted in a laparotomy with right salpingo-oophorectomy, peritoneal washing, careful inspection of the pelvic-abdominal cavity, and multiple biopsies of the pelvic and abdominal peritoneum and omentum.

The histopathologic examination revealed the cyst to be a pure YST with thick fibrous capsule covered by serous tissue. Surgical margin was macroscopically hemorrhagic and microcystic. The mass was heterogeneous containing cystic and solid areas. Necrosis involved 15% of the mass. On immunohistochemistry, tumor cells were positive for AFP, placental alkaline phosphatase (PLAP), total cytokeratin (CK) and negative for vimentin, EMA, CD 30, CD 99, and inhibin. In the examined sections there were predominantly glandular, micro-cystic and polivesicular components. The tumor did not exceed ovarian capsule. No vascular invasion by the tumor was observed and it was classified as Stage IA according to American Joint Committee on Cancer (AJCC) in 2010. Peritoneal washing cytology was negative. Omentum, peritoneum and right tube...
were disease-free. The postoperative course was ordinary. AFP serum value on the first day after surgery was 298 UI/ml and it had completely fallen six weeks after surgery (0.8 UI/ml).

It was decided to avoid chemotherapy according to the low risk of the patient. Her menstrual cycle returned to normal at approximately eight weeks after surgery. A 36 months follow-up, clinical, radiologic, and serologic exams were executed, as outlined in the surveillance paper by Dark et al. [4]. At six weeks postoperatively, full staging was carried out including: physical examination, serum tumor markers (AFP, CA 125, β-HCG), and computed tomography (CT) of chest, abdomen, and pelvis. All these exams were negative. Follow up was performed monthly for the first year, every two months for the second year and every three months for the third year. A repeat CT scan was performed three months after surgery. Chest X-ray was executed at alternate visits and abdominal US was repeated every third clinic visit for the first two years. Serum AFP was measured every two weeks for six months, monthly for 18 months, and then at every clinic visit. No signs of tumor recurrence were detected. Currently the patient is undergoing further follow-up.

Discussion

Due to the rarity of YSTs, there are no randomized trials and most of the available literature is composed of retrospective reviews and case reports comprising all subtypes of MOGCT. The most common symptom of these tumors is abdominal pain occurring in 55% - 80% of the patients [5]. Other signs comprise the presence of abdominal or pelvic mass with abdominal enlargement. Fever is present in one-fourth of the cases. Ascites or peritonitis secondary to torsion, infection or rupture of the ovarian tumor, are other possible clinical features [6]. Few patients present with vaginal bleeding and very few with amenorrhea. The authors have found in literature only two cases of YSTs presented with amenorrhea, but in both cases yolk sac component was associated with other types of tumoral cells. In one case luteinized stromal cells were associated with the tumor. In the other case the tumor was diagnosed in a pregnant woman and it resulted composed by islands of Leydig cells mixed with the typical features of yolk sac tumor [7, 8]. Indeed our young patient presented with amenorrhea had a pure YST.

Because of the rapid growth of these tumors, a delay in diagnosis and subsequent treatment may harm the patient and sharply reduce chances of survival. Thus a diagnosis at an early stage (when five years survival reaches 95%) is fundamental, although it is generally difficult and no specific imaging distinguishes YST from other ovarian masses preoperatively [9]. Therefore, when a pelvic mass is found in children or young women, the diagnosis of YST should be considered and AFP levels should be measured also in absence of pain. It is important to make differential diagnosis with cystic teratoma, tubo-ovarian abscess, appendiceal abscess, mesenteric cyst or gastrointestinal duplication cyst.

Another particular feature of this case is the duration of symptoms that is usually brief (approximately between two to four weeks) [10], instead in the present patient symptoms lasted about 16 weeks. Nevertheless tumor mass was not so large and it was at a very early stage.

In the past, YSTs had a very poor prognosis and they were almost always fatal. In the last several decades the development of new chemotherapeutic regimes has dramatically changed survival rate. Nowadays the standard treatment for YST is fertility-sparing surgery (due to the extreme rarity of bilateral involvement of the ovaries and to reproductive age of most patients) followed by chemotherapy [11]. There are no recent studies evaluating chemotherapeutic regimen for cases of isolated YSTs. Therefore, the current management of YST is based on recent retrospective reviews comprising all subtypes of MOGCT. BEP regimen (bleomycin, etoposide, and cisplatin) has become the
current standard therapy, but it causes short- and long-term complications such as life-threatening neutropenic sepsis, deaths for acute renal failure, pulmonary fibrosis, secondary malignancies, and cardiovascular diseases [12-15]. At the present, a controversial issue concerns the role of chemotherapy in Stage IA/IB MOGCTs but last researches [16-19] have emphasized that true Stage IA could be adequately managed by only surgery followed by careful clinical, radiological, and serological surveillance and do not require adjuvant chemotherapy. The management of our patient further supports literature on this subject.

