EUROPEAN JOURNAL OF GYNAECOLOGICAL ONCOLOGY – EJGO (ISSN 0392-2936) publishes original peer reviewed work, preferably brief reports, in the fields of female genital cancers and related subjects, prevention, early detection, epidemiology, pathology, diagnosis, management, and also proceedings of Gynecologic Oncology Society global meetings. The Journal is covered by ISI Journal Master List, Index Copernicus International, Science Citation Index Expanded, Current Contents - Clinical Medicine, Web of Science, Index Medicus/MEDLINE, EMBASE Excerpta Medica, PubMed, MedSci, Pubget, Genamics JournalSeek, Sciencescape, Unbound Medicine, and PubFacts.com. EJGO is issued bimonthly in one volume per year by 7847050 CANADA Inc., Montréal (Canada). Printed in Italy by “Centro Servizi Editoriali S.r.l.” - Grisignano di Zocco - 36040 Vicenza (Italy).
Contents

European Journal of Gynaecological Oncology - Vol. XXXVI, no. 6, 2015

ORIGINAL ARTICLES

Decreased expression of SIRT6 promotes tumor cell growth and correlates closely with poor prognosis of ovarian cancer
G. Zhang, Z. Liu, S. Qin, K. Li - Jinan city, CHINA
The link between SIRT6 and cancer provides new insight for the therapeutic potential of small molecule activators.

Surgical Stage I high-grade ovarian cancer: is adjuvant chemotherapy warranted?
Y. Segev, N. Ismiil, R. McVey, A. Covens - Toronto, Ontario, CANADA
The recurrence rate of Stage I high risk epithelial ovarian cancer completely staged without adjuvant treatment, is comparable to those of treatment arm previously reported.

Which is the appropriate surgical procedure for Stage I endometrial carcinoma?
L. Sun, X.G. Sheng, L. Wei, F. Gao, X. Li, N.F. Liu, D.P. Li, X. Zhang, T.T. Zhang, P. Wei - Jinan, CHINA
The management of endometrial adenocarcinoma Stage I is evaluated in relation to risk factors.

Intraoperative subserosal approach to label sentinel nodes in intermediate and high-risk endometrial cancer
P. Valha, E. Kucera, P. Sak, O. Stepanek, M. Michal - Brno, CZECH REPUBLIC
The flexibility and the reliability of in vivo sentinel lymph node mapping and a new method of subserosal blue dye in cases of endometrial cancer were evaluated.

HPV16 infection up-regulates Piwi2, which affects cell proliferation and invasion in cervical cancer by regulating MMP-9 via the MAPK pathway
Piwi2, highly expressed in cervical cancer and stimulated by HPV, plays an important role in regulating proliferation and invasion of cervical cell.

Adjuvant treatment with a dialyzable leukocytes extract contributes to maintain HPV-infected women free of low-grade cervical lesions
The treatment with dialyzable leukocytes extract provides an important stimulus for a more favourable immune response from HPV-infected patients affected by low-grade cervical lesions.

Cytoplasmic p21 is responsible for paclitaxel resistance in ovarian cancer A2780 cells
X. Xia, T. Ji, R. Liu, Y. Weng, Y. Fang, Z. Wang, H. Xu - Shenzhen, CHINA
The role of cytoplasmic p21 as potential therapeutic target for paclitaxel-resistant ovarian tumor was evaluated.

Endometrial adenocarcinoma in young-aged women: a Turkish population study
T. Gungor, N. Cetinkaya, B. Ozdal, H. Yalcin, S. Erkaya, H.I. Yakut - Ankara, TURKEY
The incidence, clinicopathological features, treatment, and outcomes of endometrial adenocarcinoma in young patients were evaluated.

Comparison of whole-body PET/PET-CT and conventional imaging procedures for distant metastasis staging in patients with breast cancer: a meta-analysis
Zhe Sun, Yu Li Yi, Yu Liu, Jian Ping Xiong, Chao Zhu He - Nanchang, CHINA
Whole-body PET/PET-CT was compared with conventional imaging procedures in the overall assessment of distant metastasis in breast cancer patients.
Relationship between smoking, HPV infection, and risk of cervical cancer
E. Mazarico, M.D. Gómez-Roig, L. Guirado, N. Lorente, E. Gonzalez-Bosquet - Barcelona, Spain
Risk of HPV infection, CIN 2-3, and cervical cancer carcinoma increase between smoking versus non-smoking patients.

Survival in women with ovarian cancer with and without microsatellite instability
The survival of patients with ovarian cancer does not change between two groups with or without microsatellite instability.

p16INK4a as an adjunct test in cervical cytology
L. Vitković, Z. Perisic, M. Zamurović, N. Mitić, V. Piperski, G. Trajković, M. Cvejić, M. Perisic - Belgrade, Serbia
Correlation between cervical cytology, expression of p16INK4A, and HPV infection is analyzed to improve prediction and prognosis of cervical diseases.

Protein kinase D1 inhibits breast cancer cell invasion via regulating matrix metalloproteinase expression
X.J. Qin, Z.G. Gao, J.L. Huan, X.F. Pan, L. Zhu - Shanghai, China
The protein kinase D1 expression in malignant breast cancer decreases significantly.

Ki-67 antigen expression in the mammary epithelium of female rats in persistent estrus treated with raloxifene
Raloxifene treatment significantly reduced Ki-67 expression in the mammary epithelium of rats in persistent oestrus.

How to improve the preoperative staging of presumed early-stage endometrioid endometrial cancer?
G. Bleu, E. Arsène, B. Merlot, O. Kerdraon, J. Bigot, L. Boulanger, B. Dedet, D. Vinatier, P. Collinet - Lille, France
Hysteroscopy-curettage combined with MRI may improve preoperative staging of early-stage endometrial cancer, especially for presumed intermediate-risk disease.

General/epidural anesthesia in combination preserves NK cell activity and affects cytokine response in cervical carcinoma patients undergoing radical resection: a cohort prospective study
J.M. Li, J.L. Shao, W.J. Zeng, R.B. Liang - Kunming, China
Combined general/epidural anesthesia seems to be helpful to maintain the body's perioperative immune function compared to general anesthesia alone in cervical carcinoma patients receiving operation.

Placental site trophoblastic tumor: report of a tertiary center experience
O. Kuru, C. Cetin, C. İyibozkurt, E. Yavuz - Istanbul, Turkey
The diagnostic and therapeutic approaches of placental site trophoblastic tumor are discussed.

Total laparoscopic radical hysterectomy: a change in practice for the management of early stage cervical cancer in a U.K. cancer center
G. Angelopoulos, A. Etman, D.J. Cruickshank, J.P. Twigg - Middlesbrough, United Kingdom
The safety, surgical, and oncological outcomes of total laparoscopic radical hysterectomy in early cervical cancer were evaluated.

Decreased microRNA-206 and its function in cervical cancer
S. Ling, M. Ruiqin, Z. Guohong, S. Bing, C. Yanshan - Shantou, China
The expression patterns and clinical implications of miR-206 in cervical cancer are examined.

The 16, 18, and 45 HPV infection in high grade squamous cervical lesions in primary hr-HPV test screening program
The predictive value of the identification of HPV was investigated.
BEP for high-risk gestational trophoblastic tumor: results from a cohort of 45 patients
For young high-risk gestational trophoblastic disease patients, BEP may represent a safe and effective regimen.

Identification of cervical cancer markers using cDNA subtraction approach
Y. Liu, S.H. Man, X. Liu, X.Y. Ding, W.L. Xiao - Shandong, CHINA
The opportunity of finding effective cervical cancer markers for accurate detection of the disease can be realized using cDNA subtraction.

CASE REPORTS

Safety of converting a radical vaginal trachelectomy to a radical hysterectomy during pregnancy
W.A.A. Tjalma - Antwerp, BELGIUM
A radical vaginal trachelectomy is feasible in the first and second trimesters of pregnancy.

Diagnostic usefulness of FDG-PET/CT in advanced malignant lymphoma of the uterus: report of two cases
T. Okuda, S. Ijichi, S. Yamashita, T. Yoshioka, H. Nishigaki, J. Kitawaki - Kyoto, JAPAN
The experience with PET/CT in malignant lymphoma of the female genitalia suggests that it may not be as rare as previously reported.

Ovarian fibrosarcoma: case report and latest trends in diagnostic and therapeutic management
F. Grauso, E.M. Messalli, M.E. Salzillo, L. Di Martino, F. Falcone, P. Orabona, A. Caiola, G. Balbi - Caserta, ITALY
The management of a case of ovarian fibrosarcoma together with the latest experiences reported in the literature are described.

Postradiation carcinosarcoma of the corpus uteri – a case report
A. Zwierzchowska, G. Panek, M. Gajewska - Warsaw, POLAND
Radiotherapy implications, treatment, and outcome of a case of uterine carcinosarcoma are discussed.

Is gastrointestinal stromal tumor (GIST) originating from the rectovaginal septum GIST or extra-GIST (EGIST)? A case report with literature review
Y.H. Lee, G.O. Chong, D.G. Hong - Daegu, REPUBLIC OF KOREA
The exact origin of gastrointestinal tumor, diagnosed in rectovaginal septum, is important in the choice of management.

Primary melanoma of the vagina: a case report and review of literature
A. Stefanović, J. Jeremić, K. Jeremić, I. Likić, M. Mitrović, J. Stojnić - Belgrade, SERBIA
The management of a rare case of primary vaginal melanoma was reported.
Decreased expression of SIRT6 promotes tumor cell growth correlates closely with poor prognosis of ovarian cancer

G. Zhang¹, Z. Liu¹, S. Qin², K. Li²,³

¹ Department of Gynaecology and Obstetrics, Affiliated Qianfoshan Hospital of Shandong University, Jinan city
² Institute of Materia Media, Shandong Academy of Medical Sciences, Jinan city
³ Department of Gynaecology and Obstetrics, Affiliated Hospital of Shandong Academy of Medical Sciences, Jinan city (China)

Summary

Introduction: Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Sirtunins belong to a protein family and it is present in all organisms. SIRT6 is downregulated in tumor and acts as tumor suppressor. These sirtunin proteins are linked to repair DNA and metabolism. Material and Methods: To measure the role of SIRT6 in tumor cell, 20 mice were used and European Collection of Cell Cultures (ECACC) cell lines were used for the analysis. A histopathological technique showed the level of tumor cells. Results: A recent study provided exceptional insight into the mechanism of SIRT6-related chromatin regulation. According to the histopathology of cancer, SIRT1 localizes to the promoters of several aberrantly silenced tumor suppressor genes whose DNA is hypermethylated. SIRT1 has a role associated with the epigenetic hallmarks of cancer. Conclusion: The link between SIRT6 and cancer provide new insight into the therapeutic potential of small molecule activators or specific targets of SIRT6 for the prevention and treatment of cancer. Further investigation into the specific mechanism of SIRT6 is required to realize this potential.

Key words: Ovarian cancer; SIRT6 expression; Sirtunins.

Introduction

Cancer cells are characterized by the attainment of several characteristics that enable them to become tumorigenic [1]. Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is initiated by both external factors (tobacco, infectious organisms, chemicals, and radiation) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). These contributory factors may act collectively or in sequence to initiate or promote carcinogenesis [2].

Sirtunins belong to a protein family and it is present in all organisms. Acetylated lysines of various peptides and proteins are targeted by the sirtuin [3]. Sirtunins play important role in cellular stress and ageing and disease like Alzheimer’s disease [4], Parkinson’s disease [5], and cancer [6] also related to sirtuin. They have two binding sites i.e. NAD⁺ binding site and catalytic binding site [7]. Through the NAD⁺ dependent deacetylation reaction, cellular energy is produced. They also play a part in health promotion of several species [8]. It is activated through stress, caloric restriction, and pharmacological agents [9]. Metabolic state of cell and sirtuin are directly correlated with each other [10]. Deacetylation begins with the breakdown of amide from the NAD⁺ and formed nicotinamide and covalent ADP-ribose peptide imidate intermediate (ADPR) and it is NAD⁺ dependent reaction. There are seven classes of sirtuin present in humans. Each class has its own function, characteristic, and localization [11]. SIRT1, 6, and 7 are present in the nucleus, while SIRT3, 4, and 5 are present in the mitochondria [12]. SIRT2 is found in the cytoplasm [13].

Nuclear localization signals (NLS) are also present in sirtuin. SIRT6 and SIRT7 have a single nuclear localization signal while SIRT1 has two nuclear localization signals. Besides nuclear localization signals, nuclear export signals are also present in sirtuin [14]. Because of their histone deacetylase activity, sirtuin is involved in the expression of gene regulation. The role of sirtuin in disease is identified by SIRT1 by emerging as tumor, either by providing tumor suppressor or tumor promoter as their function [15]. Redox regulation of mitochondria is carried out by SIRT3 in mitochondrial matrix, where SIRT5 is present, where n-terminus is cleaved. Carbamoyl phosphate synthase-1 (CPS-1) is a main target of the
SIRT5 [16]. SIRT6 has a key role in the base excision repair (BER). DNA-dependent protein kinase is stabilized directly by SIRT6 at dsDNA breaks site and DNA repair complex is formed [17]. It is also related to chromatin [18] and also present in the promoter region of NF-kB activated proteins. The deficiency of SIRT6 is related to the shortness of lifespan and increases ageing phenotypes. Maintenance of telomereres and telomeric function is accomplished by SIRT6. Due to the removal and reduction of SIRT6, telomere dysfunction and to Werner’s syndrome is also caused by the absence of SIRT6. The cells that have low or deficient SIRT6 have increased possibility to genotoxic DNA damage. In the controlling of DNA damage and NF-kB function through SIRT6 also indicate a major role in tumorigenesis [19].

Under stress conditions, survival of the cell takes place or is promoted by SIRT1 by repressing p53 dependent apoptosis. Deacetylation of p53 which is mediated by SIRT1 has been confirmed by other groups [20]. P53 mediated tumor suppression role of SIRT1 remains unknown. In case of humans, several types of cancer, prostate cancer, acute myeloid leukemia, and colon cancer is highly expressed by SIRT1 [15]. Node-positive breast cancer versus non-malignant breast tissues is related to high level of SIRT3 [21]. As well as oral squamous cell carcinoma, SIRT1 and SIRT3 might be lost in late stages of tumor progression. This also suggests that SIRT6 is downregulated in tumor and acts as tumor suppressor. These sirtuin proteins are linked to repair of DNA and metabolism. The authors investigated the role of SIRT6 in the progression of ovarian cancer in female albino rats.

**Materials and Methods**

The entire experimental work was conducted at the Shandong Academy of Medical Sciences. All experiments were performed according to the rules and regulations of authority. This study was also conducted according to the rules and regulations of authority of Shandong Academy of Medical Sciences.

For this experiment 20 albino rats of 200-250 grams were selected to study the role of SIRT6 in the progression of ovarian cancer. The rats were housed in cage and maintained in controlled temperature at 30°C during the entire experimental work in animal cages.

The sample were processed and analyzed for the estimation of role of SIRT6 in the mice. The mice were divided into two groups: one included healthy and normal mice that constituted the control group and the other included mice suffering from ovarian cancer. The authors aimed to discover that role of SIRT6 in both groups and to assess the survival rate.

Tissue was drawn and immediately placed it on ice cold normal saline. Later, two-ml 0.15M Tris HCl and two-ml phosphate buffer was added and grinded into micro tube by micropestle. The mixture was centrifuged and stored in a cold environment.

All cells were grown in a 5% CO₂, 3% O₂ incubator at 37°C in Eagle’s minimal essential medium supplemented with 15% FBS,100 units/ml penicillin, and 100 μg/ml streptomycin.

ECACC cells were transfected with 0.1 μg of HR construct linearized by NheI. Cells were maintained in media which contained one-mg/ml G418 for eight days to select colonies with integrated reporter cassettes. After colonies were formed, cells were trypsinized, reseeded, and then cultured in one plate until they reached confluence. Three separate transfections were proficient, giving rise to three independent pools. These cells lines were named and then consecutively passaged, with split every three to four days, until they reached senescence at PD71.

**Results**

The criteria used for tumor grading and to separate borderline tumors from carcinomas was based on histologic subtype. These results were based on modern criteria for histotyping ovarian carcinomas. Serous carcinomas showed a very broad spectrum of histologic appearances, that con-
Decreased expression of SIRT6 promotes tumor cell growth correlates closely with poor prognosis of ovarian cancer.

Contrast with most other primary ovarian carcinomas in which morphologic variation is considerably less. The morphologic heterogeneity of serous carcinomas is likely an expression of the genetic and heterogeneity of these tumors and suggests that some tumors currently diagnosed as serous carcinomas represent transformation or progression from other tumor type.

A recent study provided exceptional insight into the mechanism of SIRT6-related chromatin regulation. According to the histopathology of cancer, SIRT1 localizes to the promoters of several aberrantly silenced tumor suppressor genes whose DNA is hypermethylated. SIRT1 has a role associated with the epigenetic hallmarks of cancer (Figures 1 and 2). The Kaplan-Meier survival curve shows that the survival rate was slow in case of SIRT6, as shown in Figure 3. SIRT1 negatively regulates p53-dependent apoptosis by deacetylation of p53 in response to cellular damage.

Discussion

Important intracellular signal transduction pathways that are necessary for the action of some antineoplastic agents can also be affected by oxidative stress. There are two major pathways of drug-induced apoptosis following cellular damage by anti-neoplastic agents: (1) mitochondrial pathway, initiated by release of cytochrome c; and (2) CD95 death receptor pathway, initiated by CD95L binding to its death receptor [22-25].

The sirtuin genes encode an important and complex family of proteins that participate in a wide spectrum of physiological processes. In several species, caloric restriction has been shown to increase lifespan and decrease spontaneous rates of illness, such as insulin resistance, neurodegenerative disease, and cancer. According to Table 1 the significant value is 0.03 and degree of freedom is 1. These results will be analyzed on the basis of Kaplan-Meier curve. To gain additional insight into the biochemical cascade that SIRT6 initiates to drive apoptosis in cancer cells, the present authors assessed whether SIRT6-mediated killing of cancer cells was dependent on any of the classic apoptotic pathways.

Conclusion

Over the past decade, SIRT1 has been the most investigated gene involved in diverse cellular functions. The link between SIRT6 and cancer provide new insight into the therapeutic potential of small molecule activators or specific targets of SIRT6 for the prevention and treatment of cancer. Further investigation into the specific mechanism of SIRT6 is required to realize this potential.

Acknowledgement

This study is supported by Nature Science Foundation of Shandong Province (ZR2009CL027), Science-Technology Star Foundation of Jinan Municipal Science and Technology Bureau (20100118), and Medical Foundation of Shandong Academy of Medical Sciences (201023).

References


Address reprint requests to:
K. LI, M.D.
Department of Gynaecology and Obstetrics
Affiliated Hospital of Shandong Academy of Medical Sciences
38 Wayingshan Road, Jinan city
Shandong Province, 250031 (China)
e-mail: kunli3448@gmail.com
Surgical Stage I high-grade ovarian cancer: is adjuvant chemotherapy warranted?

Y. Segev1, N. Ismiil2, R. McVey1, A. Covens1

Departments of Obstetrics and Gynecology, Division of Gynecologic Oncology1, and Pathology2, University of Toronto, Sunnybrook Hospital and Health Sciences Center, Toronto, Ontario (Canada)

Summary

Objective: To review the results of patients with high-grade Stage I ovarian cancer managed without adjuvant treatment. Materials and Methods: A retrospective chart review identified patients with newly diagnosed Stage I high-grade ovarian cancer, who underwent comprehensive surgical staging. Results: Thirty-three patients with FIGO surgical Stage I high-grade ovarian cancer were identified. After a median follow-up of 40 months, nine patients (27%) recurred. The median time to recurrence was 19 months. Of the nine patients with recurrences, four (44%) are alive with disease, three (33%) patients have no evidence of disease, and two have died of disease (22%). The two- and five-year overall survival is 100% and 90%, respectively. Conclusions: It would appear the recurrence rates of Stage I high risk epithelial ovarian cancer completely staged, without adjuvant treatment are comparable to those of treatment arms reported in the literature. A proportion of these patients can be salvaged at recurrence, yielding a high overall survival.

Key words: Ovarian cancer; Surgical staging; Adjuvant chemotherapy; High grade; Early stage.

Introduction

Ovarian cancer is a common gynecologic malignancy. Approximately 20% of patients with ovarian cancer are diagnosed with Stage I. For those diagnosed with epithelial ovarian cancer (EOC) confined to the ovary (IA or IB) and well-differentiated (grade 1) tumors, prognosis is excellent with survival of at least 90% following surgery alone [1]. Those with Stage IC, high grade or clear cell histology have a 30% risk of developing recurrent disease after surgery [2, 3].

In 2003, two large randomized clinical trials- The European Organisation for Research and Treatment of Cancer-Adjuvant ChemoTherapy in Ovarian Neoplasm Trial (EORTC-ACTION Trial) [4] and the International Collaborative Ovarian Neoplasm Trial 1 (ICON1) were published [5]. For analysis purposes, the two studies were combined as neither was able to reach the planned accrual. Both studies included Stage I and II patients, and a significant proportion of the patients were either mucinous or grade 1 tumors. No central pathologic review was done, and the extent of surgical staging was variable. While there was an overall survival benefit with the use of adjuvant chemotherapy, it was confined to those patients with incomplete surgical staging. Adjuvant chemotherapy provided no survival benefit in completely surgically staged patients.

Additional randomized studies, predominantly conducted by the Gynecology Oncology Group (GOG) without an observation only arm, have consistently demonstrated a 20-30% relapse rate [6, 7]. Importantly, in patients that have been treated with adjuvant chemotherapy, recurrence is uncommonly translated into long-term survival [6, 7].

This high recurrence rate in Stage I high-grade EOC has led many clinicians to recommend adjuvant treatment, despite the absence of level I evidence in completely surgically staged patients.

Since 2004 in the present center, the authors have not administered adjuvant therapy for completely surgical Stage I high grade ovarian cancer. The purpose of this study was to review the outcomes of these patients.

Materials and Methods

Following approval from the Research Ethics Board, patients with FIGO Stage I high grade (grade 2-3) EOC diagnosed from 2004 to 2012 were identified from the tumor registry databases at the Sunnybrook Health Sciences Center. Inclusion criteria included; newly diagnosed, Stage I, grade 2 or 3, serous, clear cell, and endometroid histologies, and comprehensive surgical staging. Comprehensive surgical staging in this study was defined as total hysterectomy, bilateral salpingo-oophorectomy, omentectomy, ipsilateral (or bilateral) pelvic and para-aortic lymphadenectomy, and biopsy of any suspicious abnormalities. All surgery was performed by a formally trained gynecologist oncologist. Patients with Stage II or greater, grade 1, mucinous histology, incomplete surgical staging procedure, neoadjuvant chemotherapy, previous radiation therapy, and use of adjuvant treatment were excluded.

Abstracted data included patient demographics, clinico-pathologic features, surgical substage, co-morbidities, date and location of recurrence, subsequent therapy, and survival.
Of the 550 cases of ovarian cancer treated at the present institution during the above-mentioned period, 33 patients met the above criteria for inclusion in this study. During this time period, while not formally evaluated, approximately twice as many patients were given adjuvant therapy due to incomplete surgical staging or one physician’s preference.

The median age of the population was 56 years (range 42-81). Sixteen patients had Stage IA disease, nine Stage IB, and eight Stage IC. Fourteen patients had pure clear cell histology, seven endometroid, five serous, and seven had mixed histology either clear cell/endometroid or serous/endometroid.

The number of lymph nodes evaluated was provided in 100% of the pathology reports. The median number of nodes removed at lymphadenectomy was 18 (range 5–68). Ten patients (30%) had only unilateral lymphadenectomy with a median number of nodes removed among this group of 13.

After a median follow up of 40 months (range 7–116), nine patients (27%) have recurred. The median time to recurrence was 19 months (range 2-69). The details of these patients are listed in Table 1. All of the patients with nodal recurrences were noted superior to the cranial extent of the dissection, none within the dissected nodal bed. Of the three patients that had undergone a unilateral lymphadenectomy only at primary surgery, none recurred in the contralateral non-dissected side.

Of the nine patients with recurrence, four (44%) were alive with disease at last contact, and three (33%) have been rendered free of disease at last contact (23, 59, and 70 months post recurrence). Two patients have died of disease (14 and 28 months post recurrence). The five-year progression-free and overall survival is 70% and 90%, respectively (Figures 1, 2). Treatment for recurrence consisted of chemotherapy alone [4], chemotherapy + surgery [2], and chemotherapy + radiation therapy [2], and one patient is under observation only (patient choice).

Table 1. — Characteristics of patients with recurrence.

<table>
<thead>
<tr>
<th>Histology</th>
<th>Stage</th>
<th>Sites of recurrence</th>
<th>Time to recurrence</th>
<th>Treatment for recurrence</th>
<th>Last (months)</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell</td>
<td>IB</td>
<td>Vaginal vault</td>
<td>19</td>
<td>Carboplatin - Taxol</td>
<td>AWD*</td>
<td>35</td>
</tr>
<tr>
<td>Serous</td>
<td>IB</td>
<td>Intraperitoneal</td>
<td>13</td>
<td>Carboplatin - Taxol</td>
<td>AWD</td>
<td>20</td>
</tr>
<tr>
<td>Clear cell</td>
<td>IA</td>
<td>Intraperitoneal</td>
<td>2</td>
<td>Carboplatin - Taxol</td>
<td>NED**</td>
<td>71</td>
</tr>
<tr>
<td>Serous</td>
<td>IA</td>
<td>Left pelvic side wall - intraperitoneal</td>
<td>41</td>
<td>Surgery + carboplatin and taxol</td>
<td>NED</td>
<td>108</td>
</tr>
<tr>
<td>Clear cell</td>
<td>IA</td>
<td>Supraclavicular and mediastinal node</td>
<td>5</td>
<td>Carboplatin and taxol + radiation</td>
<td>DOD***</td>
<td>33</td>
</tr>
<tr>
<td>Clear cell</td>
<td>IC</td>
<td>Aortocaval- above the area of dissection</td>
<td>23</td>
<td>Carboplatin and taxol + radiation</td>
<td>DOD</td>
<td>37</td>
</tr>
<tr>
<td>Clear cell</td>
<td>IC</td>
<td>Pelvis-intrapitoneal</td>
<td>9</td>
<td>Carboplatin-taxol</td>
<td>NED</td>
<td>32</td>
</tr>
<tr>
<td>Clear cell</td>
<td>IA</td>
<td>Left para-aortic node above the area of dissection</td>
<td>30</td>
<td>No treatment</td>
<td>AWD</td>
<td>30</td>
</tr>
<tr>
<td>Serous</td>
<td>IB</td>
<td>Colon mucosa and abdominal wall</td>
<td>69</td>
<td>Surgery + carboplatin and taxol</td>
<td>AWD</td>
<td>90</td>
</tr>
</tbody>
</table>

*AWD: alive with disease; **NED: no evidence of disease; ***DOD: dead of disease.

Figure 1. — Recurrence free survival of the cohort.

Figure 2. — Overall survival of the cohort.

Results

Of the 550 cases of ovarian cancer treated at the present institution during the above-mentioned period, 33 patients met the above criteria for inclusion in this study. During this time period, while not formally evaluated, approximately twice as many patients were given adjuvant therapy due to incomplete surgical staging or one physician’s preference.

The median age of the population was 56 years (range 42-81). Sixteen patients had Stage IA disease, nine Stage IB, and eight Stage IC. Fourteen patients had pure clear cell histology, seven endometroid, five serous, and seven had mixed histology either clear cell/endometroid or serous/endometroid.

The number of lymph nodes evaluated was provided in 100% of the pathology reports. The median number of nodes removed at lymphadenectomy was 18 (range 5–68). Ten patients (30%) had only unilateral lymphadenectomy with a median number of nodes removed among this group of 13.

After a median follow up of 40 months (range 7–116), nine patients (27%) have recurred. The median time to recurrence was 19 months (range 2-69). The details of these patients are listed in Table 1. All of the patients with nodal recurrences were noted superior to the cranial extent of the dissection, none within the dissected nodal bed. Of the three patients that had undergone a unilateral lymphadenectomy only at primary surgery, none recurred in the contralateral non-dissected side.

Of the nine patients with recurrence, four (44%) were alive with disease at last contact, and three (33%) have been rendered free of disease at last contact (23, 59, and 70 months post recurrence). Two patients have died of disease (14 and 28 months post recurrence). The five-year progression-free and overall survival is 70% and 90%, respectively (Figures 1, 2). Treatment for recurrence consisted of chemotherapy alone [4], chemotherapy + surgery [2], and chemotherapy + radiation therapy [2], and one patient is under observation only (patient choice).

Discussion

There is no clear consensus, on what, nor if any treatment should be given to surgical Stage I EOC patients with high risk features.
Surgical Stage I high-grade ovarian cancer: is adjuvant chemotherapy warranted?

The GOG conducted the first randomized study of adjuvant chemotherapy in early stage moderately or well-differentiated ovarian cancer [8]. Eighty one patients were randomized to oral melphalan or no adjuvant therapy. After a median follow-up of more than six years, there were no statistically significant differences in the five-year disease-free survival and overall survival (94% vs 98% for the observed and treated patients, respectively, \( p = 0.43 \)) [8]. In the same study they also assessed 141 patients with poorly differentiated Stage I and II ovarian cancer and compared treatment with either a single intraperitoneal dose of \(^{32}\)P or melphalan. The recurrence rates (19% each group), and five-year overall survival were similar between groups (78% and 81%, respectively, \( p = 0.48 \)) [8].

The GOG conducted several studies comparing various regimens of adjuvant therapy in early stage EOC. While these studies tended to include grade I tumours, mucinous histology, and Stage II patients, all were associated with a recurrence rate in the order of 20-35% [6-8].

Bolis et al. conducted two randomized studies to evaluate the impact of cisplatin versus no treatment in patients with EOC Stages Ia/Ib, grades 2/3, and cisplatin versus IP chromic phosphate (\(^{32}\)P). In both studies cisplatin significantly reduced the recurrence free survival (HR 0.35; \( p = 0.028 \) and 0.39; \( p = 0.007 \), respectively). However, while both were statistically significant, no overall survival benefit from adjuvant therapy was observed (the first study: five-year overall survival was \(88\% \) and \(82\% \) (HR = 1.15; 95% CI = 0.44-2.98; \( p = 0.773 \)) for cisplatin and controls, respectively; for the second study five-year overall survival was \(81\% \) and \(79\% \) (HR = 0.72; 95% CI = 0.37-1.43; \( p = 0.354 \)) for cisplatin and \(^{32}\)P respectively [9].

Tropé et al. randomized patients with Stage I EOC (all grades, including clear cell carcinomas) to either six cycles of carboplatin or no treatment [10]. Surgical staging was not mandatory, and mucinous histology was included. No statistically significant differences in disease free [71% vs 70% in the control and treatment groups, respectively (HR = 0.98; 95% CI = 0.52-1.83)] or disease specific survival [85% vs 86% in the control and treatment groups respectively (HR = 0.94; 95%CI = 0.37-2.36)] was identified.

The EORTC-ACTION Trial included patients in Stages Ia/b, grades 2/3, Stages Ic and IIa (all grades) [4]. Approximately 18% of the patients had mucinous histology. A multivariate analysis demonstrated that histologic cell type was a statistically significant prognostic factor for overall survival. After ten years of follow-up, the multivariate analysis found no association between cancer-specific survival and histological cell type, however, surgical staging was a significant predictor for survival and recurrence. [10]. The recurrence rate for the entire study was 18% and 27% in the adjuvant chemotherapy and observation groups, respectively. Only 34% were considered to have optimal surgical staging. In the optimally staged patients, there was no difference in recurrence-free (HR = 1.14; 95% CI = 0.54-2.93, \( p = 0.7 \)) and overall survival (HR = 0.81; 95% CI = 0.32-2.05, \( p = 0.7 \)) between the observation and chemotherapy groups [4]. In the optimally staged patients, no difference in cancer specific survival between the observation and treatment groups was observed after ten years of follow-up (HR = 1.58, 95% CI = 0.61 - 4.08, \( p = 0.34 \)) [11].

The ICON 1 randomized 477 patients with early-stage EOC to receive either adjuvant chemotherapy immediately following surgery or no adjuvant chemotherapy. However, it is unclear how many of the patients were completely surgically staged, likely a minority. Moreover, 23% of the cohort had mucinous tumour histology, and 32% of the tumours were grade 1. This study showed improved overall survival (HR = 0.66, 95% CI = 0.45 -0.97; \( p = 0.03 \)) and recurrence free survival (HR = 0.65; 95% CI = 0.46 to 0.91; \( p = 0.01 \)) for patients receiving platinum-based chemotherapy [5].

The benefit of adjuvant chemotherapy in patients with early-stage EOC has been further evaluated in two meta-analyses [11, 12]. Elit et al. examined 13 trials conducted between 1965 and 2004 [12]. Only eight of these studies were performed exclusively in stage I EOC. Women with stage I EOC showed a benefit for adjuvant treatment in terms of recurrence-free survival (RR = 0.70, 95% CI = 0.58-0.86) and overall survival (RR = 0.74, 95% CI = 0.58-0.94). Five-year overall survival was improved with the use of adjuvant platinum-based therapy (HR = 0.67, 95% CI = 0.50-0.90). No subset analysis for completely staged patients was performed, although the authors mentioned the lack of surgical staging for many of the women as a limitation [12].

Winter –Roach et al. looked at five randomized trials conducted between 1990 and 2003 involving 1,277 women [13]. Adjuvant chemotherapy was associated with benefit in terms of both progression-free survival (HR = 0.67, 95% CI = 0.52-0.84) and overall survival (HR = 0.71, 95% CI = 0.53-0.93). In this meta-analysis, women who had optimal staging, did not have an improved overall survival with chemotherapy compared to those observed (HR = 1.22, 95% CI = 0.63-2.37). By comparison, for women who had sub-optimal staging, chemotherapy resulted in superior survival when compared with observation (HR = 0.63, 95% CI = 0.46-0.85). Women with high-risk tumours (both optimally and non-optimally staged) had a survival advantage with the use of adjuvant chemotherapy compared to observation (HR = 0.48, 95% CI = 0.32-0.72) Patients with low-risk tumours, (both optimally and non-optimally staged), did not benefit from chemotherapy (HR = 0.95, 95% CI = 0.54-1.66) [12].

Two studies looked at recurrence of early-stage ovarian cancer. The first one by the GOG included patients with Stage IA-IB grade 3, Stage IC and II [14]. All patients underwent complete surgical staging. In this study all patients received adjuvant chemotherapy. The five-year recurrence-free and overall survivals were 75.5% and 81.7%, respectively [14]. For patients that recurred, the median time from completion of primary chemotherapy to recurrence was 21 months, and the median survival after recurrence was 24 months.
months [15]. Kolomainen et al. evaluated recurrence among 194 patients with Stage I ovarian cancer [15]. The cohort included mucinous histology and a significant proportion of grade 1 tumors. Complete surgical staging did not include complete pelvic and para-aortic lymphadenectomy, but only nodal examination and biopsy of macroscopically abnormal nodes. The recurrence rate was 31%. All were treated with platinum–based chemotherapy. The overall survival for all 194 patients was 72% at ten years [16].

The present study is unique in that all the patients had high-grade histology (confirmed by a gynaecologic pathologist), mucinous tumors were excluded, and all patients were surgically staged. This is the first study evaluating recurrence and survival among high-grade, Stage I epithelial ovarian cancer after complete surgical staging without adjuvant therapy. As a significant proportion (33%) of patients with recurrence can be rendered disease-free with chemotherapy (+/- surgery and radiation), the differences in recurrence and survival among high-grade, Stage I epithelial ovarian cancer after complete surgical staging without adjuvant therapy is warranted.

References


Address reprint requests to:
A. COVENS, M.D., FRCS
Division of Gynecologic Oncology
Sunnybrook Health Sciences
2075 Bayview Ave., T2051
Toronto, Ontario, M4N 3M5 (Canada)
e-mail: Al.Covens@sunnybrook.ca
Introduction

Endometrial carcinoma (EC) is one of the commonest gynecological cancers worldwide. Surgical evaluation and staging have been the cornerstone of management since 1988, when the International Federation of Gynecology and Obstetrics (FIGO) system was changed from clinical to surgical staging, and FIGO modified its staging system in 2009 [1]. Generally, the surgical procedure should include an adequate vertical abdominal incision, peritoneal fluid sampling for cytological evaluation, and meticulous exploration of the whole abdominal cavity. Then proceeding to extrafascial hysterectomy with bilateral salpingo-oophorectomy (BSO), peritoneal biopsy, and pelvic and para-aortic lymphadenectomy. The newly introduced staging incorporated pathological risk factors in order to better define the extent of disease, estimate prognosis, and guide adjuvant treatment recommendations. Despite implementation of this more accurate staging system, the optimal surgical procedure for the management of EC remains controversial, particularly in clinical early-stage patients. Within Stage I disease, 3-5% of women with well differentiated tumours and superficial myometrial invasion will have lymph-node involvement. This proportion rises to roughly 20% of women with poorly differentiated tumours and deep myometrial invasion. The decision to treat depends on whether the patient has risk factors such as histologic grade, myometrial invasion, extrauterine and lymph node involvement, and the age of the patient. Several retrospective series have suggested that these factors are important in determining the recurrence and death rate [2]. Management varies widely, particularly in Stage I EC patients with different risk factors. The initial treatment of Stage I EC is usually surgery involving a total abdominal hysterectomy (TAH) and BSO [3]. However, evidence is scarce of the therapeutic benefit for lymphadenectomy in terms of survival. The present study is focused on identifying the appropriate surgical procedure for Stage I EC, by considering the impact of ovarian preservation, high-risk factors, clinical and pathological features, and prognosis.

Materials and Methods

A retrospective analysis was performed on 277 patients with Stage I EC who received surgery in X hospital from January 1, 2000 to March 31, 2008. These patients were treated primarily by surgery and certified with pathology and without pre-operative chemotherapy, radiotherapy or hormonal therapy. Staging was based retrospectively on the surgical and pathology reports and according to the FIGO EC staging [4]. On the basis of their etiologic and pathologic features, EC is classified into type I and type II [3,4]. In the study, 225 patients were EC type I (125 grade 1, 75 grade 2 and 55 grade 3) and 52 were EC type II. Table 1 outlines the distribution of clinical pathologic features for the EC groups.
Table 1. — **Four surgical groups based on the surgical pathologic stage.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Procedure I</th>
<th>Procedure II</th>
<th>Procedure III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>112</td>
<td>55 (31%)</td>
<td>36</td>
</tr>
<tr>
<td>Ib</td>
<td>165</td>
<td>32</td>
<td>59</td>
</tr>
<tr>
<td>Total</td>
<td>277</td>
<td>87</td>
<td>95</td>
</tr>
</tbody>
</table>

*Number of patients with ovarian preserved.

Table 2. — **Clinicopathologic characteristics in 277 EC patients.**

<table>
<thead>
<tr>
<th>Clinicopathologic Characteristics</th>
<th>N</th>
<th>Operative pathologic stage</th>
<th>Stage Ia</th>
<th>Stage Ib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterine basal part</td>
<td>137</td>
<td>59</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Uterine cavity or inferior segment</td>
<td>140</td>
<td>53</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Tumor diameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 4cm</td>
<td>142</td>
<td>67</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>&gt; 4cm</td>
<td>135</td>
<td>49</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Depth of invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrium only</td>
<td>70</td>
<td>70</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Inner half of myometrium</td>
<td>127</td>
<td>127</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Deep myometrial invasion</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Serous membrane invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>233</td>
<td>84</td>
<td>149</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>44</td>
<td>0</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Cervical invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>254</td>
<td>112</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>0</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Differentiation or grade adenocarcinomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>110</td>
<td>49</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>65</td>
<td>24</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>45</td>
<td>16</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Undifferentiation</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Non-adenocarcinomas</td>
<td>42</td>
<td>18</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Ascites positive cytology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>245</td>
<td>97</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>12</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

Of the 277 patients included in this retrospective analysis, the surgical procedures were divided into three types according to the different therapeutics. Procedure I was TAH and BSO or unilateral ovary preserved for young patient who wanted to ovarian preservation. Procedure II was subradical hysterectomy with pelvic lymph node biopsy or sampling. Procedure III was radical hysterectomy with pelvic lymphadenectomy. Patients were divided into 4 groups according to the different pathologic stages (as shown in Table 1) in order to choose the most appropriate surgical procedure. Patients were stratified on the basis of whether bilateral salpingo-oophorectomy (BSO) was performed (BSO group) or whether the ovaries were retained (Ovary preserved group).

Patients with clinical stage I EC were surgically staged and were stratified into three risk categories. In the high-risk patients (stage Ib, high tumor grade, deep myometrial invasion, cervical involvement, pelvic and para-aortic lymph node involvement and serous or clear cell histology), pelvic external beam radiotherapy and/or vaginal brachytherapy and chemotherapy as well as hormone treatment can be given as an adjuvant treatment to reduce the risk of recurrence. The different operative pathologic stages between falling ill, high-risk factors, pathologic type, grade and ovaries or extraterine as well as lymph node invasion and prognosis were analyzed.

Data are expressed as the median and range. All statistical tests were two-tailed. Survival was analyzed by the log-rank test and Cox multivariable regression analysis. Cox proportional hazards models were fit with potential factors associated with falling ill, high-risk factors, pathologic type, grade, ovarian or parametrium invasive, as well as lymph node metastasis and prognosis. Kaplan-Meier curves were generated to examine overall survival based on whether a BSO was performed. A \( p < 0.05 \) was set as the level of statistically significant difference. All statistical analysis was done with SPSS 11.3 (Statistical Package for Social Science).

**Results**

From 1 January 1, 2000 to March 31, 2008, 277 patients were diagnosed with endometrioid uterine cancer and underwent a surgical staging procedure including lymph node assessment and stage assignment based on the 1988 FIGO staging system [1]. Because FIGO modified its staging system in 2009, the authors retrospectively analyzed and staged their patients under the new version. The clinical pathologic characteristics of the study population are given in Table 2. With informed consent, confirmation of the absence of known risk factors, and intraoperative evidence suggesting the absence of advanced disease, BSO with or without lymph node dissection, and preserved ovaries were performed. Factors of ovaries, extraterine involvement, lymphovascular space invasion, and lymph node metastases involvement analysis are given in Tables 3 and 4.

The survival of the patients whose ovarian preserved versus those who underwent BSO were compared. Of the 277 patients, 246 (88.9%) underwent BSO, and the remaining 31 (11.2%) had their ovaries preserved. A Kaplan-Meier analysis revealed no difference in overall survival between the two groups (\( p > 0.05 \)) (Figure 1). Ovarian preservation had no significant influence on disease-free survival and metastatic tumor in patients with grade I EC (\( p > 0.05 \)).

Data on chemotherapy, tumor invasion of the cervix and deep muscularis, as well as extraterine involvement, EC Stage Ib grade 3, and ascites had carcinoma cells, and a raised Ca 125 blood test (\( p < 0.05 \)) were high-risk factors of EC metastasis to the parametrium and ovary. Cervical involvement and deep myometrial invasion as well as parametrium, EC Stage Ib grade 3, and ascites had carcinoma cells, and were high-risk factors of EC metastasis to the retroperitoneum (\( p < 0.05 \)). Estrogen and progesterone (ER/PR) positivity was not significantly correlated to the retroperitoneum and lymph node metastasis (\( p > 0.05 \)). There was no statistically significant difference in overall survival between Stage Ia patients with BSO or unilateral salpingo-oophorectomy (\( p < 0.05 \)).

The three-year and five-year survival rates of EC Stage Ia were 98.21% and 93.46%, respectively. The operation procedure I (TAH and BSO or unilateral salpingo-
Which is the appropriate surgical procedure for Stage I endometrial carcinoma?

The three- and five-year survival rates of EC Stage Ib were 97.74% and 93.26%, respectively. The survival rates of surgical procedures II and III were significantly higher than that of procedure I \((p < 0.05)\). The survival rates of the surgical procedures II and III were not significantly correlated. The three- and five-year survival rates of the surgical procedures I, II, and III were significantly correlated \((p < 0.05)\).

The three-year survival rate was not significantly correlated to postoperative chemotherapy, radiotherapy or hormone treatment, but the five-year survival rate was significantly higher than that without postoperative chemotherapy and radiotherapy or without hormone treatment \((p < 0.05)\).

**Discussion**

EC is the commonest gynecologic malignancy worldwide and more than 40,000 new cases are diagnosed each year [5]. Because vaginal bleeding is commonly associated with the presence of disease, more than 75% of patients with EC are diagnosed at an early stage, resulting in overall favorable prognosis, with a five-year overall survival rate of 80-85% and a cancer-specific survival rate of 90-95% [6]. Traditional management of women with Stage I EC has been surgery, typically combined with adjuvant radiotherapy for women whose pathological features suggest an increased risk of nodal metastases. The cornerstone of curative therapy for patients with EC is surgical treatment, including complete hysterectomy, removal of remaining adnexal structures, and appropriate surgical staging in patients considered at risk for extraterine disease [7]. Treatment of EC has a generally favorable outcome when patients present in the early-stage of the disease. The need for a radical and complete surgical staging procedure is clinically important but has been poorly studied. On the basis of retrospective findings, the present study was focused on patients with Stage I EC as defined by the high-risk factors, clinical and pathologic features, and prognosis factors in the surgical specimen combined with respective adjuvant assessment. The present objective was to identify appropriate surgical procedure and adjuvant treatment for Stage I EC.

Since FIGO introduced surgical staging of EC in 1988, various questions have remained unanswered [8]. One of the potential challenges for defining the most effective treatment of EC arises from inconsistency in the surgical staging. The staging system includes tumor grade, depth of myometrial invasion, occult extension to the cervix, adnexal invasion, and lymphovascular space involvement. The present study aimed to identify factors that could predict lymphovascular space involvement and identify appropriate surgical procedure for Stage I EC.

**Table 3. — Factors of ovaries, parametrium and lymphovascular space invasion analysis**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Ovarian metastases (n=8)</th>
<th>Multivariable analysis</th>
<th>Parametrium metastases or lymphovascular space invasion (n=11)</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 45 years old</td>
<td>80</td>
<td>2 (2.50)</td>
<td>2 (2.50)</td>
<td></td>
</tr>
<tr>
<td>&gt; 45 years old</td>
<td>197</td>
<td>6 (3.05)</td>
<td>9 (4.57)</td>
<td>0.29 0.59</td>
</tr>
<tr>
<td>Lesion region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterine basal part</td>
<td>137</td>
<td>3 (2.19)</td>
<td>6 (4.38)</td>
<td></td>
</tr>
<tr>
<td>Uterine cavity or inferior segment</td>
<td>140</td>
<td>5 (3.57)</td>
<td>5 (3.57)</td>
<td>0.18 0.67</td>
</tr>
<tr>
<td>Tumor diameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 4cm</td>
<td>142</td>
<td>3 (2.11)</td>
<td>3 (2.12)</td>
<td></td>
</tr>
<tr>
<td>&gt; 4cm</td>
<td>135</td>
<td>5 (3.70)</td>
<td>8 (5.93)</td>
<td>1.60 0.21</td>
</tr>
<tr>
<td>Depth of myometrial invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>70</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>≤ 1/2</td>
<td>127</td>
<td>1 (0.78)</td>
<td>1 (0.78)</td>
<td>12.32 0.02</td>
</tr>
<tr>
<td>&gt; 1/2</td>
<td>80</td>
<td>7 (8.75)</td>
<td>10 (12.50)</td>
<td></td>
</tr>
<tr>
<td>Serous membrane invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>233</td>
<td>2 (0.86)</td>
<td>2 (2.45)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>44</td>
<td>6 (13.64)</td>
<td>9 (20.45)</td>
<td>12.22 0.01</td>
</tr>
<tr>
<td>Cervical invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>205</td>
<td>1 (0.49)</td>
<td>5 (2.44)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>72</td>
<td>7 (9.72)</td>
<td>6 (8.33)</td>
<td>2.71 0.10</td>
</tr>
<tr>
<td>Histopathological types</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrioid adenocarcinomas</td>
<td>225</td>
<td>6 (2.67)</td>
<td>8 (3.56)</td>
<td></td>
</tr>
<tr>
<td>Non-endometrioid histology</td>
<td>42</td>
<td>2 (4.76)</td>
<td>3 (7.14)</td>
<td>6.64 0.01</td>
</tr>
<tr>
<td>Differentiation grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>8</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>122</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>80</td>
<td>3 (3.75)</td>
<td>1 (12.5)</td>
<td>11.09 0.01</td>
</tr>
<tr>
<td>G3</td>
<td>55</td>
<td>3 (5.45)</td>
<td>7 (12.7)</td>
<td></td>
</tr>
<tr>
<td>Undifferentiation</td>
<td>12</td>
<td>1 (8.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascites positive cytology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>245</td>
<td>3 (12.2)</td>
<td>4 (16.3)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>5 (15.6)</td>
<td>7 (21.8)</td>
<td>13.38 0.01</td>
</tr>
<tr>
<td>Serum CA125 level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>92</td>
<td>4 (4.34)</td>
<td>3 (3.26)</td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>185</td>
<td>4 (2.16)</td>
<td>8 (4.32)</td>
<td>3.42 0.06</td>
</tr>
</tbody>
</table>
nexal involvement, peritoneal cytology, pelvic and periaortic lymph node involvement, and vaginal, inguinal or distant metastases. In particular, controversy has been focused on FIGO Stage I endometroid adenocarcinoma with different grades. Currently, the standard of care for patients with no contraindication to surgical intervention is TAH and BSO, pelvic and periaortic lymph node sampling or lymphadenectomy [9]. Patients and physicians are confronted with the dilemma of whether to follow the standard surgical guidelines or to accommodate the desire of the patients to avoid surgical menopause. Careful patient selection and surgical competence are instrumental in ensuring successful treatment. The concomitant use of surgical staging, hysterectomy, and resection of adnexal structures is currently recommended for most patients with endometrial malignancies [4, 5]. However, the extent of dissection necessary for adequate staging has not been standardized. On the basis of retrospective study of 277 patients, it is suggested that the surgical extent was determined by the preoperative evaluation of lesion region, depth of myometrial invasion, and presence of cervical invasion. When imaging is necessary for medically inoperable patients, magnetic resonance imaging (MRI) of the pelvis is superior to computed tomography (CT) for visualizing the uterus and surrounding tissues [10, 11]. Baseline cancer antigen levels can be useful for predicting extrauterine spread but are not sufficiently sensitive to replace surgical staging. In this study, the authors found that hysterectomy was appropriate for Stage Ia patients. To avoid the short- and long-term consequences of surgical menopause, there is a strong rationale for ovarian preservation in young women. The major risk factor of preserving the ovaries in young women with early-stage endometrial cancer is the risk of coexisting adnexal malignancy. In women with early-stage cervical cancers, the incidence of ovarian metastasis has been reported to be < 1% by several large-scale studies [12]. In the present study, ovarian preservation has not been shown to increase the risk of recurrence. There was no significant difference of overall survival whether the ovary was preserved or not.

For Stage Ib cases, however, univariate analysis demonstrated that radical hysterectomy or subradical hysterectomy was necessary according to the high-risk factors. Subradical hysterectomy with pelvic and para-aortic node sampling should be used for EC Stage Ib patients. On the basis of this study, the risk factors that were predictive for distant recurrence were cervical involvement, deep myometrial invasion, tumor diameter > two cm, serous membrane invasion, ascites positivity, and high blood CA125 level.

Lee et al. reviewed 272 patients with a mean age of 51.8 years. They identified a non-endometrioid histologic subtype, intraoperative extrauterine disease, lymph node metastases, and age as independent risk factors for adnexal metastases in women with early-stage and grade of endometrial carcinoma [13]. They also concluded that after

<table>
<thead>
<tr>
<th>Predictor</th>
<th>N</th>
<th>N Positive rate (%)</th>
<th></th>
<th></th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 45 years</td>
<td>80</td>
<td>3</td>
<td>3.75</td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Age &gt; 45 years</td>
<td>197</td>
<td>12</td>
<td>6.09</td>
<td></td>
<td>0.37</td>
</tr>
<tr>
<td>Lesion region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterine basal part</td>
<td>137</td>
<td>12</td>
<td>8.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterine cavity or inferior segment</td>
<td>140</td>
<td>9</td>
<td>6.43</td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td>Depth of myometrial invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>70</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1/2</td>
<td>127</td>
<td>8</td>
<td>7.87</td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td>&gt; 1/2</td>
<td>80</td>
<td>13</td>
<td>16.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical invasion</td>
<td>No</td>
<td>205</td>
<td>7</td>
<td>3.41</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>72</td>
<td>13</td>
<td>18.05</td>
<td></td>
<td>1.29</td>
</tr>
<tr>
<td>Histopathological types</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrioid adenocarcinomas</td>
<td>115</td>
<td>8</td>
<td>6.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-endometrioid histology</td>
<td>42</td>
<td>11</td>
<td>26.19</td>
<td></td>
<td>1.13</td>
</tr>
<tr>
<td>Differentiation grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>122</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>80</td>
<td>3</td>
<td>3.75</td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td>G3</td>
<td>55</td>
<td>10</td>
<td>18.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undifferentiation</td>
<td>12</td>
<td>1</td>
<td>8.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphovascular space involvement</td>
<td>No</td>
<td>258</td>
<td>15</td>
<td>5.82</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19</td>
<td>11</td>
<td>57.89</td>
<td></td>
<td>24.25</td>
</tr>
<tr>
<td>Acites positive cytology</td>
<td>Negative</td>
<td>245</td>
<td>13</td>
<td>5.31</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>12</td>
<td>37.50</td>
<td></td>
<td>7.57</td>
</tr>
<tr>
<td>ER</td>
<td>Positive</td>
<td>105</td>
<td>8</td>
<td>7.62</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>172</td>
<td>9</td>
<td>5.23</td>
<td></td>
<td>0.56</td>
</tr>
<tr>
<td>PR</td>
<td>Positive</td>
<td>97</td>
<td>4</td>
<td>4.12</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>180</td>
<td>13</td>
<td>7.22</td>
<td></td>
<td>3.05</td>
</tr>
</tbody>
</table>

Figure 1. — Kaplan-Meier analysis of overall survival of patients with early-stage endometrial cancer stratified by BSO or preservation of the ovaries.
extensive preoperative and intraoperative evaluation, and in the absence of risk factors, ovarian preservation might be an option in early-stage EC. Bilateral oophorectomy in pre-menopausal women causes significant adverse long-term effects in bone, heart, and neurologic health as well as in quality of life [14]. In the present study, the ovaries were not removed from any young EC Stage I patient who wanted to ovarian preservation and metastatic tumor was not discovered in ovaries at follow-up.