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Intraparenchymal metastasis to the accessory spleen from ovarian cancer: a case report

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Summary
Splenic metastasis from ovarian cancer is a rare entity. A few case reports are present in the literature, but to the authors’ knowledge, intraparenchymal metastasis to the accessory spleen has never been reported in the literature. The authors report a case of accessory splenic metastasis from ovarian carcinoma.

Key words: Cancer; Ovary; Metastasis; Accessory spleen.

Introduction
Distant metastases of ovarian cancer are present in eight percent of patients at the time of diagnosis, and develop in 22% of patients during the course of the disease [1]. Parenchymal metastases in patients with ovarian cancer represent hematogenous spread of the disease, and are present in two to three percent of patients [2]. Solitary splenic metastasis of carcinoma of ovarian origin is exceedingly rare [3]. The authors present a case of a primary ovarian cancer with intraparenchymal metastasis to the accessory spleen.

Case Report
A 65-year-old woman was admitted to this present hospital for evaluation of a bilateral adnexal mass. The level of CA-125 was 1630 U/ml. Her history included splenectomy which was performed due to a traffic accident. Laparotomy revealed right 9 x 7 x 7 cm and left 5 x 4 x 3 cm solid ovarian masses which showed extensive adhesions to the surrounding tissues and pelvic organs. In addition, a solid mass of 8.5 x 5 x 3.5 cm was detected on the mesentery of transverse colon (Figure 1). Peritoneal and serosal surfaces, omentum stomach, and liver were macroscopically intact. After peritoneal washing for cytology, total abdominal hysterectomy and bilateral salpingo-oophorectomy with omentectomy, and excision of the mass on the mesentery of transverse colon was performed. Final pathologic report showed serous cystadenocarcinoma on both ovaries without capsule invasion. Histopathology of omentum and cytology of peritoneal washing showed no tumor. Histological examination of the mass on the mesentery of transverse colon was evaluated as accessory spleen and pathological examination of this mass revealed intraparenchymal adenocarcinoma metastasis (Figure 2). Results of the immunohistochemical examination performed on both the ovarian and the accessory splenic tumor cells were CK7 (+), CA125 (+), vimentin (−), and CK20 (−).

Discussion
Isolated splenic metastasis is rare. Hypotheses that can explain the rarity of splenic metastasis include the role of the splenic capsule acting as a shield, the lack of an afferent lymphatic route to the spleen, the tortuosity of the splenic artery, the contractile properties of the spleen, and the immune competence of the splenic parenchyma [4, 5]. The splenic artery, the splenic vein, and the lymphatic system are the three major metastatic pathways with the splenic artery being thought to be the most common route of spread. Isolated intraparenchymal metastases are not common and represent hematogenous dissemination [1].

Accessory spleens are congenital anomalous foci of healthy splenic tissues that are separate from the main body of the spleen [6]. Accessory spleens are common and affect between 10% and 30% of the population. They are usually asymptomatic and diagnosed incidentally in radiological examinations performed for other reasons [7]. Splenic hilum is the most common site of an accessory spleen followed by pancreatic tail, gastro-splenic, spleno-colic, and gastrocolic ligaments. Other rare locations are splenorenal ligament, greater omentum, mesentery as in the present case, presacral area, adnexal region, and scrotum [8].

Splenectomy is a proper therapeutic approach for an isolated splenic metastasis, and it may play a principal role in the management of such cases with increased patient survival [9].