Several authors concluded in their study that lymphadenectomy can be omitted in patient without risk factors such as grade 3 cancer, deep myometrial invasion, age > 60 years, and clear cell or papillary serous histology [15, 16]. In the setting of significant morbidity related to premature menopause secondary to the BSO, and the effects of lymphadenectomy, hysterectomy with ovarian preservation seems to lead to better disease-free survival in young endometrial cancer patients, especially with Stage Ia EC as in the present study. Subradical hysterectomy with or without pelvic and para-aortic biopsy was used for endometrial carcinoma Stage Ib EC.

Numerous studies have demonstrated that cell type, histologic grade, depth of myometrial invasion, cervical involvement, and lymphovascular involvement can predict recurrence and survival in patients with EC [17]. A number of recent retrospective studies have suggested that Stage I EC patients with negative lymph nodes after systematic surgical staging could have been treated with vaginal brachytherapy alone; historically, these women received adjuvant pelvic radiotherapy [18, 19]. Adjuvant therapy is necessary for a patient with high-risk factors that include high tumor grade, deep myometrial invasion, cervical extension, and serous or clear cell histology consists of vaginal brachytherapy, teletherapy, systemic chemotherapy or some combination thereof [20,21].

Survival is heavily dependent on surgical stage, which is determined at present using the classification system adopted by FIGO in 2009 [22]. Patients commonly present with postmenopausal bleeding and those with early-stage EC generally have an excellent prognosis. The five-year relative survival rate for Stage I disease is 97.4%. Without adjuvant chemotherapy or vaginal brachytherapy, the recurrence rate is 23% in patients with stage I disease [23]. The three- and five-year survival rates of operation procedures II and III were significantly higher than those of procedure I ($p < 0.05$). The three- and five-year survival rates of procedure III were significantly higher than those of procedures I and II. The extent of dissection might improve the chance of survival by removing micrometastatic disease and decreasing recurrence and metastases. Positron emission tomography (PET)/CT, MRI, and dilation and curettage preoperative studies are not accurate methods for the evaluation of lymph nodes. Likewise, intraoperative assessments such as lymph node palpation and examination of frozen sections have been shown to be inadequate. It appears that comprehensive surgical staging allows the surgeon to identify high-risk EC patients who would benefit from adjuvant therapy.

Treatment options for EC differ according to the disease status and vary from a primary surgical treatment to a combination of surgery and adjuvant radiotherapy or chemotherapy [24]. External pelvic radiotherapy and/or vaginal brachytherapy should be used postoperatively for patients with tumor characteristics that predict a high risk of local recurrence and a poor prognosis [25].

In conclusion, ovarian preservation in young Stage I EC patients may be safe and not associated with an increased risk of mortality. Continuous follow-up would be necessary for patients with preserved ovaries after hysterectomy. Subradical hysterectomy should be approached to Stage Ib EC patients, and subradical hysterectomy with pelvic biopsy should be performed to those patients with high-risk factors. There is no evidence of benefit in terms of overall or recurrence-free survival for radical hysterectomy plus pelvic lymphadenectomy in women with Stage I EC.

Acknowledgements

The project was supported by grants from the Medical Science and Technology Project of Shandong (2011HZ097), the Natural Science Foundation of Shandong (ZR2012HM010), and partly by Shandong Cancer Hospital, Shandong Academy of Medical Sciences, Shandong University, Jinan, China.

References


Address reprint requests to:
X.G. SHENG, Ph.D, M.D.
Department of Gynecologic Oncology
ShanDong Cancer Hospital & Cancer Institute
Shandong University
440# Ji Yan Road, Jinan 250117 (China)
e-mail: guishengxiu@163.com
Intraoperative subserosal approach to label sentinel nodes in intermediate and high-risk endometrial cancer

P. Valha¹,², E. Kucera¹, P. Sak², O. Stepanek², M. Michal²
¹ 3rd Medical Faculty Charles University, Prague; ² Hospital Ceske, Budejovice a.s. (Czech Republic)

Summary

Design: prospective experimental study. Purpose of investigation: The purpose of this study was to evaluate feasibility and reliability of in vivo sentinel lymph node (SLN) mapping in patients with endometrial cancer and to verify a modified method of application of subserosal blue dye. Detection substance was applied subserosally in the uterine edges vicinity the round ligament of uterus and uterine vessels in the isthmic portion of the uterus. Materials and Methods: Eighteen patients with intermediate and high-risk endometrial cancer Stages I-II were subjected to staging laparotomy with intraoperative detection of SLNs and subsequent completion of the pelvic and para-aortic lymphadenectomies. Harvested SLN was routinely examined by classical haematoxylin eosin staining and in case of negativity, immunohistochemistry with anti-keratin antibodies AE1/AE3 was applied. Results: Total of 773 lymph nodes were removed in 18 patients: pelvic 420 (54%) and para-aortic 353 (46%). SLNs were detected in 16 of 18 patients totalling 59 nodes (7.6% of all nodes). Forty-eight were identified in the pelvic area (81%) and 11 nodes (19%) in the para-aortic area. Three metastatic SLNs were found in two patients (11%). No false negative nodes were demonstrated. Conclusion: Experimental study results indicate that the proposed modified approach to label SLNs is applicable. The presented modified approach brings the highest added value namely in women with a myomatous uterus and scars from previous surgical procedures on the uterus.

Key words: Endometrial cancer; Sentinel node; Blue dye; Lymphadenectomy; Staging; Laparotomy.
cal data were obtained in all patients and all patients underwent experimental modelling in the detection of Slants. The patients were operated on by two surgeons. Patients underwent low mid-line incision with extension above navel under general anesthesia. After exploration of the abdominal cavity, lavage was performed by default. The uterus was fixed neither by fundus nor by the uterine edges and it was only supported by two fingers during the application of the detection substance (Figure 1). Four ml of Patent Blau (2.5%) was used as a detection agent. It was split into two syringes - one per each half of the uterus. Subcutaneous needle was used for substernal application. Application location was subserosally in the uterine edges from dorsal side of uterus body in the level of ligamentum ovarii proprium and uterine vascular bundles in the isthmic part of the uterus. Injection depth was approximately one mm and the substance was inserted gradually. The location was spot coagulated immediately after the injection to prevent the leakage of detection substance and subsequent contamination of the surgical field. Same application scheme was used for the second half of the uterus. Only four punctures to the uterus were applied. A ten-minute delay aimed to allow for sufficient uptake of lymphotropic agents followed. The procedure then continued by the dissection of pelvic peritoneum to inspect retroperitoneal spaces like pararectal, paravesical, and obturator fossa. Subsequently the retroperitoneum in the radix of mesentery was digested to visualize para-aortic space. Blue node was removed and identified as sentinel describing the anatomical area of location and laterality. Standard extrafascial hysterectomy with bilateral adnexectomy and systematic pelvic and para-aortal lymphadenectomies to the level of the renal veins was completed.

Histological processing

A pathologist evaluated SLNs at first with haematoxilin eosin staining and then all negative sentinel nodes were processed by sentinel node ultra-section technique. Six sections in one node at 200 μm intervals were performed. An additional cut was ammended between the third and fourth section upon which immunohistochemistry examinations were applied; it was namely a mouse monoclonal antibody anti AE 1/AE 3 cytokeratine. Non SLNs were stained only by haematoxilin eosin.

Results

Altogether 18 patients (mean age 66 ± 7.68 years (range 52 - 77) with intermediate and high-grade endometrial cancer Stage I-II underwent surgical intervention ranging hysterectomy, bilateral adnexectomy, detection of SLNs and subsequent complete pelvic and para-aortic lymphadenectomy during the period from June 2012 through February 2014. Average BMI 32.5 ± 5.4 (min 23 - max 44). Total of 773 lymph nodes were removed, out of which 420 were pelvic lymph nodes (54%) and 353 (46%) were para-aortic lymph nodes. The average harvest of the pelvic lymph nodes was 28.4 ± (min 15 - max 34) and para-aortic lymph nodes was 24.5 (min 11 - max 28) (Table 2).

SLNs were detected in 16 of 18 patients (detection rate 88%). In total there were 48 pelvic SLNs. Eleven para-aortic SLNs were removed in nine patients (detection rate 50%), two para-aortic nodes were detected in one patient. Para-aortic SLNs were always detected in combination with pelvic and para-aortic lymphadenectomy; no isolated para-aortic SLNs were found. Three metastatic involvements of lymph nodes, two pelvic, and one para-aortic, were found in two patients (11.1%). Metastases to one pelvic and one para-aortic SLNs were found in one patient. Metastatic lymph nodes were confirmed by classical staining haematoxilin eosin and all negative sentinel nodes also proven by immunohistochemi-
Intraoperative subserosal approach to label sentinel nodes in intermediate and high-risk endometrial cancer

Intraoperative subserosal approach to label sentinel nodes in intermediate and high-risk endometrial cancer

Discussion

Despite the fact that SLN mapping in endometrial cancer recently underwent intensive development, each of the currently used methods has some limitations moreover patient sample set was small and therefore a question of SLN detection optimisation approach still remains. SLN labelling approach techniques in endometrial cancer according to recent publications are: hysteroscopic application [8-13], subserosal application to the uterine body [14-18], cervical application [19-25], and combination of subserosal and cervical applications.

The authors present their data on a modified method of subserosal application of blue dye in 18 women who underwent surgery for intermediate and high-risk patients. SLNs were detected in 16 of 18 patients (detection rate 88%). Para-aortic SLNs were detected always together with pelvic nodes and detection rate for sentinel para-aortic lymph nodes was 50%. After SLN detection, complete pelvic and para-aortic lymphadenectomies were performed. In two patients positive lymph nodes were found (11.1%).

SLN identification with subserosal injection into the uterine body had been researched in six studies. The detection rate ranged from 45-92%.

Burke et al. in 1996 first presented SLN detection protocol with subserosal blue dye application in endometrial cancer [14]. In 15 patients blue dye was applied subserosally in three sites in the sagittal line of the uterine body in total amount of three millilitres. Fallopian tubes were occluded. After ten minutes of dye uptake, SLNs were detected and complete pelvic and para-aortic lymphadenectomies were performed. Detection rates of SLNs were 67% and 27% lymph nodes were positive.

Lopes et al. in 2007 applied three ml of blue dye in 40 women [15]. Detection rate of SLNs was 78%. Complete surgical staging, including pelvic and para-aortic lymphadenectomy were performed.

Altgassen et al. in 25 patients used four ml of blue dye subserosally by applying eight injections into the uterine body, four from the ventral and four from the dorsal site, with minimal manipulation with uterine body and any occlusion of the fallopian tubes were performed [16]. Detection rate was 92% which is the highest achieved and 12% of positive nodes were diagnosed. Only in selected patients para-aortic lymphadenectomy was performed.

Li et al. in 20 patients administered four ml of blue dye subserosally in five sites [17]. SLNs detection rate was 75% and 10% of nodes were involved. Para-aortic lymphadenectomy was also only performed in selected patients.

Frumovitz et al. in 18 patients described combination of subserosally applied blue dye and 99mTc [18]. Injections were applied in three sites. Detection rate of SLNs was 45%. This is the lowest detection rate in the subserosal technique and the reason in unclear. Positive lymphatic nodes were not diagnosed.

Robova et al. in 67 patients also used a combination of blue dye and 99mTc [12]. Higher detection rate of 73% was achieved by using same technique as Frumovitz et al. Positive lymphatic nodes were diagnosed in 5.5%.

In the present study, the authors achieved a detection rate of 88% which is comparable with other studies. They consider a benefit that this study included only patients with intermediate and high-risk endometrial cancer and that negative sentinel nodes were examined by immunohistochemistry. After detection of sentinel nodes, pelvic and para-aortic lymphadenectomies were completed.

Four injections were administered from the dorsal side of uterus body close to the uterine edges. This technique of application eliminates the question of how deeply and where to subserosally inject detection substance at various thicknesses of the myometrium, especially in myomatous uterus, adenomyosis, previous surgical interventions on the uterus, and the adhesive process in the pelvis. In the present authors’ application scheme, the detection substance is injected from the dorsal side of the uterus, and they see an advantage in that it eliminates handling and application through vesicouterine fold in the isthmic portion of the uterus and subsequently diffuse blue stain, which reduces the clarity of the surgery field.

From the experience of the previous application of blue dye, short term coagulation at the place after application it appears to be practical, which leads to minimize backflow of detection substance and prevents contamination of the surgical field. The advantages include the simplicity of the method without the need for other associated methods like lymphoscintigraphy, hysteroscopy, and is a technique with the shortest learning curve.

The limitation of this study is that the presented method is based only on blue dye detection and small number of patients. Application with usage of another dye for example indocyanine-green or combination with 99mTc nanocolloid should increases the detection rate [22, 24, 25]. Based on the multicenters prospective studies SENTI ENDO [26] and the study Khoury Collado et al. [27] show that immunohistochemistry evaluation of the SLN and ultra-staging of the SLN may be even more sensitive than a full lymphadenectomy,
with lymph nodes evaluated by conventional pathology. However, the clinical importance of isolated tumor cells discovered in a lymph node that is negative by traditional histological analysis is still not known.

Conclusion

The present experimental study offers an alternative to the already published application schemas. It appears particularly advantageous for patients with myomatous uterus with scars after surgical procedures and during the adhesive process in the pelvis. Recent literature survey indicates that pathologist and immunohistochemical processing play a crucial role in SLNs examination.

Acknowledgments

The study was supported by the Research Project, Charles University, Prague, PRVOUK 27/LF3, with the support of the Hospital Ceske Budejovice a.s., 3rd. Medical Faculty Charles University Prague, and also with the approval of the local ethics committee.

References


Address reprint requests to:
P. VALHA, M.D.
Hospital Ceske Budejovice a.s.
3rd Medical Faculty of Charles University Prague
Dept. of Oncogynecology
B.Nemcove 585/54, 370 01 (Czech Republic)
e-mail: petrvalha@seznam.cz
HPV16 infection up-regulates Piwil2, which affects cell proliferation and invasion in cervical cancer by regulating MMP-9 via the MAPK pathway


1 Department of Gynaecology and Obstetrics, General Hospital of Lanzhou Military Region, Lanzhou
2 Division of Medical Administration, General Hospital of Lanzhou Military Region, Lanzhou
3 Department of Gynaecology and Obstetrics, Southwest Hospital of the Third Military Medical University, Chongqing (China)

Summary
Purpose of investigation: The present study aimed to investigate the effect of Piwil2 on proliferation and invasion of cervical cancer cells. Materials and Methods: Thirty-two HPV-positive or negative cervical cancer tissues and corresponding normal adjacent cervical tissues were obtained from General Hospital of Lanzhou Military Region. Piwil2 expression in these tissue samples, as well as two cervical cell lines were evaluated by quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemical. A specific short hairpin RNA (shRNA) was used to knockdown the Piwil2 gene in SiHa cells. CCK-8 assay and flow cytometry (FCM) was used to evaluate cell proliferation. Cell invasion was detected by transwell chambers assays. Immunoblotting was used to assess the effect on relevant proteins. Result: In the early stage (IA1 – IB1) of curvival, 84.4% (27/32) tumor tissues have a more predominant expression of Piwil2 than the normal adjacent samples. Piwil2 overexpression was correlated with HPV16 infection ($p < 0.05$). Knockdown of Piwil2 gene in SiHa cells inhibited cell growth and invasion, and downregulated matrix metalloproteinase-9 (MMP-9) compared to scrambled shRNA transfected cells. Further analysis revealed that downregulation of Piwil2 gene induced inhibition of the MAPK signaling pathway activity. Conclusion: Piwil2, which stimulated by HPV16 infection, plays an important role in regulating proliferation and invasion of cervical cells by regulating MMP-9 expression via alternation of the MAPK signaling pathway.

Key words: Cervical cancer; Piwil2; Cell growth; Invasion; MMP-9.

Introduction
Cervical cancer, as the third most common cancer and the fourth that leads to death in women worldwide, is the most frequent cancer of females in developing countries. There are about 529,826 new cases and 275,125 of them die of cervical cancer every year [1, 2]. Although developments and progresses have been made in treatment with early diagnosis, many patients are threatened by tumor recurrence [3]. It is known that human papilloma virus (HPV) infection is a high risk factor in the progression and development of cervical cancer [4]. Infection of those high risk HPVs, such as HPV16, HPV18, causing cell immortalization, and when followed by further mutations, leads to cervical cancer and other anogenital tumors. [5-7]. Many clinical studies have revealed that HPV infection is related to prognosis and recurrence of carcinomas. In addition, the recurrence-free survival of those who remained HPV-positive after treatment was significantly lower than those who turned negative [8-10]. Piwi-like RNA-mediated gene silencing 2 (Piwil2) was essential in the initial phase of spermatogenesis: regulating RNA silencing and transcrip-
Materials and Methods

Tumor specimens and cell lines

Thirty-two patients with cervical cancer in Stage IA1 – IB1 underwent radical tracheectomy from May 2011 to November 2012 in General Hospital of Lanzhou Military Region. Carcinomatous and adjacent tissues were confirmed by two experienced pathologists according to the 2009 Edition’s WHO classification guideline of cervical cancer. HPV16 infected was determined by E7 gene expression or not [22]. Informed consent was signed by all patients and the study was approved by ethics committee of General Hospital of Lanzhou Military Region, Lanzhou, China. Human cervical cancer cell line SiHa and normal human keratinocyte line HaCaT were obtained from American Type Culture Collection and were cultured in RPMI-1640 complete medium supplemented with 10% fetal bovine serum (FBS) containing 100 U/ml of penicillin and 100 μg/ml of streptomycin.

SiHa cell transfection with shRNA

Following the manufacturer’s protocol, the green fluorescent protein (GFP) tagged lentiviral vector integrated with Piwil2-specific shRNA (sequence: 5’-AAAGCCAGAGGAACCAGCAC-3’, named as lv-Piwil2-KD) or integrated with the scrambled shRNA (sequence: 5’-GTACCGCACGTCATTCGTATC-3’, named as lv-Piwil2-scr) was transfected into SiHa cells in the 24-well plate. Then the serum-free medium in the 24-well plate was replaced with the complete medium after six hours. The GFP positive cells were harvested through the flow cell cytometry (FCM) on the next day. SiHa cells transfected with lv-Piwil2-KD or lv-Piwil2-scr were referred to as the Piwil2-shRNA group and the Mock group, respectively.

Quantitative real-time polymerase chain reaction (qRT-PCR) and immunoblotting assay

Total RNA was extracted from tissues or adherent cells using RNAiso plus and then was reversely transcribed to cDNA using TaKaRa RNA-PCR Kit. The qRT-PCR was performed using the Maxima SYBR Green qPCR Master Mix Kit. The expression level of concerned genes was calculated based on the formula: 2−ΔΔCt, calibrated with β-actin and GAPDH as reference genes. The Piwil2 expression was related to the expression of GAPDH. Immunoblotting was performed according to the protocol as below. Protein extraction of adherent cells was performed as described by Gierke et al. [23]. Briefly, cells were cultured in free-serum starvation for 24 hours to detect the level of protein phosphorylation and the total protein concentration was determined in accordance with the Pierce BCA Protein Assay Kit protocol. Thirty micrograms of protein were loaded into each lane, and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After separated proteins were electrophoretically transferred onto polyvinylidene difluoride (PVDF) membrane, blocked with 5% skimmed milk for 30 minutes, and then incubated with the primary antibodies against Piwil2 (1:400), β-actin (1:1500), Erk1/2 (1:800), JNK (1:1000), p38 (1:800), MMP-9 (1:500 dilution), phospho-Erk1/2 (Thr202/Tyr204) (1:1500), phospho-JNK (Thr183/Tyr185) (1:1000), phospho-p38 (1:1000) overnight at 4°C. Followed by incubation for two hours at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-mouse or anti-rabbit for the host species of primary antibody). Immunoreactive protein bands were detected with enhanced chemiluminescence (ECL) substrate.

Statistical analysis

All data were analyzed by SPSS 15.0. The data are shown as mean ± SEM. The chi-square test was used to evaluate the relationship between Piwil2 expression and clinicopathological parameter, and Independent-Samples T-test was used for comparison between the Piwil2-shRNA group and the Mock. A p < 0.05 was considered as statistically significant.

Results

Piwil2 expression differed in cervical cancer tissue and cancer cells

The expression of Piwil2 and TP53 mRNA was detected by qRT-PCR. Notably, the result showed that 27 of 32 patients who underwent radical tracheectomy had a higher level expression of Piwil2 than corresponding adjacent tissues (Figure 1A). Furthermore, Piwil2 in HPV16-positive patients (9/32) had an over-expression compared with that in HPV16-negative patients (23/32) (Figure 1A, p < 0.05). Interestingly, there was a significant decrease in TP53 expression following the overexpression of Piwil2 in mRNA level (Figure 1 B).
SiHa are HPV16-positive human cervical carcinoma cell line, while HaCaT cells are HPV16-negative. qRT-PCR and Immunoblotting results revealed that SiHa cells highly expressed PiwiL2, with a low level of TP53, while the immortal human keratinocyte HaCaT had a low expression of PiwiL2 (Figure 2) and high level of TP53. Knockdown of PiwiL2 in SiHa cells increased TP53 level (Figure 3A). The silencing efficient of PiwiL2 gene was also evaluated in SiHa cell by qRT-PCR assay and Immunoblotting (Figure 3A and B). No difference of PiwiL2 expression with Mock and untransfected SiHa cells, while siRNA of PiwiL2 mediated down-regulation of PiwiL2.

PiwiL2 knockdown significantly suppressed SiHa cells growth via trapping cells in G0/G1 phase.

PiwiL2 knockdown significantly suppressed the growth of SiHa cells. Compared with the mock group, the number of cells in G0/G1 phase significantly increased in sh-PiwiL2 group (p < 0.05), while G2/M and S phase cells were significantly reduced (p < 0.05) (Table 1). The result indicated that downregulation of PiwiL2 expression made SiHa cells stasis in G0/G1 phase. The CCK-8 assay was applied to evaluate the proliferation of transfected SiHa cells. The inhibition rate of cell proliferation was 29% at fourth day, 32% at fifth day, 40% at sixth day, and 41% at seventh day (Figure 4C).

PiwiL2 knockdown inhibited SiHa cells migration and invasion.

The present authors assessed whether PiwiL2 had an effect on cell migration and invasion. As shown in Figure 5A and B, comparing to Mock, downregulation of PiwiL2 inhibited SiHa cells migration and invasion. It indicated that silencing PiwiL2 gene reduced the ability of cell migration and invasion in the SiHa cell.

PiwiL2 downregulated MMP-9 via regulating the MAPK signaling pathway in SiHa cells.

Matrix metalloproteinases (MMPs), which degrade the extracellular matrix [24] having an essential role in inva-
sion and metastasis of tumor. The aforementioned results also demonstrated that Piwil2 downregulation effectively inhibited migration and invasion in SiHa cells. Therefore, the present authors evaluated the expression of MMPs by qRT-PCR assay and Immunoblotting (Figures 4C and D). Knockdown Piwil2 by shRNA rapidly decreased MMP-9 expression.

MAPK signaling pathway was involved in a variety of functions in cell migration, invasion, and metastasis, and the stress-activated c-Jun NH2-terminal kinase (JNK), the p38 kinase (p38), and the extracellular signal-regulated kinase1/2 (ERK1/2) are the most important members of MAPK pathway. To the present authors’ knowledge, activation of MAPK pathway could promote the transcription of MMPs. Therefore they investigated the expression of JNK, p38, ERK1/2 phosphorylation by immunoblotting after Piwil2 knockdown. Western-blot demonstrated that downregulating Piwil2 expression significantly inhibited protein phosphorylation of ERK1/2 and JNK (p-ERK1/2, p-JNK), while phosphorylation of p38 (p-p38) had no change (Figure 3A). p-JNK specific inhibitor SP600125 and p-ERK1/2 specific inhibitor PD98059 were applied to cervical cancer cell and resulted in down-regulation of MMP-9 protein (Figures 3B and C).

Additionally, in sh-Piwil2 cells, the present authors detected a lower expression of cyclin D1 and cyclin E which were downstream effectors of the ERK1/2 signaling pathway. By using p-ERK1/2 specific inhibitor PD98059 in SiHa cells, the expression of cyclin D1 and cyclin E remarkably decreased (Figure 3C). Bizari et al. studies also revealed that the high level expression of Cyclin D1 and cyclin E could prevent cell cycle arrested in the G0/G1 phase and accelerate cell out through S phase [25]. According to the above results, Piwil2 gene probably regulated cell proliferation via the ERK1/2 pathway.

Discussion

Piwi-like RNA-mediated gene silencing 2 (Piwil2) belongs to Ago/Piwi family proteins, which are comprised of Piwi1/Hiwi, Piwil2/Hili, Piwil3 and Piwil4/Hiwi2. Piwil2 serves as the candidate oncogene and was involved in genital tumors, such as ovarian and endometrial cancer [26]. Increasing evidences show overexpression of Piwil2 in several type of cancers, and correlation with tumorigenicity [13-17]. He et al. found that Piwil2 gene was expressed in various stages of cervical neoplasia, and was an essential supplement to screening for HPV16-infected patients with cervical cancer [14], which was consistent with the present study. In this study, the authors investigated that Piwil2 overexpressed in cervical cancer tissue (27/32) as compared to adjacent normal tissue, weakly expressed in human keratinocyte HaCaT in contrast to cervical cancer cell line SiHa cells.

The mRNA expression level of Piwil2 was significant correlated with HPV16 infection. Consistently, there is in-
HPV16 infection up-regulates Piwil2, which affects cell proliferation and invasion in cervical cancer by regulating MMP-9 etc.

Figure 3. — Piwil2 gene downregulated MMP-9 transcription activities via regulating the MAPK signaling pathway in the SiHa cell. (A) Deleting Piwil2 gene significantly decreased p-ERK1/2, p-JNK protein in sh-Piwil2 group compared with mock group, but no significant alternation in p-p38. (B) p-JNK specific inhibitor SP600125 resulted in downregulation of MMP-9 protein in cervical cancer cell. (C) p-ERK1/2 specific inhibitor PD98059 induced downregulation of cyclin D1, cyclin E and MMP-9 protein in cervical cancer cell. * indicates \( p < 0.05 \).

Figure 4. — Specific shRNA knockdown Piwil2 expression in the cervical cancer cell line. (A, B) Compared with scrambled shRNA group (Mock), Piwil2-targeted shRNA group (sh-Piwil2) significant downexpression of Piwil2 mRNA and protein in SiHa cells, silencing efficient was detected by qRT-PCR and immunoblotting assay. (C) The CCK-8 assay was performed to evaluate the proliferation of in Mock and sh-Piwil2 group. The sh-Piwil2 significantly inhibited cell proliferation in SiHa cells. * indicates \( p < 0.05 \).

Figure 5. — Knockdown Piwil2 reduced cervical cancer cell invasion and migration in vitro. (A, B) The invasion and migration were evaluated in the sh-Piwil2 and the Mock group in Transwell chamber. Downregulation of Piwil2 in SiHa cells reduced the ability of invasion and migration. (C, D) Knockdown Piwil2 induced significantly decreased the MMP-9 expression in the mRNA and protein level, using qRT-PCR and Immunoblotting assay in the sh-Piwil2 group compared with the mock group. * indicates \( p < 0.05 \).
creasing evidence demonstrated that HPV is a risk factor in cervical cancer; about 70.9% of cervical cancers are attributed to HPV types 16 and/or 18 worldwide [4, 27, 28]. However, how HPV16 and Piwil2 interact mutually in the cervical cancer remains unclear. Notably, the present result showed that HPV-positive tumor tissues get a higher level of Piwil2 than negative ones. It has been proved HPV16 infected cells lost TP53 activation gradually, which may be responsible for the anti-apoptosis and immortalization of cells [29, 30]. Interestingly, the present authors investigated TP53 level of HPV-positive and negative tumor tissues, finding that high level of Piwil2 always accompany with a low level of TP53, however, a low level of Piwil2 did not signify a high level of TP53. Similar pattern was shown in HPV16-positive SiHa cells but not in HPV16-negative HaCaT cells. Since Piwil2 was reported to suppress TP53 by regulating STAT3 signaling pathway and plays a role in anti-apoptosis in tumor cells [31]. It was reasonable to believe that HPV-positive may be responsible for increasing Piwil2, which downregulated TP53. However, further studies will be needed for a comprehensive understanding.

Invasion and metastasis are included in biological behavior of malignancies, which are also important factors related to prognosis and survival rate of patients with the result of interaction of multiple factors, and also involved in many biological events, including cell adhesion, angiogenesis, and growth [32]. The present authors found that Piwil2 expression was closely related to tumor migration and invasion, and making Piwil2 gene silence in SiHa cells, significantly inhibited the ability of cell migration and invasion. Piwil2 was also capable in regulating the expression of MMP-9. In SiHa cells, both mRNA and protein expression of MMP-9 rapidly decreased in the sh-Piwil2 group when compared with the mock group. MMP family members are required for increasing motility of the epithelial cancer cells [33]. Overexpression of MMP-9 plays an important role in malignant tumors progression and development [34-36]. It has been reported in several studies that upregulation of MMP-2 and MMP-9 in cervical neoplasias were significantly correlated with poor prognosis [37, 38]. Moreover, it is the MAPK pathway that plays crucial role in tumorigenesis and development [39-44]. JNK, p38, and ERK1/2 are three most important members in the MAPK signaling pathway, which are involved in the regulation of cell growth, differentiation, migration, invasion, metastasis, inflammation, and cell apoptosis process [45, 46]. When JNK signaling pathway is activated by upstream signals, translocation of JNK from the cytoplasm to the nucleus induces the nuclear transcription factor c-Jun N-terminal phosphorylation of 63 and 73 serine residue [47]. The transcription factor c-Jun is phosphorylated and the c-Fos and activator protein-1 (AP-1) complex is formed, which regulates downstream genes [48]. ERK1/2 inhibitor PD98059 integrated with liposomes and significantly reduced invasive ability of oral cancer, which was associated with a decreased ERK1/2 activity and MMP-9 downregulation [49]. These reports are consistent with the present study, in which downregulation of piwil2 expression inhibited phosphorylation of ERK 1/2 and JNK, and when p-JNK specific inhibitor SP600125 and p-ERK1/2 specific inhibitor PD98059 were respectively applied to cervical cancer cell, and MMP-9 protein expression was significantly downregulated. The above result suggested that Piwil2 could regulate the MMP-9 activity via ERK1/2 and JNK pathway, but not p38. Furthermore, cyclin D1 and cyclin E were also involved in Piwil2-induced cell proliferation. It has been reported that CDK2 and CDK4 are two important cyclin-dependent kinases, which can interact with cyclin D1 and cyclin E to form a complex leading to uncontrolled cell cycle regulation and cell proliferation [50].

In conclusion, Piwil2 gene was identified to be highly expressed in cervical cancer tissues and SiHa cells. HPV-positive tissues with a low level of TP53 while highly expressed Piwil2, indicating HPV-16 infection may be responsible for the increase of Piwil2, which downregulated TP53, in HPV-positive tumors. In addition, Piwil2 was involved in regulating cell proliferation via regulating cyclin D1 and cyclin E, and it also increases invasion of cervical cancer cell by regulating MMP-9 via MAPK pathway. Finally, the present study provides evidence that Piwil2 was an essential supplement to screening for cervical cancer cell by regulating MMP-9 via MAPK pathway and plays a role in anti-apoptosis in tumor cells.

Invasion and metastasis are included in biological behavior of malignancies, which are also important factors related to prognosis and survival rate of patients with the result of interaction of multiple factors, and also involved in many biological events, including cell adhesion, angiogenesis, and growth [32]. The present authors found that Piwil2 expression was closely related to tumor migration and invasion, and making Piwil2 gene silence in SiHa cells, significantly inhibited the ability of cell migration and invasion. Piwil2 was also capable in regulating the expression of MMP-9. In SiHa cells, both mRNA and protein expression of MMP-9 rapidly decreased in the sh-Piwil2 group when compared with the mock group. MMP family members are required for increasing motility of the epithelial cancer cells [33]. Overexpression of MMP-9 plays an important role in malignant tumors progression and development [34-36]. It has been reported in several studies that upregulation of MMP-2 and MMP-9 in cervical neoplasias were significantly correlated with poor prognosis [37, 38]. Moreover, it is the MAPK pathway that plays crucial role in tumorigenesis and development [39-44]. JNK, p38, and ERK1/2 are three most important members in the MAPK signaling pathway, which are involved in the regulation of cell growth, differentiation, migration, invasion, metastasis, inflammation, and cell apoptosis process [45, 46]. When JNK signaling pathway is activated by upstream signals, translocation of JNK from the cytoplasm to the nucleus induces the nuclear transcription factor c-Jun N-terminal phosphorylation of 63 and 73 serine residue [47]. The transcription factor c-Jun is phosphorylated and the c-Fos and activator protein-1 (AP-1) complex is formed, which regulates downstream genes [48]. ERK1/2 inhibitor PD98059 integrated with liposomes and significantly reduced invasive ability of oral cancer, which was associated with a decreased ERK1/2 activity and MMP-9 downregulation [49]. These reports are consistent with the present study, in which downregulation of piwil2 expression inhibited phosphorylation of ERK 1/2 and JNK, and when p-JNK specific inhibitor SP600125 and p-ERK1/2 specific inhibitor PD98059 were respectively applied to cervical cancer cell, and MMP-9 protein expression was significantly downregulated. The above result suggested that Piwil2 could regulate the MMP-9 activity via ERK1/2 and JNK pathway, but not p38. Furthermore, cyclin D1 and cyclin E were also involved in Piwil2-induced cell proliferation. It has been reported that CDK2 and CDK4 are two important cyclin-dependent kinases, which can interact with cyclin D1 and cyclin E to form a complex leading to uncontrolled cell cycle regulation and cell proliferation [50].

In conclusion, Piwil2 gene was identified to be highly expressed in cervical cancer tissues and SiHa cells. HPV-positive tissues with a low level of TP53 while highly expressed Piwil2, indicating HPV-16 infection may be responsible for the increase of Piwil2, which downregulated TP53, in HPV-positive tumors. In addition, Piwil2 was involved in regulating cell proliferation via regulating cyclin D1 and cyclin E, and it also increases invasion of cervical cancer cell by regulating MMP-9 via MAPK pathway. Finally, the present study provides evidence that Piwil2 was an essential supplement to screening for HPV16-infected patients with cervical cancer and should be considered as a potential target for therapy.

Acknowledgments
This study was supported by the Science and Technology Planning Project of Gansu Province (No. 1204FKCA174).

References
HPV16 infection up-regulates Piwil2, which affects cell proliferation and invasion in cervical cancer by regulating MMP-9 etc.


[10] Inaba K., Nagasaka K., Kawana K., Arimoto T., Matsumoto Y., Tsu- 

ruga T., et al.: “High-risk human papillomavirus correlates with re- 
currence after laser ablation for treatment of patients with cervical 
 intraepithelial neoplasia 3: A long-term follow-up retrospective 


hiwi gene in human gastric cancer was associated with proliferation of 


expressed in various stages of cervical neoplasia is a potential com- 


pressed in various stages of breast cancers and has the potential to be 


pressed by bone marrow-derived cells contributes to skin carcinogen- 

esis”. Cell, 2000, 103, 481.


signaling”.

driven in breast cancer stem cells by stem cell protein piwi2”. Can- 
cer Res., 2010, 70, 4569.


Address reprint requests to:
L. WANG, M.D.
Department of Gynaecology and Obstetrics
General Hospital of Lanzhou Military Region
98 Xiaoxihu West Street
Lanzhou 730050 (China)
e-mail: linowang@163.com
Adjuvant treatment with a dialyzable leukocytes extract contributes to maintain HPV-infected women free of low-grade cervical lesions

A. Rodriguez-Flores¹, G. Nuñez-Fernandez², I. Estrada-Garcia¹, M. Aguilar-Santelises¹,³, O. Rojas-Espinosa¹, S. Estrada-Parra¹

¹ Department of Immunology, National School of Biological Sciences, IPN, Mexico City (Mexico)
² Cervical Dysplasias Clinic, Angeles Hospital, México City (Mexico); ³ CMM, Karolinska Institute, Stockholm (Sweden)

Summary

Purpose of investigation: To investigate if adjuvant treatment with a dialyzable extract of leukocytes (DLE), may help HPV-infected patients with low-grade intraepithelial squamous cervical lesions (LIS) to get free of HPV infection and cervical lesions. Materials and Methods: Patients with untreated, low-grade cervical lesions were treated either with surgery (Group A) or with DLE (Group B). Patients with low-grade but recurrent cervical lesions were newly treated with surgery plus DLE (Group C). Results: A decreased or absent cervical lesion correlated with a diminished or absent HPV viral load at one year of treatment ($r = 0.6, p < 0.05$). Seventy-nine percent of Group B but only 50 % of Group C and 38 % of Group A patients were free of cervical lesion after 24 months of treatment ($p < 0.05$). Conclusion: The present data support the benefit of adding DLE as adjuvant for treating HPV-infected women with LIS.

Key words: Dialyzable leukocytes extract, low-grade cervical lesions, HPV.

Introduction

High-risk human papilloma virus (HR-HPV) infection is the prevalent risk factor for development of cervical cancer [1, 2]. A global HPV burden as high as 10.4% of the world population has contributed to establish cervical cancer as the second cause of cancer death of women worldwide [1, 3]. Eighteen of the 200 known HPV subtypes are high-risk subtypes HPV that may alter the uterine cervix transitional epithelium cells’ growth and favour the appearance of squamous epithelial lesions and cervical cancer [4 - 7]. In Mexico, up to 43.6% of the adult female population may be HPV-infected and most of them are infected with high-risk (16, 18, 31, 33, 35, 52, 58) HPV subtypes [4, 8-10].

Most HPV infections are cleared without treatment, even if they are caused by high-risk HPV subtypes [11]. However, up to 15 % of women with high-risk HPV infections may be unable to remove the virus from the cervix, which facilitates epithelial squamous lesions’ progression from low- to high-grade and to cancerous lesions [12]. Cervical intraepithelial neoplasias (CIN) grade 1, 2 or 3 pre-cancerous lesions are currently treated with different approaches. A simple follow-up is often exercised without any treatment for mild pre-cancerous cervical lesions such as CIN 1, until further evaluation [13]. However, moderate dysplasia (CIN 2) and severe pre-cancerous cervical lesions (CIN 3) usually associated with high-risk HPV infection require either cryo- or laser-surgical removal of the area of abnormality. Although recurrence rates vary, patients who have been treated for CIN are considered to be at high-risk of developing invasive cervical cancer for many years after treatment, particularly in association with high-risk HPV persistence [14 - 16]. Whereas a Th1 immune response could be important to achieve regression of HPV infection, recurrence may be facilitated by preoperative factors negatively influencing the immune status such as HIV infection or pregnancy and postoperative factors, such as positive surgical margins and high-grade pathology on the excision specimens [17 - 19].

Dysplasia and tumour progression are often accompanied by a poor immune response, viral evasion mechanisms or both [2]. CD3+ CD4+, CD3+ CD8+, and CD3+ CD4+ CD25+ peripheral blood T cells may not be capable of containing HPV infection in spite of its normal or even high numbers in HPV-infected women [3, 12, 20]. IL-10 and IL-10 producer cells may also be in high numbers in these patients, contributing to a decreased Th1 cells function and lesion transformation into cervical neoplasia [21 -22]. These alterations strongly suggest the need to evaluate the immune status of HPV-infected women with low-grade cervical lesions and to test new means to restore a more suitable cell immunity balance in these patients [3, 4, 23].
Lawrence and Borkowsky were the first to report that a dialyzable extract of leukocytes (DLE) from donors that were sensitized and immune responsive to specific antigens could be administered to unresponsive recipients and make them become responsive to those particular antigens [24]. Kirkpatrick et al. determined that DLE contains more than 200 molecules with <10 kDa MW and proposed that the DLE specificity might be due not to one, but many small peptides [25]. Numerous investigators have used DLE to treat various kinds of diseases with variable success and the present authors have earlier demonstrated that DLE may increase in vivo production of TNFα and IFNγ, helping patients affected by acute infection of herpes zoster to achieve a more favourable clinical course [33]. Now, the present authors report on a group of HPV-infected women having low-grade cervical lesions that were treated with surgery, DLE, or surgery plus DLE, to determine if DLE may modify their numbers of circulating regulatory T cells and their cytokine profile in order to achieve a proper immune cell response against HPV, and to prevent their lesions’ progression to cervical cancer.

Materials and Methods

Study subjects

The protocol of this project was evaluated and approved by the Hospital Ethical Committee. All the women included in the study signed informed consent before initiating the study and treatment protocols. Patients receiving any kind of immune modifier therapy were excluded from the study. Pregnant, menopausal, chronically ill women suffering diabetes, allergies, autoimmunity, AIDS or other sex transmitted diseases were also excluded. A cervical biopsy was taken from the affected tissue in every patient to determine the type of intraepithelial cervical lesion they had (Table 1). Women with high-grade cervical cell abnormalities were also excluded. Fifty-four sexually active women with a mean age of 31 ± 6.9 years old, ** Inflammatory process in addition to the cervical lesion, *** diameter in cm, Group A (n = 17) had first-time lesion and received surgical treatment, Group B (n = 18) had first-time lesions and was treated only with dialyzable extract of leukocytes (DLE), Group C (n = 19) had recurrent lesions and was treated both with surgery and DLE. Group D was a control group of non-HPV-infected, age-matched, healthy volunteers. HR = high-risk HPV 16, 18, 33, 39, 51, 52, 56, 58, 59 or 66. LR = low-risk HPV 11, 43, 44, 81 or 61. NDR = non-determined-risk HPV 20, 69, 90 or 102. Negative = non-viral protein detection. LIS = low-grade intraepithelial squamous lesion. HIS = high-grade intraepithelial squamous lesion.

Dialyzable extract of leukocytes (DLE)

DLE was prepared by repeated freezing and thawing of leukocytes isolated from buffy coats of healthy blood donors. The diazlyzed extract of leukocytes was adjusted by protein content and stored frozen until use. One unit (2.2 mg of protein in five ml of bi-distilled sterile water) was orally administered to patients from Group B and C during five weeks. One more unit was at the same time, topically applied to these patients, every 72 hours during the first two weeks. This schedule of treatment was repeated when a persistent lesion was found during colposcopy examination at three, six, nine, and 12 months of the study. Additional schedules of DLE were orally and topically administered to eight patients of Group B and 12 patients from Group C during the first year.

<table>
<thead>
<tr>
<th>Table 1. Study groups’ features.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Age*</td>
</tr>
<tr>
<td>Sex partners</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>≥ 3</td>
</tr>
<tr>
<td>HR-HPV</td>
</tr>
<tr>
<td>LR-HPV</td>
</tr>
<tr>
<td>NDR</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>LIS</td>
</tr>
<tr>
<td>HIS</td>
</tr>
<tr>
<td>Cervicitis**</td>
</tr>
<tr>
<td>Lesion size***</td>
</tr>
</tbody>
</table>

* Mean ± SD years old, ** Inflammatory process in addition to the cervical lesion, *** diameter in cm, Group A (n = 17) had first-time lesion and received surgical treatment, Group B (n = 18) had first-time lesions and was treated only with dialyzable extract of leukocytes (DLE), Group C (n = 19) had recurrent lesions and was treated both with surgery and DLE. Group D was a control group of non-HPV-infected, age-matched, healthy volunteers. HR = high-risk HPV 16, 18, 33, 39, 51, 52, 56, 58, 59 or 66. LR = low-risk HPV 11, 43, 44, 81 or 61. NDR = non-determined-risk HPV 20, 69, 90 or 102. Negative = non-viral protein detection. LIS = low-grade intraepithelial squamous lesion. HIS = high-grade intraepithelial squamous lesion.

HPV subtypes and viral load

Cervicovaginal exudates were collected by brushing and sent to the Investigation and Molecular Analysis Laboratory (LIAM) for PCR determination of HPV infection and viral subtype. The MY09/MY11 consensus-primer set served to obtain a 450 bp L1 gene fragment that was automatic sequenced. The highly conserved MY09/MY11 primer set allowed detection of 42 HPV subtypes, including high-risk 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73 HPV subtypes and low-risk 2a, 3, 6, 7, 10, 11, 13, 26, 27, 28, 29, 30, 32, 34, 40, 42, 44, 53, 54, 55, 57, 61, 62, 66, 67, 70, 72, and 74 HPV subtypes. Cervicovaginal samples were also obtained by scraping and brushing and analysed by the hybrid capture 2 assay at LIAM as complementary analysis for detection of high-risk 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 HPV subtypes. Results of the hybrid capture two assays are given in relative light units (RLU), which provide a semi-quantitative estimate of viral load in the specimen, indicating also if the HPV is in replicative or latent phase [34].

Flow cytometry

Venous blood was collected in vacutainer tubes with EDTA at 0, 15, 30, 90, 180, and 365 days of study. Peripheral blood cells were phenotyped by single or double immunofluorescence staining with fluoresceinated anti-CD 3, -CD 4, -CD 8, -CD 16, -CD 56, -CD 25, or -CD 19 monoclonal antibodies (Mab) to identify total T, helper T and cytotoxic T cells, NK cells, and B cells. Non-stained cells and cells treated with FITC- or PE-conjugates with matched isotypes were also stained with FITC- or PE-conjugates with matched isotypes.
were used as controls. Thirty μl of leukocytes-containing plasma were incubated 20 minutes at room temperature in the dark with five μl of one or two of the above listed monoclonal antibodies for determination of cell surface markers. Erythrocytes were lysed by 12 minutes incubation with FACS lysing solution. Cells were then washed with PBS, resuspended in 400 μl paraformaldehyde 2% and analysed by flow cytometry. In addition, 100 μl heparinized blood were incubated for 20 minutes with five μl anti-CD3-Percp. After lysing erythrocytes, these cells were washed and incubated for 20 minutes with permeabilizing solution and ten μg/ml brefeldin A, washed, further incubated for 30 minutes more with five μl anti-IL-10-PE, anti-IFN-γ-FITC, anti-IL-4-PE or their respective isotype-matched FITC- or PE-conjugated Mab, washed and PFA fixed. Stained samples were analysed by flow cytometry, acquiring 10,000 cells for each surface marker or 20,000 cells for cytokines intracellular determination with an acquisition rate < 400 events/sec in a system equipped with an argon laser and CELLQuest pro software.

Statistical analysis
Mean values were compared using the Student’s t-test for paired and unpaired samples. Pearson’s coefficient of correlation and ANOVA test were used to analyse the correlation between independent observations and two-tailed statistical significances were determined. Values without normal distribution were analysed with the non-parametric Mann Whitney U test.

Results

Analysis before treatment
Age-matched healthy controls were not different to the patients in respect to number of sex partners and socioeconomic status. None of the healthy controls were HPV-infected whereas all the patients but one were HPV-infected (Table 1). Lesion size was also similar in the different groups of patients and there was no significant difference either between the numbers of total leukocytes and total lymphocytes from patients and healthy controls (Table 2). Patients from Groups A and B had lower amounts of B lymphocytes (289 ± 44 and 321 ± 57, respectively) than healthy controls (477 ± 64, \( p < 0.05 \)), whereas the numbers of T lymphocytes (1849 ± 201) and CD3+ CD4+ (777 ± 107) were slightly higher in patients from the Group C than in healthy controls (1350 ± 118 and 507 ± 71, \( p < 0.05 \)). In addition, numbers of CD3+ CD4+ CD25+ peripheral blood cells in the three groups of patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Total leukocytes</th>
<th>Total lymphocytes</th>
<th>CD3+</th>
<th>CD19+</th>
<th>CD3+CD4+</th>
<th>CD3+CD8+</th>
<th>CD3+ CD4+CD25+</th>
<th>CD3+CD56+</th>
<th>CD3+ IL10+</th>
<th>CD3+ IFN-γ+</th>
<th>Number of sex partners</th>
<th>Lesion size</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6818 ± 600</td>
<td>2487 ± 322</td>
<td>1595 ± 232</td>
<td>321 ± 57</td>
<td>426 ± 99</td>
<td>144 ± 30</td>
<td>72 ± 36</td>
<td>7 ± 4</td>
<td>7 ± 4</td>
<td>4 ± 2</td>
<td>3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>5673 ± 426</td>
<td>2505 ± 289</td>
<td>1693 ± 207</td>
<td>321 ± 57</td>
<td>426 ± 99</td>
<td>144 ± 30</td>
<td>72 ± 36</td>
<td>7 ± 4</td>
<td>7 ± 4</td>
<td>4 ± 2</td>
<td>3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6463 ± 479</td>
<td>2595 ± 269</td>
<td>1849 ± 201</td>
<td>321 ± 57</td>
<td>426 ± 99</td>
<td>144 ± 30</td>
<td>72 ± 36</td>
<td>7 ± 4</td>
<td>7 ± 4</td>
<td>4 ± 2</td>
<td>3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>6297 ± 421</td>
<td>2160 ± 162</td>
<td>1350 ± 118</td>
<td>321 ± 57</td>
<td>426 ± 99</td>
<td>144 ± 30</td>
<td>72 ± 36</td>
<td>7 ± 4</td>
<td>7 ± 4</td>
<td>4 ± 2</td>
<td>3 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

N.A. = not applicable. * \( p < 0.05 \) vs Group D.

Figure 1. — A) absolute numbers of CD3+ CD4+ CD25+ peripheral blood cells of patients with first-time diagnosed low grade cervical lesions before treatment with surgery (Group A), patients with first-time diagnosed low grade cervical lesion before treatment with the dialyzable extract of leukocytes (DLE, Group B), and recurrent patients before treatment with surgery plus DLE (Group C) and non-HPV infected, age-matched, healthy women (Group D), B) absolute numbers of CD3+ CD4+ CD25+ peripheral blood cells during a 365 days follow up of patients in Group A (○), Group B (●), and Group C (▲). CD3+ CD4+ CD25+ cells were highest in the group C before and after treatment (\( p < 0.05 \)) as compared to Groups A, B, and D.

and those of CD3+ IL-10+ cells in the group C were significantly ($p < 0.05$) elevated as compared to normal values from Group D (Table 2).

**Lymphocyte populations after treatment**

CD3+ CD4+ CD25+ and CD3+ IL-10+ cells were highest in the Group C before treatment ($144 \pm 28$ vs $30 \pm 6$ and $31 \pm 10$ vs $5 \pm 0.8$, $p < 0.05$) and remained elevated after treatment (Table 2 and Figure 1). The amount of CD3+ IFNγ+ cells increased from $0.63 \pm 1.2$ to $5.3 \pm 6.4$ in patients from group B ($p < 0.05$, Figure 2) but not in patients from the other groups. The remaining cell populations had minor variations during the follow up period of 365 days (Figure 2).

---

**Figure 2.** — Percentages of CD3+ IL-10+ (2A), CD3+ IFNγ+ (2B), and CD3+ IL4+ (2C) cells in peripheral blood of HPV-infected women, before (left hand side) and during treatment (right hand side). Patients with first time lesions that were treated with surgery (○, Group A), patients with first time lesions that were treated only with DLE (•, Group B), and recurrent patients that were treated with surgery plus DLE (△, Group C). Group D (◊) was formed by non-HPV infected, age-matched, healthy women. CD3+ IL-10+ cells were highest in Group C before and after treatment ($p < 0.05$).
Lesion size and viral load

Thirty-eight percent of the patients from Group A, 79% of the patients from group B, and 50% of the patients from Group C were free of cervical lesion after two years of treatment, all of which were significantly different from their initial conditions \((p < 0.05\), Table 3). The difference in success of keeping the patients without cervical lesion was also significant between Groups B and A \((p < 0.05\), Table 3), indicating that the treatment with the DLE alone was at least as good as surgical treatment for women with LIS, diagnosed for the first time. The outcome of half of the patients from Group C also suggests that even recurrent patients, originally treated with surgery may benefit from receiving DLE as adjuvant treatment, together with a surgical removal of the new cervical lesion (Tables 3 and 4).

The predominance of HR-HPV subtypes was confirmed by the presence of the subtype 16 in most of the patients (not shown). Viral load was quantified by the hybrid capture 2 assay and reported as RLU. Viral load decreased significantly in most of the 20 patients that were tested after one year of treatment and seven of these patients were at the same time, entirely free of cervical lesion and viral load. Moreover, a decreased or absent cervical lesion correlated with a diminished or absent HPV viral load at one year of treatment \((r = 0.6, p < 0.05\), Table 4).