To the best of the authors’ knowledge, there is no reported case of intraparenchymal metastasis to an accessory spleen from the ovary in the literature. Here, the authors report a case of bilateral ovarian cystadenocarcinoma without peritoneal dissemination with an isolated synchronous intraparenchymal metastasis in accessory spleen detected during surgery on the mesentery of transverse colon.
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Polymorphisms of glutathione-s-transferase M1, T1, and P1 genes in endometrial carcinoma - K. Ozerkan, M.A. Atalay, T. Yakut, Y. Doster, E. Yilmaz, M. Karkucak
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CASE REPORTS
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CASE REPORTS

Endometrial adenocarcinoma in a young woman - D. Caserta, G. Bordi, S. Scarani, M. Moscarini

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No. 2, March-April
An undescribed coexistence of benign metastasizing leiomyoma in the lung with serous borderline tumor of the ovary - M.F. Gan, H.S. Lu

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CASE REPORTS


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Late recurrence of malignant melanoma mimicking primary peritoneal cancer - M. Sano, Y. Hashiguchi, T. Yasui, T. Sumi, K. Wakasa, O. Ishiko

Amelanotic malignant melanoma of the perineum: a case report - M. Zamurovic, V. Soldo, N. Cutura, Z. Perisic


Multiorgan thrombotic disorder in a young patient with primary antiphospholipid syndrome (APS) and ovarian tumor - K. Jeremic, A. Stefanovic, A. Ljubic, P. Miljic, J. Stojnic, B. Kastratovic, L.J. Arsenijevic


Contents - Volume XXXIV, 2013
A rare case of lymphangiomyomatosis treated with leuprolide acetate: five-years follow-up - E. Bastu, S.E. Akhan, B. Karamustafaoğlu, F. Gungor-Uğurlucan, H. Sozen, A.C. iyibozkurt ................................................................. 278
Endometrial stromal sarcoma with intracaval extension at initial presentation - V. Boskovic, T. Bozanovic, A. Ljubic, I. Likic-Ladjevic, T. Janjic, S. Milicevic ................................................................. 280

No. 4, July-August

EXPERT SERIES

An improved, disposable indwelling intrauterine tube (“Smit Sleeve”) not requiring retaining stitches for brachy-radiotherapy for carcinoma of the cervix - B.J. Smit, A.L. van Wijk ................................................................. 289

ORIGINAL ARTICLES

Clinical outcome of patients with microinvasive adenocarcinoma of the uterine cervix - L. Meglič, R. Košir Pogačnik, S. Rakar, Š. Smrkolj ................................................................. 296
Photodynamic therapy effectively palliates gynecologic malignancies - H. Godoy, P. Vaddadi, M. Cooper, P.J. Frederick, K. Odunsi, S. Lele ................................................................. 300
Development of antiangiogenic therapies for ovarian cancer - A. Markowska, J. Lubin, R. Mađry, J. Markowska ................................................................. 303
Clinicopathological characteristics and outcome of patients with small cell neuroendocrine carcinoma of the uterine cervix: case series and literature review - Y. Wang, K. Mei, M.F. Xiang, J.M. Li, R.M. Xie ................................................................. 307
Epidemiological characteristics of HPV-related female cancers in the Umbria region of Italy: pre-vaccination period - D. D’Alò, I. Bernardini, F. Cioccoloni, G. Calagreti, S. Leite, M. Saba Petrucci, F. Bianconi, V. Brunori, F. La Rosa, F. Stracci ................................................................. 311
Role of omentectomy and appendectomy in surgical staging of endometrioid endometrial cancer - B. Ozdal, B.S. Unlu, H.R. Yalcın, O.L. Tapisiz, H. Energin, M. Besli, T. Gungor ................................................................. 322

CASE REPORTS

Carcinosarcoma of the uterus in advanced stage: a case report - L. Tasic, M. Vasiljevic, M. Prorocić, A. Jurisic, S. Dragojevic-Dikic, S. Jankovic-Raznatovic ................................................................. 343
Successful treatment of isolated tubular bone metastasis in a uterine endometrial cancer of clear cell carcinoma - C.Y. Chen, K.G. Huang, N.A. Abdullah, S.H. Ueng, C.L. Lee ................................................................. 347
Primary signet-ring cell adenocarcinoma of the uterine cervix: case report and review of the literature - O. Kaidar-Person, A. Amit, A. Berniger, R. Ben-Yosef, A. Kuten, R. Bortnyak-Abdah ................................................................. 353
Primary squamous cell carcinoma of endometrium: clinicopathologic analysis of two cases with review of the literature - Y. Li, H.J. Lu, Y. Yang ................................................................. 355
Transition of low-grade to high-grade endometrial stromal sarcoma: a case report - M. Kanda, A. Sonoyama, H. Hirano, T. Kizaki, N. Ohara ................................................................. 358
No. 5, September-October