Discussion

The high prevalence of HPV-infection is a global public health concern that relates both to cervical cancer and precancerous lesions world incidence \([1, 3]\). Only 1% from the 10% of patients that become chronically infected with HPV develops cervical cancer, apparently because there usually is a pro-inflammatory microenvironment that leads to an effective immune response capable of eliminating HPV-infected cells \([17]\). However, HPV viruses have developed strategies to achieve host immunity suppression and viral DNA integration into the host DNA \([35 - 36]\). In such cases, stimulation of the immune response with adjuvants may be helpful to reverse the anti-inflammatory microenvironment.

HPV association with cervical cancer is matter of particular great concern in Mexico, since half of the Mexican female population may be infected with high-risk \((16, 31, 18, 35, 52, 33,\) and \(56\) HPV subtypes \([4, 8 - 10, 37]\). Additional risk factors such as low nutritional and socioeconomic status, long-term intake of contraceptives, sex activity at early age, sex transmitted diseases, high number of pregnancies, and the expression of certain HLA Class II antigens are features that characterize a large number of Mexican women and contribute to maintain the high cervical cancer prevalence that afflicts a country where one woman may be dying of cervical cancer every two hours \([37 - 38]\). HPV persistence and additional risk factors most probably also contribute to the elevated recurrence observed in our low-income group of patients \([38]\). Nearly half of our patients were positive for viral load one year after diagnosis and this percentage most probably augments with longer follow up after treatment, which may account for the high recurrence of LIS observed in patients followed up to two to five years of diagnosis \([39]\). The lack of an appropriate immune response against infectious agents, often associated with dietary deficiencies and inadequate healthcare also make HPV-infection become a first priority. Sex education, national screenings, and other preventive measurements such as HPV vaccine applications are therefore, as important as the availability of inexpensive and effica-
cious measures for early treatment of women with cervical lesions [10].

Increased levels of CD3+ CD4+ CD25+, and IL-10 as observed in the present recurrent patients are in agreement with other reports showing an increased activity of regulatory T cells and generation of an anti-inflammatory profile [40–42]. IL-10 production and other Th2 cytokines are associated with significantly longer times to HPV clearance [43]. High percentages of infection with high-risk subtypes HPV and the predominance of subtypes 16, 18, 31, 33, 35, and 58 in the present patients are in agreement with data reported on prevalent HPV subtypes infection in Mexican women with cervical intraepithelial lesions [44].

The hybrid capture 2 assay has higher negative predictive value than the Papanicolaou test for detection of CIN. A correlation exists between RLU values and the severity of cervical lesions, with lower RLU values for patients without lesion and increasingly higher values for patients with low-grade and high-grade lesions [45]. In the present work, a diminished or negative viral load after one year from initiation of the treatment of all the patients correlated to a simultaneously diminished or absent cervical lesion. The cervical lesion had also a decreased size or was totally absent in many patients from the three study groups after two years of treatment. However, recurrence may come along with HPV reinfection and the highest success in keeping more patients free of cervical lesion at two years of treatment initiation was observed in patients treated with DLE either alone or as adjuvant to surgery during the first year after diagnosis.

Conclusion

Treatment with DLE as adjuvant may provide an important stimulus to incline the balance towards a more favourable immune response from HPV-infected patients having low-grade cervical lesions, who live in permanent risk due to their poor socioeconomic status and persistent HPV infection or continuous reinfection. Results of this pilot study suggest that DLE has a therapeutic potential for HPV-infected women. Since no negative side effects have been reported following the administration of DLE and because it is a biological product with very low cost of production, the authors suggest that the administration of DLE should be considered a suitable addition to the currently established treatment for HPV-infected women, either with first or recurrent low-grade cervical lesions. Although many unanswered questions exist regarding DLE composition and mechanisms of action, its beneficial effects deserve further investigation.

References

Adjuvant treatment with a dialyzable leukocytes extract contributes to maintain HPV-infected women free of low-grade cervical lesions


[38] Bernal-Silva S., Granados J., Gómez-Ferrete J.C., Alaez C., Flores-Aguilar H., Cerda-Flores RM., et al.: “HLA-DRB1 Class II antigen level alleles are associated with persistent HPV infection in Mexican women; a pilot study”. Infect. Agent Cancer., 2013, 8, 31. doi: 10.1186/1750-9378-8-31


Introduction

Ovarian cancer represents the sixth most commonly diagnosed cancer among women in the world and it causes more deaths per year than any other cancer of the female reproductive system [1, 2]. The major obstacle to successful therapy is drug resistance which leads to low five-year survival rates [3]. It is widely acknowledged that the sensitivity to chemotherapy is determined by the apoptotic response of cancer cells to chemotherapeutic drugs [4]. In the previous study, many molecules, such as PI3K/Akt [5, 6], PTEN [7], BRCA [8], and MDR [9], have been confirmed to be involved in the regulation of apoptosis and in the complicated signaling network that determines the fate of cancer cells, i.e., either “death” or “survival.” More importantly, the present authors’ recent study has also found that cytoplasmic p21 was not only a novel biomarker of cisplatin resistance but also represented a potential therapeutic target for ovarian tumors that are resistant to cisplatin treatment [10].

In addition to cisplatin, there is another conventional anticancer agent paclitaxel (PTX), which is widely used for the chemotherapeutic treatment of ovarian cancer patients [11]. Nevertheless, less than 50% of ovarian cancers exhibit a satisfactory response to PTX and effective strategies are needed to enhance its sensitivity [12]. Given that multidrug resistance and high cross-resistance occurred in the ovarian cancer treatment [9, 13], this study was further conducted to investigate whether p21 could act as a similar role in regulating the PTX resistance, just as cisplatin resistance. Here, the authors report that in the ovarian cancer cell line A2780, induction of p21 translocation into the cytoplasm via constitutively active Akt2 transfection in A2780 enhanced the resistance to PTX, while inhibition of p21 translocation into the cytoplasm via Akt2 shRNA transfection in A2780 cells significantly increased PTX treatment sensitivity. Furthermore, knockdown of cytoplasmic p21 by direct p21 siRNA transfection in Akt2 overexpressed A2780 cells notably increased PTX-induced apoptosis. Conclusion: Cytoplasmic p21 may represent a potential therapeutic target for ovarian tumors that are resistant to PTX treatment.

Materials and Methods

Cell lines and cell culture

The A2780 ovarian cancer cell line was obtained from a European manufacturer. Cells were cultured in RPMI-1640 supplemented with two mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, and 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere containing 5% CO2.

Construction of plasmids

A constitutively active Akt expression vector (AAkt2), short hairpin RNA targeting Akt2 (Akt2Sh), small p21 RNA interfering
fragment (p21si), and their corresponding control plasmids were constructed as described previously [5, 10].

Establishment of stable-expression cell lines of AAkt2 and Akt2sh in A2780 cells
A2780 cancer cells were stably transfected with AAkt2 and Akt2sh vectors using lipofectamine 2000. The empty vectors of pcDNA3.1 and pEGFP-C1 were transfected as negative controls. The cells were selected with 600 μg/μl G418. After 21 days the G418-resistant cell pools were established and inoculated into 100 mm dishes for further propagation.

Transient transfection for RNAi targeting
The Akt2 stably transfected A2780 cancer cells were transfected with small p21 RNA interfering fragment (p21si) using lipofectamine 2000. After incubation for six hours, the transfection solution was replaced with fresh complete growth medium. Then 48 hours post-transfection, the cells were assayed for the expression of p21, Akt2, and p-akt, and treated with PTX for further experiment.

Western blot analysis
Total proteins were extracted by lysing cells in buffer containing 50 mM Tris pH 7.4, 150 mM NaCl, 50 mM NaF, 0.5% NP-40, one mM Na2VO4, one mM phenylmethylsulfonyl fluoride, 25 mg/ml leupeptin, and 25 mg/ml aprotinin. The lysates were cleared by centrifugation, and the supernatants were obtained. Cytoplasmic proteins were extracted using the N-PER kit according to manufacturer’s instructions. Equal amounts of protein lysate were used for Western blot analyses using the indicated antibodies. Specific signals were visualized with NBT/BCIP.

Analysis of apoptosis
Cells were harvested, washed with PBS, and stained with the annexin-V/PI apoptosis kit according to manufacturer’s instructions. Apoptosis rates were evaluated using a flow cytometer, and the data were analyzed using cell fit software.

Statistical analysis
All experiments were repeated three times. Results expressed as mean ± SD were analyzed using the Student t test. Differences were considered significant when p < 0.05. Data was analyzed using SPSS software version 13.0.

Results
Induction of p21 translocation into the cytoplasm decreases sensitivity to PTX in A2780
The plasmid of Akt2 (A2780/AAkt2) and empty plasmid (A2780/NC) were stably transfected into A2780 cells. Total and cytoplasmic protein were extracted from the cells and assessed by Western blot. As is shown in Figure 1A, the expression levels of Akt and p-Akt were significantly enhanced in A2780/AAkt2 cells when compared to A2780/NC cells and A2780 cells. Moreover, cytoplasmic p21 protein levels were markedly increased in A2780/AAkt2 cells compared to the non-transfected control cells and vector-transfected cells (Figure 1A). Flow cytometric analysis of cells treated with 100 nmol/L PTX for 48 hours showed that A2780/AAkt2 cells exhibited lower levels of apoptosis rate (31% ± 5.5%) than A2780/NC cells (49.2% ± 8.4%) and A2780 cells (47.2 ± 8.8%) (p < 0.05, Figure 1B). However, there were no significant differences in the apoptotic rates among the three groups when treated for 24 h (p > 0.05, Figure 1B). Based on the aforementioned results, it is demonstrated that accumulation of p21 in cytoplasm through activation of Akt2 impairs the sensitivity of A2780 cells to PTX.

Inhibition of p21 translocation into cytoplasm restores the sensitivity to PTX in A2780 cells
Short hairpin RNA targeting Akt2 and its vector control plasmid Sh-Scr were stably transfected into A2780 cells (A2780/Sh-Akt2 cells and A2780/Sh-Scr cells). Total and cytoplasmic protein was extracted and detected by West-
Cytoplasmic p21 is responsible for paclitaxel resistance in ovarian cancer A2780 cells

ern blot analysis. Compared to the non-transfected control cells and vector-transfected cells, the protein levels of Akt and p-Akt in A2780/Sh-Akt2 cells were significantly decreased (Figure 2A). Additionally, there was also a remarkable decrease in cytoplasmic p21 in A2780/Sh-Akt2 cells when compared with the controls (Figure 2A). As is shown in Figure 2B, flow cytometric analysis of cells treated with 100 nmol/L PTX for 48 hours demonstrated that A2780/Sh-Akt2 cells exhibited 65.1% ± 5.9% apoptosis rate, which was higher than vector-transfected cell (47.9% ± 8.0%) and non-transfected control cells (45.7% ± 9.5%) (p < 0.05). However, there were no significant differences in the apoptotic rates among the three groups when treated with 100 nmol/L PTX for 24 hours (p > 0.05, Figure 2B). Collectively, these results demonstrate that inhibition of cytoplasmic p21 through inactivation of Akt2 increases the sensitivity of A2780 cells to PTX.

Knockdown of cytoplasmic p21 restores the sensitivity to PTX in A2780/AAkt2 cells

To further clarify whether cytoplasmic p21 contributes to PTX resistance, RNA interference assay in A2780/AAkt2 was applied to decrease p21 that was mainly in the cytoplasm. P21si and its mismatched fragment of p21sm were transiently transfected in A2780/AAkt2 cells. As is shown in Figure 3A, p21si transfection exhibited a notable decrease in cytoplasmic p21 compared with control groups, however there was no significant change in the expression of Akt2 and p-Akt as evaluated by Western blot. After transfection, these cells were exposed to 100 nmol/L PTX for 48 hours. As shown in Figure 3B, the apoptosis rate was 49.6% ± 10.0% in A2780/AAkt2/p21si cells, which was notably higher than A2780/AAkt2/p21sm cells (32.7% ± 5.9%) and A2780/AAkt2 cells (30.2% ± 3.2%) (p < 0.05, Figure 3B) as assessed by flow cytometry. No significant differences in the apoptotic rates among the three groups were found to be treated with PTX for 24 hours (data not shown). These results demonstrate that the reduction of cytoplasmic p21 contributes to the increased sensitivity to PTX in A2780/AAkt2 cells.
Discussion

Previous studies demonstrated that p21 could act as a “tumor suppressor” by binding to cyclin/CDK complexes and proliferating cell nuclear antigen [14]. However, other studies [15] have revealed that p21 can be a paradoxical tumor promoting agent and has been positively related to the poor prognosis in cancer patients due to its accumulation in the cytoplasm. Besson et al. [16] reported that control of the p21 subcellular localization could represent an important regulatory switch from nuclear tumor suppressor to cytoplasmic tumor booster. Nevertheless little has been known about the role of p21 in cancer cell chemoresistance until Koster et al. reported cytoplasmic p21 expression determined cisplatin resistance in testicular cancer [17]. Recently, the present authors’ study has further validated that not only cytoplasmic p21 was a novel clinical biomarker of cisplatin resistance but it represents a potential therapeutic target for ovarian tumors that were refractory to cisplatin-based treatment [10], which supplemented their previous studies [5, 7] and verified that p21 was a downstream effector in the PI3K/Akt2 pathway that contributes to cisplatin resistance.

Besides cisplatin, there is another regular anticancer agent PTX, which has been widely used for the first-line chemotherapeutic treatment of ovarian cancer patients [11]. However, there were more than 50% ovarian cancer patients who showed a impaired sensitivity to the drug [12]. Effective strategies have been made to investigate the mechanism by which mediated the PTX resistance and many molecules such as kallikrein-related-peptidase 4 (KLK4) [18], multidrug resistance gene MDR1 [9], NFxB [6], and p27 [12] have been found to confer to the drug resistance. Given that multi-drug resistance and high cross-resistance occurred in the ovarian cancer treatment [9, 13], it could be easily speculated whether different chemotherapeutic drugs resistance could be reversed in a similar way. Therefore, this study was conducted to investigate whether PTX shared a similar drug resistance mechanism with cisplatin. In addition, the present authors sought to determine whether interfering with cytoplasmic p21 could enhance the susceptibility of cancer cells to PTX.

It was reported that the activation of phosphate dylinositol 3-kinase (PI3K)/Akt signaling could stimulate the accumulation of p21 in the cytoplasm [19]. There are three isoforms of Akt including Akt1, Akt2, and Akt3. All three isoforms share a high degree of amino acid sequence identity, especially within the kinase domain [20], and are activated by similar pathways. As a member of Akt family, Akt2 has been shown to be increased in approximately 30% of ovarian cancers [21], and it has been acknowledged to be involved as an anti-apoptotic factor in a number of different cell death paradigms. Similar to the previous investigation [10], the modulation of cytoplasmic p21 in this study was accomplished through activation or inactivation of the expression of Akt2. Induction of p21 translocation into the cytoplasm by transfection of constitutively active Akt2 in A2780 led to the increased resistance to PTX, while inhibition of p21 translocation into the cytoplasm by transfection of Akt2 shRNA into A2780 cells significantly increased PTX-induced apoptosis. To further validate this, PTX resistance is directly regulated by cytoplasmic p21, knockdown of cytoplasmic p21 by p21 siRNA transfection in Akt2 overexpressed cells in which p21 localized mainly in the cytoplasm was performed. As anticipated, p21si transfection resulted in a notable decrease in cytoplasmic p21 compared with control groups, while there was no significant change in the expression of Akt2 and p-Akt. Therefore, it can be safely concluded that cytoplasmic p21 represent a novel therapeutic target for PTX resistance in ovarian tumor.

Conclusion

The present study demonstrated that the accumulation of cytoplasmic p21 diminishes the sensitivity of A2780 ovarian cancer cells to PTX. This may provide a new target for reversing resistance to PTX in ovarian cancer in the future clinical practice.

Acknowledgement

This work was supported by the National Science Foundation (No. 81101971; No. 81471508; No. 81001151), Guangdong Natural Science Foundation (No. B2011295, No. S2011040006012), and Shenzhen Scientific Program (No. 20110422597, No. 201002006).

References

Cytoplasmic p21 is responsible for paclitaxel resistance in ovarian cancer A2780 cells


Address reprint requests to:
H. XU, M.D.
Department of Gynecology & Obstetrics
Shenzhen People’s Hospital,
Second Clinical Medical College,
Jinan University,
No. 1017 Dongmen Road, Shenzhen (China)
e-mail: crazy332@126.com
Introduction

The prevalence of endometrial adenocarcinoma (EC) in women at or below the 40 years of age is known to be distinct in different studies and is reported approximately between 2.9% and 14.4% [1-3]. Nulliparity, increased estrogen level, insulin resistance (with or without overt diabetes), hypertension, polycystic ovarian syndrome (PCOS), infertility, early onset of menstruation, and late menopause are mostly encountered causative factors for EC. However young women with the diagnosis of this disease are often obese or overweight with anovulation [4].

Menometrorrhagia with or without pain, intermenstrual bleeding, and the presence of risk factors may demonstrate cancer, and endometrial investigation is recommended. The disease is often well-differentiated and diagnosed frequently in early stage with limited myometrial invasion in young women [1, 4, 5].

Up to 20% of EC cases occur between 40 and 50 years and the remaining 75% occur in patients over 50 years. Thus endometrial sampling is recommended in patients with abnormal uterine bleeding at ≥ 35 or ≥ 40 years in different studies [3, 4, 6, 7].

EC is staged surgically according to International Federation of Gynecology and Obstetrics (FIGO) guidelines in 2009 [8]. The staging operation is composed of total abdominal hysterectomy with bilateral salpingo-oophorectomy, peritoneal washing, omentectomy, and pelvic and para-aortic lymphadenectomy. Depending on disease stage and tumor grade, surgery, radiation therapy, hormone therapy, and chemotherapy are used either alone or sequentially [9]. A crucial point is the age of the women at the diagnosis when the fertility issue is extremely important. Administration of high-dose progesterone has been recommended in women with clinical Stage IA and grade-I tumors that request to preserve fertility [10].

The purpose of this retrospective study was to investigate the incidence, clinicopathological features, and experience of treatment outcomes of patients with endometrial adenocarcinoma (EC) at ≤ 40 years of age in a gynecologic oncology reference center in Ankara, Turkey. Materials and Methods: This retrospective study included 577 patients with EC, diagnosed and treated between 2007 and 2013. Results: The incidence of EC ≤ 40 years of age was 5.1% (n: 30). The mean age at diagnosis was 35.5 (range: 27-40). Most of the patients with EC were overweight or obese. However, 23% had normal body mass index (BMI). Infertility was seen as a risk factor in 38.4%. The mean duration of postoperative follow-up was 38.3 months with rates of disease persistence and recurrence 14.2% and 28.5%, respectively. Conclusion: The disease is diagnosed usually in its early stage and has a good prognosis. Appropriately selected patients with fertility desire have the opportunity to conceive with conservative management.

Key words: Endometrial adenocarcinoma; Gynecological cancer; Young women; Infertility; Obesity.
index (BMI), gravida, and parity were evaluated. Moreover, result of the last Papanicolaou (Pap) smear, prediagnostic endometrial thickness, levels of CA-125 (cancer antigen-125), CA-199 (cancer antigen-199), CA-153 (cancer antigen-153), CEA (carcinoembryonic antigen),AFP (alpha-fetoprotein), endometrial sampling results, tumor grade, disease stage with findings in surgical specimens, treatment approaches, and postoperative follow-up periods were investigated.

The patients were staged according to FIGO 2009 guidelines with total abdominal hysterectomy, bilateral salpingo-oophorectomy, peritoneal washing, omentectomy, and pelvic and para-aortic lymphadenectomy. The patients that refused the surgery due their fertility desire were staged clinically and treated with oral course of megestrol acetate 160 mg/day 1x1 for six months, with or without the levonorgestrel-containing intrauterine device (IUD).

Statistical Analysis
The descriptive statistical analysis was performed with SPSS version 20.

Results
Based on the present data, there were 30 patients that had the diagnoses of EC at or less than 40 years of age with the incidence of 5.1%. The minimum age at diagnosis was 27 years (mean: 35.5, range: 27-40) and the mean weight was 78.4 kg (range: 64-98). Table 1 presents the clinical data of all patients with accompanying medical illnesses.

There were 12 nulligravid patients (46.2%), eight of which were infertile, 14 multigravid patients (53.8%) 12 of which had given at least one birth, and 14 nulliparous patients (53.8%) two of which had recurrent pregnancy loss. Infertility was seen as a risk factor only in ten patients (38.4%).

Twenty patients (76.9%) had a primary symptom of menometrorrhagia, three (11.5%) had pelvic pain, and two had (7.6%) pelvic pain with hypermenorrhea. There was only one patient without apparent symptom and her diagnosis was made after endometrial sampling due to increased endometrial thickness on the transvaginal ultrasonographic evaluation shortly after her menstruation.

On the preoperative diagnostic workup, only two patients had adnexal mass; one patient with bilateral 5x6 cm solid-cystic ovarian and the other patient with right ovarian cystic mass. The Papanicolaou tests (Pap test) were evaluated in 22 patients (84%). Eleven patients’ tests (50%) were reported as normal and the other ten patients (45%) with an inflammatory reaction. Only one patient (4%) had the presence of abnormal glandular cells on the Pap test evaluation. The mean thickness of the endometrium on transvaginal ultrasonography was 15.8 mm (range: 7 - 57 mm). PCOS was diagnosed in seven patients (26%) according to the Rotterdam 2003 criteria [12].

Endometrial sampling revealed grade-I endometrioid EC in 12 patients (46%). Up to 23% (n = 6) of patients’ pathologic diagnoses were complex atypical hyperplasia (CAH) with grade-I EC could not be excluded. Two patients’ biop-

<table>
<thead>
<tr>
<th>Table 1. — Clinical details of all patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (27-40 yrs)</td>
</tr>
<tr>
<td>&lt; 30</td>
</tr>
<tr>
<td>&lt; 30</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Normal (20 - 25)</td>
</tr>
<tr>
<td>Overweight (25 - 30)</td>
</tr>
<tr>
<td>Obese (&gt; 30)</td>
</tr>
<tr>
<td>Morbid-obese (&gt; 40)</td>
</tr>
<tr>
<td>Gravida</td>
</tr>
<tr>
<td>&gt; 1</td>
</tr>
<tr>
<td>Parity</td>
</tr>
<tr>
<td>&gt; 1</td>
</tr>
<tr>
<td>Medical history</td>
</tr>
<tr>
<td>Hypothyroidism</td>
</tr>
<tr>
<td>Hypercortisolism</td>
</tr>
<tr>
<td>HT</td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
</tr>
<tr>
<td>Hyperprolactinemia</td>
</tr>
<tr>
<td>Rheumoid arthritis</td>
</tr>
<tr>
<td>Decreased protein C, S activity</td>
</tr>
<tr>
<td>PCOS</td>
</tr>
<tr>
<td>Infiltrity</td>
</tr>
<tr>
<td>Cervical cytology</td>
</tr>
<tr>
<td>Inflammatory/reactive</td>
</tr>
<tr>
<td>AGC</td>
</tr>
<tr>
<td>CA-125</td>
</tr>
<tr>
<td>CA-199</td>
</tr>
<tr>
<td>CA-153</td>
</tr>
<tr>
<td>Endometrial sampling</td>
</tr>
<tr>
<td>GII EC</td>
</tr>
<tr>
<td>GIII</td>
</tr>
<tr>
<td>CAH with GI EC could not be excluded</td>
</tr>
<tr>
<td>CH with GI EC could not be excluded</td>
</tr>
<tr>
<td>CAH</td>
</tr>
<tr>
<td>Initial treatment</td>
</tr>
<tr>
<td>Medical</td>
</tr>
<tr>
<td>2nd course treatment</td>
</tr>
<tr>
<td>Medical</td>
</tr>
<tr>
<td>Histology at staging</td>
</tr>
<tr>
<td>Endometrioid with focal clear cell component</td>
</tr>
<tr>
<td>Endometrioid with serous and clear cell components</td>
</tr>
<tr>
<td>Serous</td>
</tr>
<tr>
<td>No tumor</td>
</tr>
<tr>
<td>Surgical Stage</td>
</tr>
<tr>
<td>IB</td>
</tr>
<tr>
<td>IIC2</td>
</tr>
<tr>
<td>Adjuvant therapy</td>
</tr>
<tr>
<td>CT</td>
</tr>
<tr>
<td>Follow-up (months)</td>
</tr>
<tr>
<td>25 - 48</td>
</tr>
<tr>
<td>&gt; 48</td>
</tr>
</tbody>
</table>

BMI: body mass index (kg/m²); PCOS: polycystic ovarian syndrome; CA-1: cancer antigen; HT: hypertension; G: grade; EC: endometrial adenocarcinoma; CAH: complex atypical hyperplasia; CH: complex hyperplasia; RT: radiotherapy; CT: chemotherapy.
Endometrial adenocarcinoma in young-aged women: a Turkish population study

669

sies (7%) were reported as CAH. Another patient that had an endometrial polyp extending throughout the vaginal lumen resulted in an adenomyoma with components of complex hyperplasia without atypia with grade-I EC that could not be excluded. Three patients, one with villoglandular differentiation, had grade-II endometrioid EC. Two other patients, one with grade-I endometrioid and the other with grade-III EC, also had focal clear cell and serous and clear cell components. There was only one patient with serous EC.

The preoperative mean CA-125, CA-199, CA-153, CEA, and AFP tumor marker levels were 66.8 U/ml (range: 4.6 - 762.0), 22.1 U/ml (range: 0.6 - 209.5), 14.2 U/ml (range: 0.4 - 29.1), 1.4 ng/ml (range: 0.1 - 4.3), and 2.2 ng/ml (range: 0.8 - 5.3), respectively.

The disease stages at surgery were Stage IA (n= 16, 80%), Stage IB (n= 3, 15%), and Stage IIC2 disease with para-aortic lymph node involvement (n= 1, 5%) respectively. Three of the infertile patients were surgically staged immediately after the diagnosis of EC according to patients’ desire. The other three infertile patients were treated with six months of oral progesterone therapy. Only one of them used the levonorgestrel-containing IUD in addition to oral therapy. However, staging surgery was the end point in these patients due to disease persistence at six months, disease recurrence in the form of CAH after assisted reproductive technology (ART) trial at 18 months and abortion after ART trial at 12 months, respectively. The other four patients who desired future fertility and grade-I, clinical Stage IA EC had no surgery and were treated with oral and progesterone containing IUD treatment with negative endometrial biopsy. After treatment, one of them dropped out from the follow-up at three months. One of the patients had two spontaneous pregnancies and deliveries. Another one had two ART trials and had one delivery, and developed CAH at 36 months. She was treated with a second course of medical therapy with the same protocol and is now disease-free. The last patient had yet no ART trial.