DISTINGUISHED EXPERT SERIES

The status of radiotherapy in the management of breast cancer 2013 - B. Smit 373

REVIEW ARTICLES

Sentinel node biopsy in endometrial cancer: systematic review and meta-analysis of the literature - M. Ansari, M.A. Ghodsirad, M. Hassanzadeh, H. Gholami, Z. Yousefi, V.R. Dabbagh, R. Sadeghi 387

ORIGINAL ARTICLES

Adjuvant treatment for uterine leiomyosarcoma - S.N. Akers, A. Groman, K. Odunsi, S. Lele, P.J. Frederick 409
Laparoscopic management of early stage ovarian cancer: is it feasible, safe, and adequate? A retrospective study - G. Montanari, N. Di Donato, S. Del Forno, A. Benfenati, V. Bertoldo, C. Vincenzi, P. Casadio, R. Seracchioli 415
Nationwide screening program for breast and cervical cancers in Hungary: special challenges, outcomes, and the role of the primary care provider - L.R. Kolozsvári, Z. Langmár, I. Kurik 419
Effectiveness of radiotherapy in patients with primary invasive vaginal carcinoma - P. Blecharz, M. Reinfuss, J. Jakubowicz, P. Skotnicki, T. Walasek, E. Luczyńska 436
High pathologic misdiagnosis of cervical adenocarcinoma in situ - S.M. Jordan, D.M. Chase, T. Watanabe; K. Osann; B.J. Monk, J.K.L. Rutgers 446
DNA damage in peripheral blood lymphocytes of ovarian cancer patients after radiotherapy - H. Zhu, S.Y. Han, X.G. Li, X.G. Zhou, Q.F. Zhang 450
Correlation of subclinical HPV infection with genital warts and cervical erosion - X. Cao, N. Gao, L. Huang, J. Yao 462

CASE REPORTS

IL-17 and IL-22 serum cytokine levels in patients with squamous intraepithelial lesion and invasive cervical carcinoma - J.M. Souza, B.F. Matias, C.M. Rodrigues, E.F.C Murta, M.A Michelin 466
Immunoreactivity for Ca 125 and INI 1 loss of expression are useful markers in the diagnosis of vulvar proximal-type epithelioid sarcomas: report of two cases - A. Cossu, P. Paliogiannis, G. Capobianco, M.C. Sini, M. Dessole, S. Desole, G. Palmieri 469
All vertebral body metastases of breast cancer: a case report and literature review - P. Cheng, X. Su, H. Gao, T. Zhang 473
Ovarian cancer presenting as a metastasis to a trocar tract used for a gasless lift-laparoscopy to resect a benign ovarian cyst: an unusual case report - H. Matsushita, T. Takayanagi, H. Ikarashi, M. Fukase 480
Laparoscopic para-aortic and pelvic lymphadenectomy and radical hysterectomy in a patient with cervical cancer, six months after primary chemoradiation - D. Zygouris, I.C. Kotsopoulos, N. Chalvatzas, T. Maltaris, V. Kartsiounis, A. Kavallaris 484


No. 6, November-December

ORIGINAL ARTICLES


Attitudes and practices of Korean gynecologists towards hormone replacement therapy in endometrial cancer survivors - S.J. Lee, S.G. Yeo, S.B. Kang, D.C. Park

Research on sequence variations analysis of HPV-16 type in Southwestern China - Y. Wang, X. Ding, Y. Zhu

Evaluation of the outcome benefit conferred by intensive surveillance strategies in women with early-stage endometrial cancer - G. Kiran, J.P. Kesterson, K. Ozerkan, M. Kanis, A. Groman, S. Lele

Response to neoadjuvant chemotherapy with paclitaxel and cisplatin in locally advanced cervical cancer - A.S. Mousavia, S. Vahidi, M. Karimi-Zarchi, M. Modarress-Gilania, F. Ghaemmaghamia


Correlation of cervical intraepithelial neoplasia with expressions of p16 and Ki67 in exfoliated cervical cells in fluid-based thin-layer samples - K. You, Y.L. Guo, L. Geng, J. Qiao

Evaluation of the importance of the serum levels of CA-125, CA15-3, CA-19-9, carcinoembryonic antigen and alpha fetoprotein for distinguishing benign and malignant adnexal masses and contribution of different test combinations to diagnostic accuracy - M. Bozkurt, A.E. Yumru, I. Aral

The effect of coexisting uterine myomas on clinico-pathological variables of endometrial carcinoma - J. Menczer, E. Ben-Shem, A. Golan, T. Levy


Comparing letrozole with medroxyprogesterone acetate (MPA) as hormonal therapy for simple endometrial hyperplasia without atypia in adult and middle-aged women - A. Tabatabaie, M. Karimi Zarchi, M. Dehghani-Tafti, A. Miratashi-Yazdi, S. Teimoori, A. Dehghani

Is the 2009 FIGO staging system really valuable for Stage I endometrial cancer? - F. Atalay, K. Cetinkaya, A. Bacinoğlu

In vitro chemosensitivity assay of ascites in epithelial ovarian cancer - X. Xu, H. Dai, Y. Zhao, Y. Wang, X. Xu, Z. Qian, X. Chen

Characteristics of diagnosis and therapy of adolescent malignant ovarian tumors - M. Su, W. Chang, T. Xu, M. Cui, S. Wu, P. Su

CASE REPORTS

A case of ovarian psammocarcinoma with homolateral serous cystoadenofibroma and thecoma associated with Brenner tumour and cystoadenofibroma of the contralateral ovary - G. Giordano, F. Brigati, E. Varotti


Bilateral poorly differentiated Sertoli-Leydig ovarian tumor associated with dysgerminoma: case report - M. Zamurovic, V. Soldo, N. Cутара

An unusual clinical presentation of a pure yolk sac tumor of the ovary: case report - D. Caserta, E. Ralli, G. Bordi, M. Moscarini

Intraparenchymal metastasis to the accessory spleen from ovarian cancer: a case report - V. Mihmanli, G. Toprakci, N. Cetinkaya, A. Kilickaya, G. Kamali
Index of Authors in alphabetical order