The entire endometrial cavity was affected by the tumor in five patients with ischemic invasion without endocervical canal entrapment. Moreover one patient with Stage IIC2 disease had positive peritoneal washing, cervical stroma, and right adnexal invasion with omentum and sigmoid colon involvement. Omentectomy evaluations revealed normal adipose tissue in 18 patients and chronic inflammatory reaction in one patient. Appendectomy specimens demonstrated normal appendix tissue (n= 15), periappendicitis (n= 3), obliteration (n= 1), and lymphoid hyperplasia (n= 1). The lymphovascular space involvement was diagnosed in five patients with Stage IA (n= 1), IB (n= 3), and IIC2 (n= 1) disease. The mean pelvic and para-aortic lymph node counts were 74.75 (range: 37 - 133). Only one patient with serous tumor had para-aortic lymph node involvement.

Two patients (9%) who had had biopsy proven EC had no malignancy in the hysterectomy specimens after staging. Endometrial sampling results of these patients were complex atypical hyperplasia with grade-I endometrioid EC and grade-I endometrioid EC, respectively. These patients were 31 vs. 40 years of age one with a history of infertility. The permanent pathology after staging surgery revealed simple endometrial hyperplasia with atypia and normal endometrium with bilateral mucinous cystadenoma in these patients.

The mean duration of postoperative follow-up was 38.3 months (range: 3-75). Only one patient on progesterone treatment dropped out from the regular check visits at three months. There are two patients in close follow-up with the pelvic lymphocyst formation after the staging surgery. Three patients with Stage IB disease had adjuvant radiotherapy. Another patient with serous tumor and positive para-aortic lymph nodes had adjuvant chemotherapy with carboplatin and paclitaxel.

Discussion

EC is more frequently seen in older, postmenopausal women. However women have changed their lifestyles in the last decades. Due to westernization of diet, getting married in later reproductive years, decreased attempts on childbearing and breast-feeding, increased use of oral contraceptives, and genetic tendency predispose the women more vulnerable to EC. The incidence of EC in young aged women at ≤ 40 years of age is reported to be roughly 5% and reaches as high as 14% in different studies [1-3]. The present authors found a 5.1% incidence of EC in young aged women at ≤ 40 years similar to literature. Moreover the incidence of infertility complicating the treatment decision was 1.7% in the present study.

The mean weight and the BMI values of the present patients supported the data in the literature related with the increased incidence of EC in overweight and obese patients with the effects of hyperestrogenism [13]. Nevertheless 23% patients with EC had normal BMI. The accompanying medical conditions with EC and normal BMI were nulliparity, PCOS, hypothyroidism, impaired fasting glucose levels, hyperprolactinemia, presence of myoma uteri and history of previous ART trials. Hypercortisolism and hypertension were the associated risk factors for EC in patients with higher BMI.

In this study, 76.9% of the patients had menometrorrhagia and the other 7.6% had hypermenorrhea with pelvic pain. However the patients with infertility had no recognized bleeding abnormality by themselves until detailed questioning after the diagnosis of EC due to endometrial irregularity on transvaginal ultrasonography or hysterosonography (H/S) during infertility work-up diagnosed. The diagnosis of EC may be difficult since the dysfunctional uterine bleeding is common in reproductive ages and the underlining pathology may be overlooked if the appropriate evaluation is not performed [14].
There are studies in the literature supporting increased endometrial pathology in the presence of endometrial glandular cells in the Pap test of women over 40 years age. The reproductive aged women have the increased possibility of desquamated endometrial cells on Pap tests due to cyclic alterations of the endometrium throughout the menstrual cycle. However the importance of the presence of these cells in respect to endometrial pathologies in women at ≤ 40 years of age is not known [15, 16]. Only one of the patients in this study had abnormal glandular cells on the Pap test evaluation and the 45% patients had inflammatory reactive alterations. Moreover the increased endometrial thickness was the most remarkable sign of endometrial pathology necessitating endometrial sampling or H/S in the preoperative diagnostic evaluation.

The endometrial sampling results and the endometrial pathologies diagnosed in the hysterectomy specimens are known to show 16% discrepancy [17]. The discrepancy rate was 9% in the present study which is much less than the literature. There were two patients with the diagnosis of no malignancy in the permanent pathology. However the preoperative tissue diagnosis was CAH with grade-I EC and grade-I EC, respectively. As suggested in the literature, these patients were accepted as having focal grade-I EC possibly on a polyp structure. Postoperative regular visits were recommended to these patients.

CA-125 levels have been clinically used for EC and increased levels correlate with the disease stage or histopathologic factors. Also it appears to predict the lymph node metastasis and advanced stage disease [18]. Elevation of CA-125 was diagnosed in 25% of patients with Stage IA and in all patients with Stage IB and IIIC2 disease. However there was only one patient with positive lymph node metastasis in the present study. The concurrent CA-199 value was increased in 6.2% and 33% in Stage IA and IB disease, respectively. The levels of CA-153, CEA or AFP levels were within normal levels in all patients.

In industrialized countries, the incidence of infertility is estimated between 8.5 - 20% [19] much more common than the 5% incidence of EC in young women. Thus evaluations during infertility work-up provide an opportunity to evaluate the endometrium and its pathologies in these patients, when the disease is in its early stage and symptom free. As mentioned in the literature conservative management of EC with oral progestational drugs in the early disease stage is effective without compromising oncological outcome in young women [20]. However there is no consensus concerning the type, dose, and duration of progestrone. The positive effects of combined treatment with oral and levonorgestrel-containing IUD are presented in the literature [21]. The oral progestational drug was megestrol acetate 1 x 160 mg for six months in the present patients. Furthermore only five patients (71.4%) had also used the levonorgestrel-containing IUD.

Dursun et al. reported the mean EC persistence and recurrence rates as 21% (range: 0% - 58%) and 33% (range: 0% - 67%), respectively, in their literature review [20]. The disease persistence and recurrence rates were 14.2% and 28.5% in the present study, similar with the literature. One of the patients had disease persistence confirmed by tissue biopsy at six months of therapy and she underwent surgery. Her definitive diagnosis was grade-II, Stage IB EC with lymphovascular space invasion (LVSI) and she had adjuvant radiotherapy. The second patient underwent surgery after a failed ART trial at 18 months due to disease recurrence in the form of CAH. Her definitive diagnosis was grade-I, Stage IA EC without LVSI and she had no adjuvant therapy. These two patients were treated only with oral progesterone therapy. Another patient with clinical Stage IA disease treated with oral progesterone and levonorgestrel-containing IUD had recurrent disease in the form of CAH at 36 months. She underwent a second course of therapy with the same regimen and is now disease-free.

The present study had some limitations. The retrospective nature of this study was one of the obstacles. Although there were infertile patients diagnosed during infertility work-up, the present results cannot present the EC incidence in the infertile women. Another limitation was that although familial cancer syndromes might predispose patients with normal BMI to EC and receptor status of the tumor affects the treatment outcome, the authors did not evaluate their patients with the genetic testing for Lynch Syndrome or the hormone receptor status.

In conclusion, it must be recognized that although the rate is low, young women are prone to EC as postmenopausal women and they are more frequently obese. The disease is noticed usually in early stage with well-differentiation. Diagnostic imaging adds benefits on gynecologic examination for the diagnosis of unexpected endometrial pathologies. The accompanying risk factors for EC should be questioned when dealing with young patients during infertility evaluations. Appropriately selected patients have change to conceive with conservative management after progesterone treatment.

References

Endometrial adenocarcinoma in young-aged women: a Turkish population study


[12] Duijkers I.J., Klipping C.: “Polycystic ovaries, as defined by the 2003 Rotterdam consensus criteria, are found to be very common in young healthy women”. Gynecol. Endocrinol., 2010, 26, 152.


Address reprint requests to:
N. CETINKAYA, M.D.
Zekai Tahir Burak Women’s Health Education and Research Hospital
Department of Gynecologic Oncology
06230, Hamamonu, Ankara (Turkey)
e-mail: cetinkayanimlufer@gmail.com
Comparison of whole-body PET/PET-CT and conventional imaging procedures for distant metastasis staging in patients with breast cancer: a meta-analysis

Zhe Sun1*, Yu Li Yi2*, Yu Liu2, Jian Ping Xiong1, Chao Zhu He2

1 Oncology Department, The First Affiliated Hospital of Nanchang University, Nanchang
2 Nursing College of Nanchang University, Nanchang (China)

Summary
Aim: To compare the performance of whole-body PET/PET-CT with that of conventional imaging procedures for the overall assessment of distant metastasis in patients with breast cancer. Materials and Methods: The authors performed a meta-analysis of all available studies of whole-body PET/PET-CT compared with conventional imaging procedures. They calculated sensitivities, specificities, positive likelihood ratios, negative likelihood ratios, and constructed summary receiver operating characteristic (ROC) curves using bivariate regression models for whole-body PET/PET-CT and conventional imaging procedures, respectively. Results: Across six studies (609 patients), sensitivity, specificity, positive likelihood ratios, and negative likelihood ratios of whole-body PET/PET-CT were 0.99 (95% confidence interval [CI] = 0.88–1.00), 0.95 (95% CI = 0.89–0.98), 21.1 (95% CI = 8.2–55.5), and 0.02 (95% CI = 0.001–0.13), respectively, and of conventional imaging procedures they were 0.57 (95% CI = 0.37–0.74), 0.88 (95% CI = 0.78–0.94), 4.8 (95% CI = 2.8–8.2) and 0.49 (95% CI = 0.33–0.74), respectively. Conclusion: Compared with conventional imaging procedures, whole-body PET/PET-CT had excellent diagnostic performance for distant metastasis staging in patients with breast cancer.

Key words: PET; Breast cancer; Distant metastasis; Meta-analysis.
Table 1. The clinical characteristics and study quality of included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Origin</th>
<th>Design</th>
<th>Imaging system</th>
<th>No. of patients</th>
<th>Age (y)</th>
<th>Analysis method</th>
<th>Follow-up time</th>
<th>QUADAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schirrmeister et al. [6], 2001</td>
<td>Germany</td>
<td>Prosp</td>
<td>PET-CT</td>
<td>60</td>
<td>28-86</td>
<td>QL+QN</td>
<td>NR</td>
<td>11</td>
</tr>
<tr>
<td>Mahner et al. [7], 2008</td>
<td>Germany</td>
<td>Retro</td>
<td>PET</td>
<td>119</td>
<td>28-89</td>
<td>QL</td>
<td>11(mean)</td>
<td>12</td>
</tr>
<tr>
<td>Fuster et al. [8], 2008</td>
<td>Spain</td>
<td>Prosp</td>
<td>PET-CT</td>
<td>60</td>
<td>40-82</td>
<td>QL+QN</td>
<td>≥12</td>
<td>11</td>
</tr>
<tr>
<td>Aukema et al. [16], 2010</td>
<td>Netherlands</td>
<td>Prosp</td>
<td>PET-CT</td>
<td>56</td>
<td>27-74</td>
<td>QL</td>
<td>13.4(mean)</td>
<td>11</td>
</tr>
<tr>
<td>Nikura et al. [17], 2011</td>
<td>USA</td>
<td>Retro</td>
<td>PET-CT</td>
<td>225</td>
<td>23-84</td>
<td>QL</td>
<td>≥24</td>
<td>12</td>
</tr>
<tr>
<td>Koolen et al. [18], 2011</td>
<td>Netherlands</td>
<td>Prosp</td>
<td>PET-CT</td>
<td>60</td>
<td>19-75</td>
<td>QL+QN</td>
<td>≥6</td>
<td>12</td>
</tr>
</tbody>
</table>

Prosp = prospective; Retro = retrospective; QL = qualitative; QN = quantitative; QUADAS = the number of items assessed as “yes” in the QUADAS tool.

for searching relevant articles with the following combination of search terms: (a) PET OR positron emission tomography, (b) breast cancer, and (c) staging OR distant metastases. References of the retrieved articles were also screened for additional studies. Reviewers of eligible studies were contacted and asked to supplement additional data when key information relevant to the meta-analysis was missing. The authors had no language restrictions for searching and identifying relevant studies.

Study selection

Studies were eligible for inclusion based on the following criteria: (1) whole-body PET/PET-CT and conventional imaging procedures evaluated breast cancer patients of all ages in any disease stage regardless of treatment status, (2) distant metastases findings were confirmed with histopathologic analysis and/or clinical and imaging follow-up, (3) the two imaging modalities (whole-body PET/PET-CT and conventional imaging procedures) were performed within three months of one another, (4) the studies were based on a per-patient analysis, (5) the studies including at least ten patients that were selected for inclusion in this meta-analysis, and (6) when data or subsets of data were presented in more than one article, the article with the most details or the most recent article was chosen. Studies were excluded based on the following criteria: (1) only whole-body PET/PET-CT or conventional imaging procedures was performed, (2) totals of true positives, false positives, true negatives, and false negatives were not provided, and (3) no data from a sub-analysis were provided.

Data extraction and quality assessment

Two reviewers (Y.K.L. and Q.Y.L) independently extracted the relevant data from each article and recorded these data on a standardized form and any difference was resolved by consensus. Data was extracted from the studies, including authors, year of publication, study design, sample size, imaging methods (whole-body PET/PET-CT or conventional imaging procedures) and imaging technical characteristics, reference standard, and totals of true positives, false positives, true negatives, and false negatives.

The authors assessed the methodological quality of the studies using the quality assessment for studies of diagnostic accuracy (QUADAS) tool [9]. It is the first systematically developed evidence-based quality assessment tool to be used in systematic reviews of diagnostic accuracy studies. The QUADAS tool includes 14 items, each of which is assessed as “yes”, or “no”.

Statistical analysis

The authors used bivariate regression models to obtain weighted overall estimates of the sensitivity and specificity as the main outcome measures, and to construct hierarchical summary receiver operating characteristic (HSROC) curves for whole-body PET/PET-CT and conventional imaging procedures, respectively [10-11]. Based on random-effects models, this bivariate approach accounts for potential between-study heterogeneity and incorporates the possible correlation between the sensitivity and the specificity. By using the pooled sensitivities and specificities, the authors also calculated diagnostic odds ratio (DOR), positive likelihood ratios (PLR), and negative likelihood ratios (NLR) for whole-body PET/PET-CT and conventional imaging procedures, respectively [11-12]. The discriminating ability is better with higher PLR and lower NLR. Although there is no absolute cutoff, a good diagnostic test may have PLR greater than 10.0 and NLR less than 0.1. All analyses were conducted with Stata version 11.0.

Results

Study selection and description

After independent review, ten articles dealing with the diagnostic performance of whole-body PET/PET-CT were compared with conventional imaging procedures for the detection of distant metastases in breast cancer patients. Of these publications, two articles [13, 14] were excluded because insufficient data were reported to enable construction of a 2×2 table of true-positive, false-negative, false-positive, and true-negative values. Two articles [5, 15] were excluded because the data was already reported in an included article [7]. Consequently, six articles [6-8, 16-18] were eligible for meta-analysis. A total of 609 patients were analyzed for the diagnostic accuracy of whole-body PET/PET-CT and conventional imaging procedures for the detection of distant metastases in the eligible articles. In four articles (66.7%), the study design was prospective (Table 1).

Study quality

The authors assessed the quality of the six articles according to the 14-item QUADAS assessment tool. Eleven of the 14 items were scored in all included articles. No study (0%) reported that all patients received the same reference test regardless of the index test result (item 6) and the reference standard was blinded to the index test results (item 11). Representative spectrum was present in 33.3% [7, 17] of the six articles (item 1). When considering all six studies (609 patients) with data on a per-patient basis [6-8, 16-18], sensitivity, specificity, and DOR of whole-body PET/PET-CT were 0.99 (95% confidence interval [CI] = 0.88–1.00), 0.95 (95% CI = 0.89–0.98) and 1407 (95% CI = 82–24276), respec-
Comparison of whole-body PET/PET-CT and conventional imaging procedures for distant metastasis staging in patients etc.

Figure 1. — HSROC curves of whole-body PET/PET-CT for the detection of distant metastases in breast cancer patients.

Figure 2. — HSROC curves of conventional imaging procedures for the detection of distant metastases in breast cancer patients.
tively, and of conventional imaging procedures were 0.57 (95% CI = 0.37–0.74), 0.88 (95% CI = 0.78–0.94), and 8.8 (95% CI = 4.8–19.8), respectively.

Likelihood ratio syntheses gave an overall PLR of 21.1 (95% CI = 8.2–55.5) and NLR of 0.02 (95% CI = 0.001–0.13) for whole-body PET/PET-CT on a per-patient basis. The respective figures for conventional imaging procedures were 4.8 (95% CI = 2.8–8.2) and 0.49 (95% CI = 0.33–0.74), respectively.

HSROC curves showed the overall good diagnostic performance of whole-body PET/PET-CT and conventional imaging procedures for all eligible studies (Figures 1-2). Overall weight area under the HSROC curves was 0.99 (95% CI = 0.98–1.00) and 0.83 (95% CI = 0.79–0.86), respectively.

Assuming a prevalence of distant malignancies of 10%, 20%, and 30% in cancer patients on a per-patient basis, NPVs for whole-body PET/PET-CT were 99.8%, 99.6%, and 99.3%, respectively, for conventional imaging procedures were 95%, 89%, and 83%, respectively.

Discussion

The presence of distant metastases is the most important predictor of survival in patients with breast cancer. A fast, accurate, and reliable diagnostic workup before treatment is of utmost importance because of its impact on treatment and prognosis. In this meta-analysis, the authors obtained summary estimates and summary ROC curves for the diagnostic accuracy of whole-body PET/PET-CT and conventional imaging procedures for the detection of distant metastases in patients with breast cancer. Compared with conventional imaging procedures, whole-body PET/PET-CT was found to have higher sensitivity (99% vs. 57%) for the detection of distant metastases in patients with breast cancer.

The DOR is a single indicator of test accuracy that combines the data from sensitivity and specificity into a single number [19]. It is the ratio of the odds of a positive test in a patient with disease relative to the odds of positive test in a patient without disease and has a value that ranges from 0 to infinity, with higher values indicating better discriminatory test performance [19]. This meta-analysis showed that the pooled patient-level DOR for whole-body PET/PET-CT and conventional imaging procedures was 1407 and 8.8, respectively, indicating that whole-body PET/PET-CT had higher accuracy than conventional imaging procedures for the detection of distant metastases in patients with breast cancer.

Since the HSROC curves are not easy to interpret and use in clinical practice, and since likelihood ratios are considered to be more clinically meaningful, both PLR and NLR were calculated and served as the present authors’ measures of diagnostic accuracy [20, 21]. Likelihood ratios of >10 or < 0.1 indicated high accuracy. The patient-level PLR values of for whole-body PET/PET-CT and conventional imaging procedures were 21.1 and 4.8, respectively. Only the value (21.1) for whole-body PET/PET-CT was therefore high enough to diagnose distant metastases. On the other hand, the patient-level NLR values for whole-body PET/PET-CT and conventional imaging procedures were found to be 0.02 and 0.49, respectively. These data suggested that only a negative examination result of whole-body PET/PET-CT may be used alone as a justification to rule out distant metastases in patients with breast cancer.

Whole-body magnetic resonance imaging (WB-MRI), particularly with the introduction of moving patient platforms, improved integrated surface-coil technology, and ultrafast data acquisition, had become clinically feasible for the assessment of distant malignancies in patients with malignant tumors [22, 23]. One study [24] regarding the accuracy of 1.5 and 3.0T WB-MRI and 18FDG-PET/CT for distant metastasis staging in patients with breast cancer also showed similar sensitivity (95% vs. 91%, p > 0.05), and specificity (92% vs. 86%, p > 0.05) on a per-lesion analysis. These results indicated that WB-MRI may be used as a first-line imaging technique for distant metastasis staging in patients with breast cancer.

The present meta-analysis had several limitations. First, the exclusion of conference abstracts, and letters to the editors may have led to publication bias. Although publication bias can be tested by using funnel plots, they were not performed in this meta-analysis because of the limited number of included studies. Second, there was no single clinical and imaging follow-up strategy, which may have affected the evaluation of whole-body PET/PET-CT and conventional imaging procedures. Actually, there is no well accepted gold standard, which is a common barrier to all studies assessing different imaging procedures for diagnostic accuracy in detection of distant metastases. Third, a wide variation in patient population, imaging techniques, study design, and quality in these selected studies may have affected the estimates of diagnostic accuracy of whole-body PET/ PET-CT and conventional imaging procedures. This was not analyzed because the number of included studies was small.

Compared with conventional imaging procedures, whole-body PET/PET-CT had excellent diagnostic performance for the detection of distant metastases in patients with breast cancer.

References

Comparison of whole-body PET/PET-CT and conventional imaging procedures for distant metastasis staging in patients etc.


Address reprint requests to:
CHAO ZHU HE, M.D.
Oncology Department
The First Affiliated Hospital of Nanchang University
Nanchang, 330006 (China)
e-mail: chaozhu_he@163.com
Introduction

The infection by HPV is considered a necessary cause but not enough to develop cervical cancer. Most women infected by HPV will never present cervical cancer. Therefore, other cofactors are needed for the progression from cervical infection by HPV to cervical cancer. Cofactors of progression that contribute to the persistence of the infection are classified in: viral, genetics, and environmental.

Smoking is one of the environmental cofactor identified with the risk of suffer precarcinogenic lesions and cervical cancer. Among women infected by HPV, tobacco is the most important cofactor of progression, it increases the risk from two to four times compared to non-smoking women. This increase is also identified in passive smokers. Moreover, stopping smoking is associated with a decrease of the size of the lesion of cervical intraepithelial neoplasia (CIN) [1-5].

The aim of this study was to confirm the relationship between smoking habits and infection by HPV and the risk of presenting cervical intraepithelial lesions and cervical carcinoma among patients who smoke.

Materials and Methods

Study population

Prospective, cross-sectional descriptive study. A total of 1,007 patients were recruited among women who visited the cervical pathology clinic of Sant Joan de Déu University Hospital in Barcelona (Spain) between January 2003 and March 2011 [6]. Patients were asked specifically about their smoking habits. Differences between groups were considered statistically significant at \( p < 0.05 \). Results: In patients studied, 48.7% were smokers. The average number of cigarettes per day among smoking patients was 7.07 (1-40). In the of patients with HPV infection, 53% were smokers versus 37% of patients without HPV infection (\( p < 0.05 \)). The average number of cigarettes per day among patients with HPV infection was 7.64 cigarettes/day versus 5.55 cigarettes/day among patients without HPV infection (\( p < 0.05 \)). In the patients with high-risk HPV genotypes infection, 54.5% were smokers versus 43.2% of patients without high-risk HPV infection (\( p < 0.05 \)). Risk of HPV infection increases 1.905 times among smoking patients versus no smoking patients (\( OR = 1.905, CI 95\% (1.426 - 2.545), p < 0.05 \)). Among patients with changes associated to HPV and atypical cells, there were 29.2% and 14.4% of smokers, respectively, versus 45.5%, 55.6%, and 48.6% of smokers among patients with grade 1 cervical intraepithelial neoplasia (CIN 1), CIN 2-3, and carcinoma, respectively (\( p < 0.05 \)). Risk of CIN 2-3 or cervical carcinoma cervical increases 1.642 times among smoking patients versus no smoking ones (\( OR = 1.642, CI 95\% (1.325 - 1.884), p < 0.05 \)). Conclusions: Smoking interferes in the increase of HPV infection prevalence and in an increased risk of CIN and cervical carcinoma. Risk also increases with more cigars smoked per day.

Key words: Human papillomavirus infection; Cervical cancer; Cervical intraepithelial neoplasia Grade 1, 2, 3; Smoking.
certain significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), and high-grade squamous intraepithelial lesions (HSIL). A colposcopy was also performed applying acetic acid solution 3-5%. The classification used was proposed by the International Federation of Cervical Pathology and Colposcopy in Barcelona 2002: normal findings (acetowhite epithelium, punctuation, mosaicism, negative to iodine stain, atypical vessels), findings suggesting invasive cancer and non-satisfactory colposcopy. Biopsy was performed in atypical zones observed in colposcopy. The sample was fixed with formaldehyde and analysed by the pathologist and classified as: negative, low-grade lesion, high-grade lesion, changes suggestive of infection by HPV, carcinoma, adenocarcinoma, vaginal intraepithelial neoplasia, and atypia.

**Genotype of human papilloma virus by PCR**

The cervical sample was obtained with a brush and was transported at room temperature to the Microbiology and Molecular Department. During the time of this study two techniques for genotype HPV were used consecutively (Linea Probe assay – LIPA – and microarray assay). In Linea Probe Assay (LIPA assay), for the extraction of cervical DNA the commercial kit Qiagen (QIAamp DNA Mini Kit) was used and samples were diluted in a final volume of 200 µl. For Microchip arrays Assay, the extraction of DNA was made using a lysis solution of proteinase (20 mg/ml). Purified DNA extracts were kept at -80°C in both cases. Linea Probe assay (LIPA assay) is based on the principle of reverse hybridization and gives us specific information for 25 different genotypes of HPV simultaneously (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74). Amplification of HPV-DNA is based on “SPF10 PCR primer set”, that amplify a piece of only 65 pairs of bases with the region “L1 open reading frame” (ORF). Part of the gene of human beta-globin (268pb) is amplified in each sample as a control. ESPF10-LIPA was used with ten µl of extracted DNA in a final volume of 100 µl.

Microchips array assay detects infections and co-infections up to 35 of most relevant genotypes of HPV (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, 71, 72, 73, 81, 82, 83, 85, and 89). It is based on microarray of low density joined to the inferior part of a classic Eppendorf two-ml tube. DNA amplification is based on a mix reaction that amplifies an extract of 450 pairs of bases with “L1 open reading frame” (ORF) region. A piece of 892 pairs of bases of human gene CFTR was amplified in each sample as a control.

**Results**

In all patients the diagnosis was confirmed histologically. In almost half of patients (47.3%, 476) a CIN 2-3 was diagnosed, in 38.7% (389) CIN 1, in 6% (61) atypical cells, in 3.7% (37) a carcinoma, and in 3.6% (36) HPV-associated changes. Most patients (77.9%, 686) were submitted to surgical treatment, of these, 23% were treated with large loop excision of transformation zone (LLETZ) and up to 54.9% with conisation (cone biopsy). In this latter group, 9.3% eventually underwent hysterectomy. A total of 740 women (73.2%) had HPV infection, among whom 86.4% (639) had a high-risk HPV genotype.

Of all patients 51.3% did not smoke and the remaining 48.7% smoked. The average number of cigarettes per day smoked by patients of the sample was 7.07 (1-40).

Of the patients with HPV infection, 53% were smokers compared to 37% of patients with no infection by HPV ($p < 0.05$). The average number of cigarettes per day among patients infected by HPV was 7.64 cigarettes/day, in contrast with an average of 5.55 cigarettes/day in patients with no HPV infection ($p < 0.01$) (Table 1). The risk of infection by HPV increased by 1.905 times among smoking women compared to non-smoking ($OR = 1.905$, I.C 95% (1.426-2.545), $p < 0.05$).