Abdullah N.A., 183, 347
Adlan A.S., 183
Agoioli L., 487
Akabay O., 75, 457
Akca A., 75
Akers S.N., 113, 409
Akhan S.E., 263, 271, 278
Akrivis G., 31
Akyol A., 457
Almeida E.Q., 48
Alpay V., 75
Ambrosini G., 227, 254
Amit A., 353
Anastassiads A., 31
Anastasopoulos C., 325
Andrijausic G., 65
Androussopoulos G., 94
Ansari M., 387
Aral İ., 540
Armosini G., 487
Arnogiannaki N., 94
Arsenijevic L.J., 273
Aslay I., 248
Atacag T., 175
Atalay F., 556
Atalay M.A., 42
Atanackovic J., 83
Aznajac G.L., 476
Bacmgola A., 556
Bae H.S., 238
Baegg A.C., 23
Baergen R., 453
Baracat E.C., 509, 532
Barczyński B., 489
Barczyński B., 489
Barczyński B., 489
Bartolucci C., 51, 231
Bashir S., 453
Bastu E., 263, 271, 278
Ben-Shem E., 545
Ben-Yosef R., 353
Benfenati A., 415
Bernardini I., 311
Berner A., 353
Bertoldo V., 415
Besli M., 322
Bianconi F., 311
Bilia J., 65
Bilecová-Rabijová M., 189
Blecharz P., 222, 436
Boni S., 496
Borahay M., 36
Bordi G., 179, 577
Borgato S., 51
Bortnyak-Abdah R., 353
Boskovic V., 113, 409
Boskovic V., 83, 280
Botas D., 325
Boutas I., 325
Bozanovic T., 280
Bozokurt M., 540
Breda E., 231, 243
Brigati G., 569
Brdusic N., 65
Brodak M., 234
Brunori V., 311
Byun S.J., 402
Byun S.J., 402
Calhong Li, 142
Calogriti G., 311
Cao X., 462
Capobianco G., 227, 254, 469
Caputo T.A., 453
Carneiro Juliano C., 532
Casadio P., 415
Casanova J., 183
Caserta D., 179, 577
Castelo-Branco M., 261
Celic H., 493
Cetin A., 175
Cetinkaya K., 556
Cetinkaya N., 580
Chalvatzas N., 484
Chang W., 565
Chang W.H., 442
Chase D.M., 446
Chen H.C., 442
Chen C.Y., 347
Chen S.Y., 548
Chen W.J., 429
Chen X., 559
Chen Z.F., 548
Cheng P., 473
Cherchi P.L., 227, 254
Chiu L.H., 442
Cho C.H., 402
Cho Y.L., 128
Choi J.S., 70
Choi M.R., 148
Chong O.O., 128
Chung Y.W., 238
Cioccoloni F., 311
Clement K.M., 28
Codroma A., 51, 243
Conte L., 51, 231
Cooper M., 300
Corona G., 5
Cossu E., 264
Cossu A., 469
Corbier R., 319
Cui M., 565
Cutura N., 269, 575
D’Agostino G., 243
D’Alò D., 311
Dabbagh V.R., 387
Dai H., 559
de Albuquerque Neto L.C., 509
De Sanctis V., 487
De Santiago J., 138
Delghani A., 552
Delghani-Tafti M., 552
Del Forno S., 415
Deligiannis D.A., 186
Dessole M., 227, 254, 469
Dessole S., 469
Di Donato N., 415
Di Iorio R., 5
Diessner J., 572
Diestro M.D., 138
Dietl J., 572
Ding X., 518
Ding X.P., 132
Ding Y., 548
Doster Y., 42
Dotlic J., 65
Dragojevic-Dikic S., 343
Du R., 548
Energin H., 322
Ermi A., 457
Erzin K., 36
Fabris A., 51
Fabris A.M., 231
Falchetto Oste M., 487
Fan L.J., 152
Farghaly S.A., 205
Fehr M., 23
Ferrari F., 213
Filimonov D., 101, 275
Fink D., 23
Focchi G.R.A., 48
Fonseca-Moutinho J.A., 261
Frederick P.J., 300, 409
Freeman D.H., 36
Frega A., 379
Frey M.K., 453
Fujitaka T., 332
Fukase M., 480
Fukushima C., 39
Furuya K., 120
Gadducci A., 213
Galandarow R., 271
Galizos G., 319
Gan M.F., 193
Gao H., 473
Gao H.J., 362
Gao Minzhi, 170
Gao N., 462
Garcia H., 261
Gedikbas A., 75
Geng L., 159, 535
Ghaemmaghamia F., 527
Ghodsi Rad M.A., 387
Gholami H., 387
Gilbraz E., 248
Giordano G., 569
Giusa-Chifferi M.G., 509
Godoy H., 300
Golan A., 545
Gonçalves W.J., 509
Gonzalez-Benitez C., 138
González-Bosquet E., 336
Goto T., 120
Graf N., 23
Granauzzo P., 532
Greco P., 5
Grigoriadis C., 94
Groman A., 113, 409, 522
Guler T., 175
Guor G., 322
Gungor Ugurlucan F., 248, 263, 271, 278
Guo Y.L., 159, 535
Gupta D., 453
Haddad H., 257
Hagiya M., 332
Haiy ing Li, 163
Hamada K., 332
Han S.Y., 450
Hashiguchi Y., 265
Hashimoto H., 332
Hassanzadeh M., 387
Hatae M., 425
Hayajneh W., 257
Hensel K.J., 453
Hernandez A., 138
Hiramatsu Y., 39
Hirano H., 358
Hirata J., 120
Hiura M., 425
Holcombe K.M., 453
Holloway R.W., 86
Hong D.G., 128
Hong L., 197
Hong Yan, 170
Hong Ye, 142
Hong O., 39
Hong I., 572
Horie K., 120
Huang K.G., 183, 347
Huang L., 462
Hrur S.Y., 148
Javazzo C.R., 218
Ikarashi H., 480
Ikeda Y., 90
Indraccolo S.R., 5
Indraccolo U., 5
Inshibashi T., 104
Ishikawa N., 104
Ishiko O., 265
Iyibozkurt A.C., 263, 271, 278
Jakovljevic S.D., 476
Jakubowicz J., 222, 436
Janjie T., 280
Jankovic-Raznatovic S., 275, 343
Jaradat S., 257
Jeong M.J., 128
Jeremic K., 273, 275
Jordan S.M., 446
Jung U.S., 70
Jurisic A., 343
Kadija S., 275
Kaidar-Person O., 353
Kalampokas T., 325
Kalampokas T.E., 218
Kamali G., 580
Kanda M., 358
Kanda M., 358
Kang S.B., 513
Kanis M., 522
Karamustraoğlu B., 263, 278
Karimí-Zarchi M., 527, 552
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terzic M.</td>
<td>65</td>
</tr>
<tr>
<td>Tian W.Y.</td>
<td>350</td>
</tr>
<tr>
<td>Tianmin X.</td>
<td>166</td>
</tr>
<tr>
<td>Toprakci G.</td>
<td>580</td>
</tr>
<tr>
<td>Tran T.A.</td>
<td>86</td>
</tr>
<tr>
<td>Trimbos J.B.M.Z.</td>
<td>208</td>
</tr>
<tr>
<td>Tsagias N.</td>
<td>319</td>
</tr>
<tr>
<td>Tsapanos V.S.</td>
<td>186</td>
</tr>
<tr>
<td>Tsikouras P.</td>
<td>319</td>
</tr>
<tr>
<td>Túma P.</td>
<td>329</td>
</tr>
<tr>
<td>Tyner J.</td>
<td>36</td>
</tr>
<tr>
<td>Tzounas N.</td>
<td>218</td>
</tr>
<tr>
<td>Ueng S.H.</td>
<td>347</td>
</tr>
<tr>
<td>Ulker V.</td>
<td>75, 457</td>
</tr>
<tr>
<td>Unlu B.S.</td>
<td>322</td>
</tr>
<tr>
<td>Urbano-Ruiz A.</td>
<td>532</td>
</tr>
<tr>
<td>Vaddadi P.</td>
<td>300</td>
</tr>
<tr>
<td>Vahidi S.</td>
<td>527</td>
</tr>
<tr>
<td>Valeriani M.</td>
<td>487</td>
</tr>
<tr>
<td>van den Tillaart S.A.H.M.</td>
<td>208</td>
</tr>
<tr>
<td>van Wijk A.L.</td>
<td>289</td>
</tr>
<tr>
<td>Varga J.</td>
<td>189</td>
</tr>
<tr>
<td>Varotti E.</td>
<td>569</td>
</tr>
<tr>
<td>Varras M.</td>
<td>31</td>
</tr>
<tr>
<td>Vasilakaki T.</td>
<td>31</td>
</tr>
<tr>
<td>Vasiljevic M.</td>
<td>343</td>
</tr>
<tr>
<td>Vieira da Motta E.</td>
<td>532</td>
</tr>
<tr>
<td>Vincenzi C.</td>
<td>415</td>
</tr>
<tr>
<td>Vrachnis N.</td>
<td>31</td>
</tr>
<tr>
<td>Wakasa K.</td>
<td>265</td>
</tr>
<tr>
<td>Walasek T.</td>
<td>436</td>
</tr>
<tr>
<td>Wang J.</td>
<td>60</td>
</tr>
<tr>
<td>Wang J.L.</td>
<td>124</td>
</tr>
<tr>
<td>Wang P.H.</td>
<td>442</td>
</tr>
<tr>
<td>Wang P.N.</td>
<td>183</td>
</tr>
<tr>
<td>Wang S.J.</td>
<td>124</td>
</tr>
<tr>
<td>Wang X.M.</td>
<td>156</td>
</tr>
<tr>
<td>Wang Y.</td>
<td>54, 307, 518, 559</td>
</tr>
<tr>
<td>Wang Y.L.</td>
<td>132</td>
</tr>
<tr>
<td>Wang Y.M.</td>
<td>350</td>
</tr>
<tr>
<td>Ward N.M.</td>
<td>453</td>
</tr>
<tr>
<td>Wasser M.N.J.M.</td>
<td>208</td>
</tr>
<tr>
<td>Watanabe T.</td>
<td>446</td>
</tr>
<tr>