Of the patients with infection by high-risk HPV genotypes (HPV-HR), 54.5% were smokers compared to 43.2% of patients with no infection by HPV-HR ($p < 0.05$). The risk of infection by HPV-HR increases by 1.886 times among smoking women in contrast with non-smoking ($OR = 1.886$, I.C 95% (1.448 - 2.457), $p < 0.05$) (Table 2). In those patients diagnosed with changes associated to HPV and atypia, the authors found 29.2% and 14.4% of

| Table 1. — **Smoking patients depending on HPV infection** |
|-----------------|-----------------|-----------------|-----------------|
|                  | **HPV infection** |                  |                  |
|                  | Yes             | No              |                  |
| Number of patients | 716             | 264             | 980             |
| Cigars/day        | 7.64 cigars     | 5.55 cigars     | $p < 0.05$      |
| Smoking patients  | Yes             | No              |                  |
|                  | 379 (53%)       | 98 (37%)        | $p < 0.05$      |
|                  | No              | 337 (47%)       | 166 (63%)        |

| Table 2. — **Smoking patients depending on HR-HPV infection** |
|-----------------|-----------------|-----------------|-----------------|
|                  | **HR-HPV infection** |                  |                  |
|                  | Yes             | No              |                  |
| Number of patients | 619             | 97              | 716             |
| Cigars/day        | 7.81 cigars     | 6.52 cigars     | $p < 0.05$      |
| Smoking patients  | Yes             | No              |                  |
|                  | 337 (54.5%)     | 42 (43.2%)      | $p < 0.05$      |
|                  | No              | 282 (45.5%)     | 55 (56.8%)       |

| Table 3. — **Smoking patients depending on cervical lesion** |
|-----------------|-----------------|-----------------|-----------------|
|                  | **Diagnosis**   |                  |                  |
|                  |                 | Smoking patients | Total           |
|                  |                 | Yes             | No              |                  |
| CIN 1            | 6.33            | 146 (45.5%)     | 175 (54.5%)     | 321             |
| CIN 2-3          | 8.32            | 247 (55.6%)     | 197 (44.4%)     | 444             |
| Carcinoma        | 7.43            | 17 (48.6%)      | 18 (51.4%)      | 35              |
| HPV-associated   | 4.38            | 7 (29.2%)       | 17 (70.8%)      | 24              |
| changes          |                 |                 |                  |                 |
| Atypical cells   | 3.10            | 6 (14.4%)       | 23 (79.3%)      | 29              |
| Total            | 423             | 430             | 853             |                 |
smokers, respectively \( (p < 0.05) \). Patients with changes associated to HPV and atypical cells smoked an average of 4.38 and 3.10 cigarettes per day, respectively. On the other hand, in patients diagnosed of CIN 1, CIN 2-3, and carcinoma, the average of cigarettes per day were 6.33, 8.32, and 7.43, respectively \( (p < 0.05) \) (Table 3). The risk of CIN 2-3 or cervical carcinoma increased 1.642 times among smoking women compared to non-smoking \( (OR = 1.642, IC\ 95\% \ (1.325 - 1.884), p = 0.05)\) (Table 3).

**Discussion**

Different references in the literature show that multiple factors can take part in cervical carcinogenesis related with smoking, especially with a direct local effect as carcinogenic, because it increases the oxidative stress, and local immunosuppression caused by smoke, because it reduces the number of Langerhans cells, as well as the affectation of systemic immunity \[7\]. The fact that nicotine and specific tobacco carcinogens have been detected in cervical mucus of smokers supports the hypothesis that there is a synergic action between tobacco and HPV in the development of high-grade cervical intraepithelial lesions and cervical carcinoma. Tobacco increases cell proliferation and turnover in transformation zone. For example, tobacco has been related with ki67, a proliferation marker and metaplasia.

An in vitro study showed that the exposition of cervical cells to benzopyrene, the main carcinogen of tobacco, increased the number of HPV copies. This fact makes an increase of HPV infection persistence needed for the cervical lesion progression \[2, 3\].

Moreover, in some studies, it has been demonstrated that the infection by HPV in smoking women lasts longer and they have less probability of clearance of the infection \[8\], and a significant relationship between the reduction of lesion size and giving up smoking in patients with low-grade lesions has been proved.

In the present study, as in most information reviewed in the literature, it is proved that tobacco increases the prevalence of HPV infection \[9-17\]. The present authors also obtained a significant relationship between the risk of infection by high-risk HPV genotypes and being a smoker. OR for tobacco in women with positive HPV goes from 1.5 to 5. In the study by Yetimalar \et al\.[16], that included patients without cervical pathology, 63% of smokers had positive HPV compared to 40% of smoking patients with no infection by HPV. In the present results, although the authors only referred to patients with cervical pathology, they also observed similar results with 53% of smokers among those with HPV infection, compared to 37% of smokers among those with no infection by HPV. Giuliano \et al\.[8] proved that smoking increases the persistence of HPV infection and decreased the probability of clearance of infection. The average duration of the infection by HPV in smokers was 10.7 months compared to 8.5 months in patients who have never smoked.

In many case-control studies, as observed in the present study where patients with serious lesions smoked more cigarettes, it is demonstrated that tobacco increases the risk of developing high-grade intraepithelial lesions like cervical carcinoma. Moreover, most studies on the risks of tobacco, according to its intensity or its duration, show an increase of cervical carcinoma with a rise in tobacco exposition \[18, 19\].

Kjellberg \et al\.[2] in his study reports an increased risk of three times of CIN 2-3 in smokers compared to patients that have never smoked. The present authors also found an increasing risk of CIN 2-3 and cervical carcinoma in smokers with OR 1.64. The longer the smoking history, and the higher the number of cigarettes smoked per day, increases the OR for CIN 2-3. Matsumo \et al\.[20], monitored 516 patients with CIN 1 with cytology and colposcopy every four months. The probability of regression in two years was significantly lower in smokers than in those that have never smoked (55% vs. 68.8%, \( p = 0.004\)), and risk of persistence increased with the higher intensity and duration of smoking \( (p = 0.003 \text{ and } p < 0.001, \text{ respectively})\). Moreover, smokers presented an increase of persistence of infection \( (OR = 2.50, IC\ 95\% \ (1.30 - 4.81), p = 0.006)\). Hildesheim \et al\.[3] also reported an increased risk of CIN 2-3 and cervical carcinoma in smokers compared to those that have never smoked \( (OR = 2.3, IC\ 95\% \ (1.3 - 4.3), p < 0.05)\) that also increases the longer the smoking history and the higher the number of cigarettes smoked per day.

Smoking also affects the progression of cervical lesion. In Szarewski \et al\.[5] study, a significant relationship between giving up smoking and a reduction in size of cervical lesion was proved. Of those patients that stop smoking, 82% showed a reduction of 20% in lesion size compared to 28% of those that continued smoking. In the present study, the authors observed an increase of the incidence of cervical carcinoma in smokers but they have not followed the progression of cervical lesions through time.

**Conclusion**

Smoking interferes in the increase of HPV infection prevalence and in an increased risk of CIN and cervical carcinoma. Risk also increases with more cigars per day smoked. With the present results and data published in the literature, smoking women with cytological alterations should be monitored with more precaution than the general population.

**References**


Address reprint requests to:
E. MAZARICO, M.D.
Gran Via Carlos III-59 8-2
08028 Barcelona (Spain)
e-mail: emazarico@hsjdbcn.org
Survival in women with ovarian cancer with and without microsatellite instability

Y. Segev¹, S. Zhang¹, M.R. Akbari¹, P. Sun¹, T.A. Sellers², J. McLaughlin³, H.A. Risch⁴, B. Rosen⁵, P. Shaw⁶, J. Schildkraut⁷, S.A. Narod¹, T. Pal²
¹Womens College Research Institute, Womens College Hospital, University of Toronto Hospital, Toronto (Canada)
²Moffitt Cancer Center, Departments of Cancer Epidemiology, Biostatistics, Anatomic Pathology, and Experimental Therapeutics, Tampa, FL (USA); ³Samuel Lunenfeld Research Institute, and Dalla Lana School of Public Health, University of Toronto, Toronto (Canada); ⁴Department of Epidemiology and Public Health, School of Public Health, School of Medicine, Yale University, New Haven, CT (USA)
⁵Department of Gynecology-Oncology, Princess Margaret Hospital, and Department of Obstetrics and Gynecology, Faculty of Medicine, University of Toronto, Toronto (Canada); ⁶Department of Pathology, Princess Margaret Hospital, Toronto (Canada)
⁷Department of Community and Family Medicine, Duke Comprehensive Cancer Center, Duke University Medical Center, Durham, NC (USA)

Summary

Purpose of investigation: Microsatellite instability (MSI) is a hallmark of defective mismatch repair and is present in approximately 20% of ovarian cancers. It is not known if the presence of MSI predicts survival in women with epithelial ovarian cancer. Materials and Methods: Cases of epithelial ovarian cancer were ascertained from a population-based study in Ontario and tumour samples were tested for MSI, using five MSI markers. Patients were divided into MSI-high and MSI-low/normal, according to National Cancer Institute criteria. The authors compared the prevalence of specific prognostic factors in the two subgroups, including age, grade, stage, and histology. They estimated the hazard ratio for death from ovarian cancer associated with MSI-high and with other prognostic factors using a multivariate analysis. Results: A total of 418 ovarian cancer patients were included. One hundred and twenty-seven (19.7%) cancers were MSI-high. Subgroup analyses did not reveal any statistically significant differences for pathologic features associated with MSI status. No survival difference was seen according to MSI status. Conclusions: The presence of MSI in ovarian cancer is not associated with survival.

Keywords: Epithelial ovarian cancer; Mismatch repair genes; Survival; Microsatellite instability.

Introduction

Ovarian cancer is an infrequent manifestation of hereditary non-polyposis colon cancer (Lynch syndrome). Germline mutations in the mismatch repair (MMR) genes (MLH1, MSH2, and MSH6) predispose to Lynch Syndrome. The most commonly seen cancers are colorectal and endometrial cancers, with lifetime risks of 60-80% [1]. The lifetime risk of ovarian cancer in Lynch syndrome is estimated to be 12% [2]. Women with hereditary susceptibility to these three tumors may be identified through germline testing for deleterious mutations in MLH1, MSH2, and MSH6 [3-5].

The various cancers which occur in women who carry one of these mutations often demonstrate microsatellite instability (MSI). Micro-satellites are widely distributed repetitive DNA sequences composed of short, tandemly-repeated nucleotide motifs. In some cancer cells, these sequences exhibit a form of genetic instability characterized by the gain or loss of repeat units at multiple independent loci throughout the genome (MSI) [6]. Such alterations have been observed to accumulate in cells defective for DNA repair and occur with high frequency in tumours found in patients with Lynch syndrome and are one of the expressions for MMR deficiency. However only a minority of women who have a cancer that demonstrates MSI carry a germline mutation in one of the MMR genes. A proportion of cases of ovarian cancer exhibit MSI in the absence of a demonstrable mutation [7].

A cancer is MSI-high if two or more of five specifically-designated genetic loci exhibit MSI [8]. Among colon cancer patients, MSI-high has been shown to be a good prognostic feature [9-11]. The purpose of this study was to evaluate the impact of MSI-high on cancer prognosis in women with ovarian cancer.

Materials and Methods

Participants

Study subjects were selected from the Familial Ovarian Tumor Study (FOTS) in Toronto which is a population-based study of epithelial ovarian cancer [12]. Cases were identified through monitoring of pathology reports submitted to the Ontario Cancer Registry for province-wide recruitment. The study protocol was approved by the
institutional review board of the University of Toronto, and written informed consent was obtained from all participants. Eligible patients were those with pathologically-confirmed invasive ovarian cancer at age 20 years or above. The authors collected questionnaire with data on clinical, demographic, and family history information, and reviewed pathology and medical records for determining tumor histopathology. Specimen collection included blood for DNA extraction and analysis. A paraffin-embedded tumour section was requested from the hospital where the patient was treated. The authors were able to obtain a specimen for MSI analysis from 418 of the 1,342 patients enrolled in the study.

MSI analyses

Tumor extracted DNA from de-paraffinized cells was analyzed by polymerase chain reaction (PCR), using the five standardized microsatellite markers developed by the National Cancer Institute (NCI) for colorectal cancers [8] with germline DNA used as the normal control DNA. The standardized markers consisted of two mononucleotide repeats (Bat25 and Bat26) and three dinucleotide repeats (D2S123, D5S346, and D17S250) [8]. Tumors were classified as demonstrating high MSI if two or more of the five biomarkers were positive for shifts in the allelic bands; in all other instances, tumors were considered MSI low/normal [8].

Statistical analyses

The authors used a Kaplan–Meier survival analysis on the members of the cohort from the date of diagnosis until the date of death from ovarian cancer. To account for the time elapsed between the date of diagnosis and the date of ascertainment (genetic testing), they performed a left-truncated survival analysis, implemented in SAS. This adjustment is done to eliminate the survivorship bias that may occur because patients who died shortly after diagnosis were often missed in the present ascertainment scheme. Patients were censored at the date of death from another cause or the end of February 2013 (based on the Ontario record linkage described above). Kaplan–Meier survival curves were constructed for subgroups defined by histopathologic type (serous, endometrioid, clear cell, mucinous, other) and by MSI status. To compare statistical significance or differences associated with the Kaplan–Meier analysis, Student’s t test was used to test for statistical significance for continuous variables, (comparing the mean of means) and the Chi-squared test was used for categorical variables. To estimate the odds ratio associated with each risk factor, a matched analysis was done using conditional logistic regression. Initially, a univariate analysis was conducted. To adjust for potential confounding a multivariable analysis was done. The multivariable adjusted odds ratios and 95% confident intervals were estimated using SAS (version 9.1.3) and $p < 0.05$ was considered to be statistically significant.

Results

In total, 418 ovarian cancer patients were included. Of these, MSI status was available on 388 patients. There were 336 (80.3%) patients with MSI-low cancers and 52 (19.7%) patients with MSI-high cancers. The two groups are com-

Table 1. — Comparison of subjects with MSI-low and MSI-high ovarian cancers*.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Low MSI</th>
<th>High MSI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=366</td>
<td>N=52</td>
<td></td>
</tr>
<tr>
<td>Year of birth</td>
<td>1941.6 (1916-80)</td>
<td>1940.4 (1918-82)</td>
<td>0.51</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>56.7 (20-79)</td>
<td>57.3 (36-78)</td>
<td>0.77</td>
</tr>
<tr>
<td>Follow up years</td>
<td>7.72 (0.28-15.7)</td>
<td>7.66 (0.91-15.6)</td>
<td>0.94</td>
</tr>
<tr>
<td>Time from diagnosis to testing</td>
<td>1.77 (0.01-5.2)</td>
<td>1.72 (0.19-3.8)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Grade</td>
<td>10 (8.3%)</td>
<td>41 (34.2%)</td>
<td>67 (55.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Stage</td>
<td>79 (21.6%)</td>
<td>68 (18.6%)</td>
<td>160 (43.7%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histology</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>202 (55.2%)</td>
<td>31 (59.6%)</td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
<td>18 (4.9%)</td>
<td>2 (3.9%)</td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>78 (21.3%)</td>
<td>11 (21.2%)</td>
<td>0.91</td>
</tr>
<tr>
<td>Clear cell/other</td>
<td>68 (18.6%)</td>
<td>8 (15.4%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Death</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>206 (56.3%)</td>
<td>29 (55.8%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>160 (43.7%)</td>
<td>23 (44.2%)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

* Missing data is not included in the frequency distributions.
Survival in women with ovarian cancer with and without microsatellite instability

pared in Table 1. No differences were found between the MSI high and MSI low in terms of grade of tumors and histology subtypes. The authors performed survival analysis using a multivariable Cox proportional hazards model to assess the association between MSI status and the risk for death (hazard ratio). In the multivariable analysis, the authors adjusted for year of birth, age at diagnosis, tumor stage and histology. The adjusted odds ratio associated with MSI-high status was 0.77 (95% CI: 0.49 - 1.19; \(p = 0.24\)) (Table 2, Figure 1). Factors which were predictors of mortality included stage and histology (Figure 2).

Discussion

MMR deficiency can be demonstrated through the presence of MSI in approximately 20% of cases of ovarian cancer. A proportion of these cases carry germline mutations in MLH1, MSH2, and MSH6 [1]. Previous studies have evaluated the prognosis of ovarian cancer patients from families with Lynch syndrome, the majority of which had MSI [13-16]. A small Dutch study of ovarian cancer patients from Lynch syndrome families indicated similar survival rates in as women with sporadic ovarian cancers [13]. Subsequently, a larger study of 144 women with Lynch-syndrome associated ovarian cancer from 11 European centers suggested a better prognosis for the hereditary cases [14].

MSI-high is associated with favorable survival in colon cancer cases from HNPCC families and in sporadic cases. Gryfe et al. tested specimens of colorectal cancer from a population-based series of 607 patients for MSI and compared the clinical features and survival of patients who had MSI high colorectal cancer with these characteristics in patients who had MSI low colorectal [9]. They found high-frequency MSI in 17 percent of the colorectal cancers, and in a multivariate analysis, MSI was associated with a significant survival advantage independently of all standard prognostic factors (HR, 0.42; 95% CI, 0.27 to 0.67; \(p < 0.001\)). The biological basis for this finding is unknown. It has been suggested that the intrinsic tumor biology leading to the extensive genetic instability associated with microsatellite alterations may ultimately compromise tumor progression, accounting for the improved survival [17].

In contrast, among patients with endometrial cancer, MSI high has been associated with high tumour grade [18]. McDonald et al. evaluated 259 endometrial carcinomas and found the five-year survival rate for the MSI high patients was 79.6%, similar to 76.2% for MSI low/normal patients (\(p = 0.6\)) [19].

Three small studies evaluated the significance of MSI status on prognosis in ovarian cancer patients. Della et al. evaluated MSI status in 66 ovarian carcinomas and 11 epithelial ovarian tumors of low malignant potential. MSI-high was found in 30% of the carcinomas. There was a trend for tumors with MSI high and MSI low to have a poor prognosis. MSI high in carcinomas was significantly associated with poor differentiation and higher clinical stage [20]. On the other hand, it was also reported that the combination chromosomal instability and MSI low or stable in ovarian cancer was associated with a shorter survival compared to MSI high patients [21]. A Danish study determined the frequency of MSI-high among ovarian cancer patients using a panel of 16 dinucleotide markers. All MSI high ovarian cancers were of the serous type, and no differences in disease specific survival according to MSI status were noticed [22]. In the present study, 60% of the MSI-high ovarian cancers were of the serous subtype. This study sample was much larger than those in previous studies. The authors found no difference in the clinical characteristics (i.e. age at diagnosis, stage, grade, and histology) and survival between MSI and MSI low/normal tumors. They did not
find a significant survival difference between patients with MSI high and MSI low/normal, although a trend towards improved survival was noted HR = 0.77 (95% CI, 0.49—1.19) (p = 0.24).

Acknowledgements

Supported by grants R01 CA111914 (TP), K07 CA108987 (TP), R01 CA063682 (HAR), R01 CA063678 (SN) and R01 CA080978 (SN) from the National Cancer Institute.

References


[25] Thibodeau S.N., Bren G., Schaid D.: “Microsatellite instability in cancer of the proximal colon”. Science, 1993, 260, 816. Address reprint requests to: S.A. NAROD, M.D. Women’s College Research Institute, 790 Bay Street, 7th Floor Toronto, Ontario M5G 1N8 (Canada) e-mail: steven.narod@wchospital.ca
Introduction

The incidence of human papilloma virus (HPV) infection has been constantly increasing.

Most researchers agree nowadays, that large families of genes, such as tumor suppressor genes, and oncogenes significantly contribute to development of malignant diseases. Molecular biology reports demonstrated that tumor suppressing gene INK4a (also known as MTS1, CDKN2, and INK4a/ARF) had a significant role in cellular regulatory mechanism for coding P16INK4а protein [1]. P16INK4а is a tumor suppressing protein, belonging to the group of cyclin-dependent kinase inhibitors (CDK4/6). In infections with high-risk HPV types, Е7 oncoprotein binds to pRb resulting in functional inactivity and hyper-phosphorylation (ppRb) and release of Е2F transcription factor. Since expression of protein gene products for cyclin-dependant inhibitor р16INK4а is under negative feedback control of functional pRb, infection of high risk HPV (which integrates its own genome into the one of the host) results in increased expression of р16INK4а protein. There are data proving that Е2F transcription factor increases expression of p16INK4a protein [2].

A strong cytoplasmatic and nuclear immunohistochemical reaction to p16INK4a protein was established in squamous carcinoma, adenocarcinoma, and high-grade squamous intraepithelial lesion (HSIL) [3, 4, 5]. Murphy et al. described increased expression of p16INK4a protein in dysplastic squamous and columnar cells with 99% sensitivity rate and 100% specificity rate [6]. Klaes et al. demonstrated that p16INK4a specific immunohistochemical reaction represents both specific and sensitive method for detection of dysplastic cervical cells in histological and cytological preparations [8]. The presence of p16INK4a protein has been also confirmed in dysplastic cells with HPV negative cell lines of cervical carcinoma and HPV-negative lesions [6,7]. This suggests the existence of HPV independent path of p16INK4a protein expression.

It is believed that p16INK4a immunohistochemistry test can identify those lesions with a high likelihood to progress to carcinoma [8, 9]. The importance of molecular biomarkers p16INK4a would be in detecting cell changes in the initial stage of the disease which is the goal of primary screening.

Materials and Methods

This was a cross-sectional study which was performed at the Department for the Early Diagnosis of Cancer in University Clinic of Obstetrics and Gynecology “Narodni front” in Belgrade. HPV DNA test for HPV types 16, 18, 31, and 33 was performed applying in...
situ hybridization method. The study included 100 patients with suspected cervical premalignant lesions. Cervical smears were taken in all subjects for cytological analysis for malignancy and immunocytochemical analysis of p16INK4a protein. In 56 patients cervical smears were taken for HPV DNA typing. The targeted cervical biopsies were taken during a colposcopy. Histology was a “gold standard” in analyzing the material. Cervical intraepithelial neoplasia (CIN) cytology kit was used for immunocytochemical analysis. CIN cytology kit contained the primary monoclonal mouse antibody E6H4 used for the detection of p16INK4a protein as antigen. DAB + was used as a chromogene. Positive reaction was demonstrated when there were both cytoplasmic and nuclear reactions in more than ten dyskaryotic or atypical cells. The reaction was evaluated semiquantitatively according to intensity of staining as: 0-negative, 1+ weak, 2+ moderate, and 3+ intense reaction.

The primary data were analyzed by means of: descriptive statistical methods, methods for testing the correlation, for statistical hypothesis testing, and evaluation of performance.

Results

The average age of subjects was 32.88 years. The youngest patient was 19 and the oldest one was 64. The average age of women with severe cervical lesions (HSIL) was 35.66. The youngest patient was 20 and the oldest one was 51. The average age of women with cervical cancer was 47.5 years.

Fifty-six women were examined for the presence of oncogenic HPV types; 47 (84%) women were HPV DNA positive, while nine (16%) were negative. In the group of 56 patients, 27 (48.2%) had HPV 16, 26 (46.4%) had HPV 31, and 18 (32.1%) had HPV 18. HPV 33 had the lowest prevalence in four (7.1%) patients.

Normal histological findings of biopsy were found in 40 (40.0%), less severe lesions on the cervix type low-grade squamous intraepithelial lesion (LSIL, CIN1) were present in 41 (41.0%), 15 (15.0%) had severe lesion types HSIL (CIN2/3), and squamous cell carcinoma was diagnosed in four (4.0%) patients.

Cytological findings of patients are shown in Table 1. The commonest cytological finding was atypical squamous cells of undetermined significance (ASC-US) in 26 (26.0%) patients, followed by normal findings in 26 (26.0%), atypical squamous cells, cannot rule out high-grade squamous intra-epithelial lesion (ASC-H, IIIa) in 15 (15.0%), LSIL (IIIb) in 12 (12.0%), and HSIL and carcinoma in 9 (9%) patients.

Cytological findings of patients are shown in Table 1. The commonest cytological finding was atypical squamous cells of undetermined significance (ASC-US) in 38 (38.0%), followed by normal findings in 26 (26.0%), atypical squamous cells, cannot rule out high-grade squamous intra-epithelial lesion (ASC-H, IIIa) in 15 (15.0%), LSIL (IIIb) in 12 (12.0%), and HSIL and carcinoma in 9 (9%).

Immunocytochemical analysis of p16INK4a protein expression in epithelial cells of cervical smears demonstrated that 36 (36.0%) patients had increased expression of p16INK4a protein (positive findings) and 64 (64.0%) had negative findings. The intensity of expression of p16INK4a protein in epithelial cells of cervical smears is shown in Table 2. In 23 (23.0%) the response was weak (value 1); nine patients (9.0%) had moderate (value 2) response, while in four (4.0%) the intensity of staining was strong (value 3). The intensity of expression of p16INK4a protein in epithelial cells in cervical smears in relation to the cytological findings is shown in Table 3.

There was a statistically significant positive correlation (Spearman r = 0.70; p < 0.001) between cytological findings and intensity of expression of p16INK4a protein in epithelial cells of cervical smears. Twenty-nine (76.3%) women with cytological findings ASC-US had negative immunohistochemical findings of p16INK4a protein in cervical smear and nine (23.7%) were positive. Increased expression of p16INK4a protein in cervical smear was not detected in eight (53.3%) patients with cytological findings of ASC-H, while it was detected in seven (46.7%) patients with LSIL.

All 12 patients with cytological findings of LSIL had increased expression of p16INK4a protein in cervical smear epithelial cells. Cytological findings of HSIL and cancer had nine women, and only one (11.1%) did not have increased expression of p16INK4a protein. Immunohisto-

### Table 1. — Cytological findings (n = 100).

<table>
<thead>
<tr>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal findings</td>
<td>26</td>
</tr>
<tr>
<td>ASC-US</td>
<td>38</td>
</tr>
<tr>
<td>ASC-H (IIIa)</td>
<td>15</td>
</tr>
<tr>
<td>LSIL (IIIb)</td>
<td>12</td>
</tr>
<tr>
<td>HSIL, Ca</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 2. — The intensity of expression of p16INK4a protein in epithelial cells of cervical smears.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 3. — The intensity of expression of p16INK4a protein in epithelial cells of cervical smears by cytological findings (n = 100).