<td>Wei L.H.</td>
<td>124</td>
</tr>
<tr>
<td>Wei S.</td>
<td>54</td>
</tr>
<tr>
<td>Weiqin C.</td>
<td>166</td>
</tr>
<tr>
<td>Weniger J.M.</td>
<td>227, 254</td>
</tr>
<tr>
<td>Wu D.B.</td>
<td>60</td>
</tr>
<tr>
<td>Wu S.</td>
<td>565</td>
</tr>
<tr>
<td>Wu X.</td>
<td>54</td>
</tr>
<tr>
<td>Xia L.P.</td>
<td>152</td>
</tr>
<tr>
<td>Xiang M.F.</td>
<td>307</td>
</tr>
<tr>
<td>Xie R.M.</td>
<td>307</td>
</tr>
<tr>
<td>Xirou P.A.</td>
<td>186</td>
</tr>
<tr>
<td>Xu D.</td>
<td>152</td>
</tr>
<tr>
<td>Xu M.</td>
<td>152</td>
</tr>
<tr>
<td>Xu T.</td>
<td>565</td>
</tr>
<tr>
<td>Xu X.</td>
<td>559</td>
</tr>
<tr>
<td>Xue F.X.</td>
<td>350</td>
</tr>
<tr>
<td>Xue J.L.</td>
<td>152</td>
</tr>
<tr>
<td>Yakut T.</td>
<td>42</td>
</tr>
<tr>
<td>Yalcin H.R.</td>
<td>322</td>
</tr>
<tr>
<td>Yalcin O.</td>
<td>248</td>
</tr>
<tr>
<td>Yamada H.</td>
<td>425</td>
</tr>
<tr>
<td>Yang L.</td>
<td>166</td>
</tr>
<tr>
<td>Yang R.</td>
<td>429</td>
</tr>
<tr>
<td>Yang W.X.</td>
<td>156</td>
</tr>
<tr>
<td>Yang Y.</td>
<td>355</td>
</tr>
<tr>
<td>Yao J.</td>
<td>462</td>
</tr>
<tr>
<td>Yasui T.</td>
<td>265</td>
</tr>
<tr>
<td>Yayci E.</td>
<td>175</td>
</tr>
<tr>
<td>Yeo S.G.</td>
<td>513</td>
</tr>
<tr>
<td>Yeom B.W.</td>
<td>238</td>
</tr>
<tr>
<td>Yildiz L.</td>
<td>493</td>
</tr>
<tr>
<td>Yilmaz E.</td>
<td>42</td>
</tr>
<tr>
<td>Yokota H.</td>
<td>120</td>
</tr>
<tr>
<td>Yoo J.Y.</td>
<td>339</td>
</tr>
<tr>
<td>Yoo S.H.</td>
<td>339</td>
</tr>
<tr>
<td>Yoon J.H.</td>
<td>148, 339</td>
</tr>
<tr>
<td>You K.</td>
<td>159, 535</td>
</tr>
<tr>
<td>Yousefi Z.</td>
<td>387</td>
</tr>
<tr>
<td>Yu J.J.</td>
<td>156</td>
</tr>
<tr>
<td>Yumru A.E.</td>
<td>540</td>
</tr>
<tr>
<td>Z.R. Xiong</td>
<td>132</td>
</tr>
<tr>
<td>Zamurovic M.</td>
<td>269, 575</td>
</tr>
<tr>
<td>Zapardiel I.</td>
<td>138</td>
</tr>
<tr>
<td>Zeng F.</td>
<td>54, 197</td>
</tr>
<tr>
<td>Zeng Y.L.</td>
<td>429</td>
</tr>
<tr>
<td>Zhang F.</td>
<td>429</td>
</tr>
<tr>
<td>Zhang G.</td>
<td>197</td>
</tr>
<tr>
<td>Zhang L.</td>
<td>54</td>
</tr>
<tr>
<td>Zhang Q.F.</td>
<td>450</td>
</tr>
<tr>
<td>Zhang T.</td>
<td>473</td>
</tr>
<tr>
<td>Zhang X.F.</td>
<td>362</td>
</tr>
<tr>
<td>Zhang X.G.</td>
<td>429</td>
</tr>
<tr>
<td>Zhang Y.</td>
<td>548</td>
</tr>
<tr>
<td>Zhao C.</td>
<td>124</td>
</tr>
<tr>
<td>Zhao L.J.</td>
<td>124</td>
</tr>
<tr>
<td>Zhao M.</td>
<td>54</td>
</tr>
<tr>
<td>Zhao X.</td>
<td>170</td>
</tr>
<tr>
<td>Zhao Y.</td>
<td>559</td>
</tr>
<tr>
<td>Zhou C.Y.</td>
<td>362</td>
</tr>
<tr>
<td>Zhou W.</td>
<td>362</td>
</tr>
<tr>
<td>Zhou X.G.</td>
<td>450</td>
</tr>
<tr>
<td>Zhu H.</td>
<td>450</td>
</tr>
<tr>
<td>Zhu Y.</td>
<td>518</td>
</tr>
<tr>
<td>Zygouris D.</td>
<td>94, 484</td>
</tr>
</tbody>
</table>


Contents


Chapter 3: Human Papillomavirus (HPV) infections. M. Branca and A. Longatto-Filho.

Chapter 4: Risk factors for cervical cancer. M. Branca.


Chapter 6: Cancer prevention in developing countries. A. Longatto-Filho.

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Chapter 8: Management of women with abnormal cytological results. M. Branca and A. Longatto-Filho.


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