<table>
<thead>
<tr>
<th>P16</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>0</td>
</tr>
<tr>
<td>Normal findings %</td>
<td>100.0%</td>
</tr>
<tr>
<td>ASC-US Number</td>
<td>29</td>
</tr>
<tr>
<td>%</td>
<td>76.3%</td>
</tr>
<tr>
<td>ASC-H (IIIa) Number</td>
<td>8</td>
</tr>
<tr>
<td>%</td>
<td>53.3%</td>
</tr>
<tr>
<td>LSIL (IIIb) Number</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>0%</td>
</tr>
<tr>
<td>HSIL, Ca Number</td>
<td>1</td>
</tr>
<tr>
<td>%</td>
<td>11.1%</td>
</tr>
<tr>
<td>Total Number</td>
<td>64</td>
</tr>
<tr>
<td>%</td>
<td>64.0%</td>
</tr>
</tbody>
</table>

Spearman r = 0.70, p < 0.001
chemical intensity of expression of p16INK4a protein in cervical smears epithelial cells and the presence of oncogenic HPV types are shown in Table 4.

Among 27 patients with positive HPV 16 findings, 18 (66.7%) had positive cervical smear for p16INK4a protein. The intensity of expression of p16INK4a protein in the cervical smear was significantly higher in patients with HPV 16 positive findings (Mann-Whitney test, \( p = 0.0065 \)).

Among 18 patients with positive HPV 18 findings, nine (50.0%) had positive immunohistochemical findings of p16INK4a protein in the cervical smear. There was no statistically significant difference between patients with positive and negative findings of HPV 18 in the intensity of expression of p16INK4a protein in cervical smear (Mann-Whitney test, \( p = 0.92 \)).

Among 26 patients with positive HPV 31 findings, 12 (46.2%) were positive for p16INK4a protein in the cervical smear. There was no statistically significant difference in the intensity of expression of p16INK4a protein in cervical smear between patients with positive and negative findings of HPV 31/33 (Mann-Whitney test, \( p = 0.56/0.64 \)). Correlation of histological and cytological findings is shown in Table 5. There was significant positive correlation (Spearman \( r = 0.71; \ p < 0.001 \)) between the histological and cytological findings. In patients with histological CIN1 type, the commonest cytological result was ASC-US in 23 (56.1%) cases and ASC-H in 12 (29.3%). In patients with histologically diagnosed HSIL (CIN2/3), the commonest cytological findings were LSIL in 11 (73.3%), and HSIL in three (20.0%).

**Discussion**

It is believed that p16INK4a protein is localized in the nucleus, but its detection in the cytoplasm can be explained by increased production of proteins or an increased transfer of protein in the cytoplasm [6]. Detection of p16INK4a protein, indirectly suggested the expression of viral oncogenes in transformed cells, while normal cells remained unchanged and did not stain. Protein p16INK4a positivity in cervical smear increases with increasing severity of the lesion. Other authors found lower detection rate of p16INK4a protein in LSIL (CIN I) compared to HSIL (CIN I/II) and cancers. The reason may be that a certain percentage of LSIL (CIN I) lesions were caused by low-risk HPV types.

Some authors consider that only a small number of cases of LSIL (CIN I) progresses to HSIL (CIN II/III), hence the small number of cases positive for p16INK4a [10-12]. A positive test result of p16INK4a protein in LSIL (CIN I) could
indirectly indicate the deregulated expression of oncogenic HPV types and the disturbed cellular control mechanisms in the basal and parabasal layer [8]. Thus, the detection of p16INK4a protein in LSIL (CIN I) lesions can be considered as a factor predictive of invasive potential, and the patients with positive p16INK4a findings are more prone to progression of lesions compared to women with negative findings [13]. A positive p16INK4a protein test result in LSIL (CIN I) could be significant for the triage of women who require more thorough monitoring and treatment.

There are suggestions that a positive finding of p16INK4a protein in LSIL (CIN I) is an indication for cervical conization [14]. The intensity of staining nuclei and cytoplasm (the degree of expression) of p16INK4a protein in increasing with the severity of histological findings [15]. The degree of expression of p16INK4a protein in cervical smear and biopsy in patients with LSIL (CIN I) was lower compared to women with HSIL (CIN II/III) and cancer, which is consistent with literature data [6, 16]. The present found a significant positive correlation between the intensity of staining, i.e. level of expression of p16INK4a protein in the cervical smear in relation to the cytological findings [16, 17]. Detection of p16INK4a protein in cervical smear increased with severity of cytological findings [18].

All the patients with findings of ASC-US, ASC-H and LSIL in which the expression of p16INK4a protein was detected in the cervical smear, had histologically proven malignant lesion on the cervix [19, 20]. The present authors proved that the use of p16INK4a in cytology test significantly reduced the percentage of false positive and false negative results and helped in the interpretation of ASC-US, ASC-H and LSIL findings [6, 17].

Immunocytochemical detection of p16INK4a can be successfully applied to the conventional Papanicolaou smears. Papanicolaou smears were decolorized and stained for p16INK4a protein. This technique can be used in retrospective immunocytochemical analysis, particularly for ASC-US, ASC-H and LSIL findings [17, 20, 21].

HPV 16 and HPV 18 are most frequently detected high risk HPV types in HSIL lesions and invasive cancers of the anogenital region [21]. In the present study the incidence of HPV 16 was significantly higher in women with HSIL (CIN II/III) 84.6%, and 100% in cancer, as compared to 29.4% in LSIL (CIN I) and 31.8% in cervicitis.

Many authors investigated the expression of p16INK4a protein as an indicator of high-risk HPV infection [10]. Sano et al. were the first ones who showed that the expression of p16INK4a protein increased in most patients with precancerous lesions [3]. Lambert et al. found increased expression of p16INK4a protein in HPV positive women [21]. The present results are consistent with results of other authors [3, 4, 16]. Also, in the present study, the authors showed that the presence of oncogenic HPV16 types were significantly higher in women with positive finding of p16INK4a protein in cervical smear [3].

In the literature, there are also data on the presence of p16INK4a protein in dysplastic cells with HPV negative cell lines of cervical carcinoma (C33A) [6]. This suggests the existence of HPV independent path of p16INK4a protein expression [7, 11, 15, 20]. From the aforementioned, the present authors can conclude that p16INK4a negative finding does not signify that a woman is not infected by an HPV virus. Also, a positive p16INK4a finding may not be associated with HPV infection. In women with precancerous cervical lesions, there is a significant positive correlation between the degree of expression of p16INK4a protein in cervical smear with cytological, histological findings, and the presence of oncogenic HPV types.

The intensity of p16INK4a protein detection in cervical smear increases with the severity of cytological findings. The use of p16INK4a in cytology test has a practical diagnostic and prognostic significance, especially in women with cytological results ASC-US, ASC-H or LSIL.

The results of the present research, as well as the data from the literature, regarding the significant correlation between the degree of expression and the severity of lesion, indicate that this test in the future could not only improve the results of cytology and HPV screening, but also the prediction and prognosis of premalignant and malignant cervical diseases.

References


Protein kinase D1 inhibits breast cancer cell invasion via regulating matrix metalloproteinase expression

X.J. Qin, Z.G. Gao, J.L. Huan, X.F. Pan, L. Zhu
Department of General Surgery, Shanghai Eighth People's Hospital, Shanghai (China)

Summary

Purpose: This study aimed to explore the role of protein kinase D1 (PKD1) in breast cancer invasion. Materials and Methods: The relative expression of PKD1 mRNA and protein in human invasive breast cancer tissue samples and normal samples, as well as breast cancer cell lines, were detected. Constitutively-active PKD1 and PKD1 specific shRNA were expressed in the MD-MB-231 and MCF-7 cells, respectively. The role of PKD1 in the invasive behavior of breast cancer cell line was evaluated by matrix metalloproteinase (MMP) expression. Results: The results showed that PKD1, as a serine/threonine kinase, is downregulated significantly in invasive ductal carcinoma and metastatic invasive ductal carcinoma tissue than the normal tissue and the low expression of PKD1 is also found in breast cancer cell line MD-MB-231. The MMP2 and MMP9 expression in PKD1 constitutively-active MD-MB-231 cells and MCF-7 knockdown cells were decreased and increased respectively. Conclusion: The authors confirmed that PKD1 was downregulated in invasive breast cancer. PKD1 can negatively regulate the MMP expression and may serve as a potential therapeutic target.

Key words: Protein kinase D1; Breast cancer; Cell invasion; Matrix metalloproteinase.

Introduction

Breast cancer is the most common malignancy in women and one of the most common causes of cancer-related mortality [1]. According to the statistic result, there were approximately 1.2 million newly diagnosed cases and more than 450,000 deaths [2]. The breast cancer metastasis contributes to most cases of deaths and the increasing risk of prevalence and mortality is observed year by year. Protein kinases are one of the most important protection mechanisms in human body. Their activities are closely related with the clinical outcome of breast cancer therapy. Therefore, it is fairly reasonable to conduct the research on them.

Protein kinase D (PKD), a kind of serine/threonine kinase, is regulated by the calcium/calmodulin kinase [3]. Increasing interest has been paid on the role of PKD in cancer biology. Trauzold et al. showed that PKD can promote the cell invasion and inhibit the apoptosis in pancreatic cancer cell line [4]. While a review suggests that PKD can inhibit the migration in multiple cancer cell lines, such as pancreatic cancer, prostate cancer, gastric cancer, and breast cancer [5]. There are three members in PKD family and they are PKD1, PKD2, and PKD3. They participate in many complex biological process, including oxidative stress, transmembrane transportation, signaling transduction, cell adhesion, etc. Among the three members, PKD1 is the most investigated one. Eiseler et al. [6] showed that PKD1 is a negative regulator protein in epithelial cell migration. Furthermore, active PKD1 can also regulate the F-actin remodeling in vitro. In fact, the wild type PKD1 can inhibit the migration of pancreas cell line and prostate cell line and PKD1 knockout will increase the invasive ability of the cells [7]. In addition, Peterburs et al. [8] showed that the migration of PKD1 knockout cells increased significantly than the control cells in MCF cell line. Another study suggested that PKD1 could increase the susceptibility of the metastasis in MD-MB-231 cell due to its lower expression [9]. Until now, the accurate mechanism of PKD in cancer growth, invasion, and migration have not yet been decided.

A potential mechanism for PKD1 regulation of cell migration is via matrix metalloproteinases (MMPs). Previous studies have confirmed that one of the most important function of MMPs is to degrade the extracellular matrix (ECM) [10-11], and there are at least 17 members in MMPs family, including collagenase (e.g. MMP1, MMP13), stromelysins (e.g. MMP-10, MMP-12), gelatinases (e.g. MMP-2, MMP-9) or membrane-type enzymes (e.g. MMP-14, MMP-16). Among them, gelatinase MMP-2 and MMP-9 are the most important ones because the experimental evidences have shown that inhibition of MMP-2 can decrease 70% of the tumor volume [12]. Furthermore, Biswas et al. [13] found that PKD1 can increase the expression of MMP-2 and MMP-9 by β3-PDK1-ERK pathway. Then the extracellular do-
main of E-cadherin will be shed by MMP-2 and MMP-9 and the proliferation of prostate cancer will be inhibited. Furthermore, Eiseler et al. [9] showed that PKD1 can downregulate the expression of MMP-2, MMP-7, MMP-9, MMP-10, MMP-11, MMP-13, MMP-14, and MMP-15 in breast cancer cell.

In present study, the authors first checked the expression of PKD1 in invasive breast cancer tissues and normal tissue, as well as in the breast cancer cell line. They also explored the role of PKD1 in the invasive behavior of breast cancer cell line by evaluating the expression of MMPs.

**Materials and Methods**

**Stable cell line construction**

All the cell lines described in the paper were maintained according to the manufacturer’s instructions. PcDNA3-based expression vector of constitutively active PKD1 (PKDactive, PKD1.Y463E) was described previously [14]. MDA-MB-231 cells were transfected with pcDNA6/TR and selected with blasticidin to generate the MDA-MB-231-TR stable cell line.

PKD1.Y463E vector was cloned into the pcDNA4/TO-B plasmid via BamHI and XhoI sites and confirmed by DNA sequencing. PcDNA4/TO-B-PKD1.Y463E vector was then transfected into the MDA-MB-231-TR and selected by Zeocin to obtain the MDA-MB-231-TR-PKD1.Y463E stable line.

The lentivirus shRNA expression vector was used to knockdown PKD1. Six shRNA sequences were obtained and one of the sequences with highest inhibition effect was selected to perform the cell experiment. The lentivirus particles were generated by using the packaging plasmids (gag-pol and vsv-g plasmids) and 293-lenti-X cells. MCF-7-PKD1.RNAi cell line was generated by infecting the cells with lentivirus particles.

**Quantification of the PKD1 expression in tissue samples**

Breast tissue samples including 15 normal breast tissues, 20 invasive ductal carcinoma (IDC), and 20 metastatic invasive ductal carcinoma (mIDC) were collected with the informed consent.

**Real-time PCR**

Total RNA extraction of myocardial tissue and the reverse transcription reaction were performed according to the manufacturer’s instructions. PKD1 specific sequences were amplified during 32 cycles of 30 seconds denaturing at 95°C, 60 seconds annealing at 59°C, and 60 seconds extension at 72°C, and the primers were TTCTCCACCTCAGGTCATC and TGCCAGAGCACATAACGAAG.

**Western-blot analysis**

The breast tissues were lysed in protein lysis buffer followed by high speed centrifugation and BCA quantification. Cellular protein was separated by electrophoresis on SDS-PAGE gel and then transferred onto PVDF membrane. After blocking, the blots were incubated with the antibodies to PKD1, MMP-2, and MMP-9 and α-tublin was used as loading control. The appropriate HPR conjugated secondary antibodies were applied. The protein bands were detected with chemiluminescent substrate on X-ray films.

**Statistical analysis**

All the statistical analyses were performed by SPSS18 software. The data was presented as mean ± SD. One way-ANOVA was used to examine the difference in three or more groups. A \( p < 0.05 \) was recognized as significant difference.
Results

The expression of the PKD1 is downregulated in invasive breast tissues and cell lines

The relative expression of PKD1 mRNA (right panel in Figure 1) was downregulated significantly in the IDC ($p < 0.01$) and mIDC ($p < 0.01$) tissues, as well as the invasive breast cancer cell line MD-MB-231 ($p < 0.01$). The authors also checked the expression of PKD1 protein by western-blot and the results showed that PKD1 expression is higher in normal and IDC tissue, and decreased in mIDC and MD-MB-231 cell line (left panel in Figure 1).

The PKD1 inhibits the breast cancer cell invasion via MMP-2 and MMP-9

According to the results of the 2-D scratch assay, the cell migration in MDA-MB-231-TR-PKD1.Y463E cells was significantly decreased than the control cells, while the invasive ability of MCF-7-PKD1.RNAi cells were increased significantly compared to the control cells (data not shown). Then the authors attempted to explore the molecular mechanism by detecting the expression levels of MMPs. As shown in Figure 2, the expression of MMP-2 and MMP-9 was decreased significantly in the MDA-MB-231-TR-PKD1.Y463E and MCF-7 cells. The exact relationship between PKD1 and MMPs is yet unknown. One possible explanation is that PKD1 may regulate an element located in the MMP promoter region. Previous studies have showed histone deacetylases (HDACs) can regulate the expression of MMPs [17-18]. PKD1 has shown to be a negative regulator of HDACs [19] and therefore PKD1 may exert its effects on these two MMPs via regulating the HDACs.

Discussion

Searching for the effective therapeutic target to inhibit the cancer metastasis and exploring the related molecular mechanism are important for patients with malignant cancer. Here, the authors presented their study on the role of PKD1 in breast cancer invasion and the results showed that expression of the PKD1 mRNA and protein was significantly downregulated in invasive breast cancer tissues and cell line MD-MB-231 (Figure 1). These results were consistent with the results on PKD1 expression in gastric and prostate cancer, where the downregulation of the PKD1 expression were also found [15-16]. Furthermore, the role of PKD1 in regulating tumour cell migration and invasion, which possess a critical role in the tumor metastasis, has not been elucidated either. In order to confirm a potential role for PKD1 in cell invasion, the present authors used a invasive breast cancer cell line MD-MB-231 and non-invasive MCF-7 to examine the PKD1 expression and found that PKD1 expression was associated with the invasive phenotype. It is higher in invasive MD-MB-231 and lower in the non-invasive MCF-7 cell lines (Figures 2). In addition, they also respectively re-introduced the constitutively active PKD1 and PKD1 shRNA into MD-MB-231 and MCF-7 to compare the ability of cell migration in 2-D scratch assay system and found that shorter migration distance was observed in the MD-MB-231 cells that constitutively expressed the active PKD1, while longer migration distance was seen in the PKD1 knockdown MCF-7 cells. Since the ECM degradation is the first step for cell migration, the present authors attempted to explore the molecular mechanism by examining the expression of gelatinase MMP-2 and MMP-9 and the results showed that expression MMP-2 and MMP-9 were also highly related with the invasive phenotype. Their expression was significantly higher in the invasive MD-MB-231 and MCF-7-PKD1. RNAi cells and lower in the invasive MDA-MB-231-TR-PKD1.Y463E and MCF-7 cells. The exact relationship between PKD1 and MMPs is yet unknown. One possible explanation is that PKD1 may regulate an element located in the MMP promoter region. Previous studies have showed histone deacetylases (HDACs) can regulate the expression of MMPs [17-18]. PKD1 has shown to be a negative regulator of HDACs [19] and therefore PKD1 may exert its effects on these two MMPs via regulating the HDACs.

In conclusion, the present study showed that PKD1 may serve as an indicator of invasive breast cancer and the authors further proved that PKD1 expression decreased significantly in the malignant breast cancer. The underlying molecular mechanism may be that PKD1 could negatively regulate the expression of MMP-2 and MMP-9. All these findings can be translated to develop novel therapeutic treatments by targeting PKD1.

Acknowledgements

This study was supported by the Shanghai Xuhui Science Foundation (No. xkkt201106).

References


Address reprint requests to:
X.J. QIN, M.D.
Department of General Surgery
Shanghai Eighth People’s Hospital
8 Caobao Road
Shanghai 200235 (China)
e-mail: xianjuqiq@gmail.com
**Introduction**

Selective estrogen receptor modulators (SERMs), particularly tamoxifen and raloxifene, are the drugs that have attracted most attention in the primary chemoprevention of breast cancer in women at a high risk of this disease [1-5]. Tamoxifen, a first-generation SERM, was the first drug approved in the United States for breast cancer chemoprevention and was found to reduce the risk of invasive cancer in around 49% of high-risk women [1]. Nevertheless, as shown in the Breast Cancer Prevention Trial (BCPT; P-1), when the drug is used for prolonged periods of time, all the animals were sacrificed and the first pair of abdominal-inguinal mammary glands was extirpated and fixed in 10% buffered formalin to investigate Ki-67 expression by immunohistochemistry. The data were analysed using Student’s *t*-test (*p* < 0.05). Results: The percentage of Ki-67-stained nuclei per 500 cells in the mammary epithelium was 42.33±6.18 and 15.51±3.71 [mean ± standard error of the mean (SEM)] in the control and experimental groups, respectively (*p* < 0.001). Conclusion: Raloxifene treatment significantly reduced Ki-67 expression in the mammary epithelium of rats in persistent estrus.

**Key words:** Rat; Persistent estrus; Raloxifene; SERMs; Ki-67.

---

**Ki-67 antigen expression in the mammary epithelium of female rats in persistent estrus treated with raloxifene**

G.V. de Sousa¹, C.S. Borges¹, P.V. Lopes-Costa¹, D.R. Costa-Silva¹, S.F. Lopes-Dias¹, A.C.P. Nazário², A.P. Alencar³, B.B. da Silva¹

¹Department of Gynecology, Federal University of Piauí, Teresina, Piauí; ²Department of Gynecology, Federal University of Sao Paulo, Sao Paulo; ³Department of Statistics, University of São Paulo, São Paulo (Brazil)

**Summary**

**Objective:** To evaluate Ki-67 antigen expression in the mammary epithelium of female rats in persistent estrus treated with raloxifene.

**Materials and Methods:** Forty-one Wistar-Hannover rats in persistent estrus induced by 1.25 mg of testosterone propionate were randomly divided into two groups: Group A (control, n=21) in which the animals received only the vehicle (propylene glycol) and Group B (experimental, n=20) in which the rats received 750 µg/day of raloxifene by gavage. After 21 days of treatment, all the animals were sacrificed and the first pair of abdominal-inguinal mammary glands was extirpated and fixed in 10% buffered formalin to investigate Ki-67 expression by immunohistochemistry. The data were analysed using Student’s *t*-test (*p* < 0.05). Results: The percentage of Ki-67-stained nuclei per 500 cells in the mammary epithelium was 42.33±6.18 and 15.51±3.71 [mean ± standard error of the mean (SEM)] in the control and experimental groups, respectively (*p* < 0.001). Conclusion: Raloxifene treatment significantly reduced Ki-67 expression in the mammary epithelium of rats in persistent estrus.

**Key words:** Rat; Persistent estrus; Raloxifene; SERMs; Ki-67.
Materials and Methods

Animals

The entire protocol was conducted in accordance with the ethical principles established by the Brazilian College for Animal Experimentation (COBEA). Forty-one virgin female Wistar-Hanover rats weighing approximately 250 grams, obtained from the Veterinary Science Laboratory of the Federal University of Piauí, were used in this study. The state of permanent estrus was achieved by administering a subcutaneous injection of 1.25 mg of testosterone propionate to the animals on their second day of life. The animals were kept in plastic cages with a metal grid lid in environmental conditions consisting of 12-hour light/dark cycles with lights on between 0700 and 1900 hours and temperature maintained by air-conditioning at 20-25°C. The animals had free access to filtered water and rat chow. When the animals were 90 days old and permanent estrus had been confirmed, they were randomly divided into two groups: Group A (the control group; n=21) and Group B (experimental, raloxifene; n=20). The state of persistent estrus was confirmed in the animals from the obliteration of the distal third of the vagina [13] and the presence of keratinization of the vaginal epithelium, the principal characteristic of permanent estrus, and also by the presence of polycystic ovaries, as later detected during histology performed at the time of autopsy. The animals in Group A received one ml of propylene glycol administered daily by gavage for 21 days, while the animals in Group B received 750 µg of raloxifene diluted in one ml of propylene glycol daily, also administered by gavage for 21 days. Gavage was always performed at the same time of the day using a metal gavage probe. On the 22nd day, the animals were beheaded and the first pair of abdominal-inguinal mammary glands was removed. The specimens were fixed in 10% buffered formalin for 12-24 hours for immunohistochemical evaluation of the mammary tissue.

Immunohistochemistry

The mammary tissue was cut into three-µm-thick sections. Next, they were processed and stained with hematoxylin and eosin for morphological evaluation, following which the sections were dehydrated in increasing concentrations of absolute and washed in buffered solution at pH 7.4 [14]. Immunohistochemical evaluation of the Ki-67 marker was performed using a detection system in combination with an antigen recovery method. For this, the sections were treated with 3% hydrogen peroxide diluted in buffered solution for five minutes to block the endogenous peroxide. After recovery of the epitopes, the tissue samples were incubated with primary mouse anti-Ki-67 monoclonal antibody (clone MIB-5, 1:100) for 16 hours, that included an overnight period in a refrigerator, at ethanol until absolute ethanol had been reached, cleared in xylol, immersed in liquid paraffin, and placed in an oven at approximately 4°C. Following washing with buffered saline solution, the sections were incubated for 45 min with a detection system. To read reaction, all sections were treated with a solution of 3,3-diaminobenzidine tetrahydrochloride at a concentration of one mg/ml of Tris buffered solution and hydrogen peroxide solution for five minutes. Next, the sections were counterstained with Harris hematoxylin or methyl green for five minutes followed by dehydration in ethyl alcohol and xylol baths. The cells were considered positive for the immunohistochemical expression of the Ki-67 antigen when the nucleus was stained a brownish color.

Quantitative method

Quantification was performed by two independent observers who were blinded with respect to the groups to which the rats belonged. Evaluation was performed using a light microscope connected to a colour digital video camera, model SCC-13, which captured the image and transmitted it to a computer equipped with an Image Lab software program, version 2.3 for image analysis. To evaluate Ki-67 expression, 500 cells of the mammary epithelium were counted on each slide, whether they were stained by the anti-Ki-67 MIB-5 antibody or not, using a magnification of x400 and beginning with the area in which Ki-67 expression was greatest. In each case, the percentage of stained cells was obtained from the ratio between the number of cells with stained nuclei and the total number of cells, and then multiplied by 100.

Table 1. — Mean percentage of Ki-67 nuclei per 500 cells in the control and experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean</th>
<th>Standard Error</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>21</td>
<td>42.33</td>
<td>6.18</td>
<td>1.30</td>
</tr>
<tr>
<td>Experimental (B)</td>
<td>20</td>
<td>15.51*</td>
<td>3.71</td>
<td>0.83</td>
</tr>
</tbody>
</table>

There was a statistically significant decrease in Ki-67-stained nuclei after raloxifene treatment (*p < 0.001).

Results

As evaluated by light microscopy, the concentration of cells expressing Ki-67 was lower in the mammary epithelial cells of the animals in Group B (raloxifene) than in the animals of Group A (control) (Figure 1). Quantitative analysis showed mean percentages of Ki-67 stained nuclei of 42.33 ± 6.19 and 15.51 ± 3.71 per 500 cells in the control and experimental groups, respectively (p<0.001) (Table 1). The box plot in Figure 2 clearly shows the difference between the mean percentage of Ki-67 stained nuclei in the control and experimental groups.

Discussion

For ethical reasons, it is difficult to evaluate the direct effects of raloxifene on normal human breast tissue. Therefore, although there are situations in which the risk of breast carcinoma is higher, such as in the case of postmenopausal women in use of hormone therapy and in those with increased breast density [12], there is scarcity of studies on the effects of raloxifene on non-neoplastic breast tissue.

In the present study, female rats in permanent estrus were used to evaluate the effects of raloxifene specifically on breast tissue. Raloxifene significantly reduced Ki-67 antigen expression in the mammary epithelium of the female rats in the experimental group compared to the control group. The drug was administered orally by gavage to the rats since the oral route of administration is the one habitually used by women. In menopausal women, raloxifene is generally used at a dose of 60 mg/day to reduce the risk of breast cancer and as a treatment for osteoporosis [2, 4, 7, 8].
Ki-67 antigen expression in the mammary epithelium of female rats in persistent estrus treated with raloxifene

A weight-equivalent dose for adult rats with a mean weight of 200–250 g would be 200–250 mg. However, the metabolism of rats is faster than that of human beings; therefore, this weight-equivalent dose would constitute an underdosage and, consequently, would not mimic the serum and tissue concentrations of raloxifene in humans. There are reports of experimental studies in the literature in which rats were given oral doses of raloxifene that varied from 1 to 10 mg/kg daily [17,18]. However, Evans et al. [19] and Turner et al. [20] showed that the administration of raloxifene at a dose of 3 mg/kg/day by gavage reversed bone loss in castrated rats. The dose used by those authors was equivalent to a dose of 600–750 mg/day of raloxifene for rats weighing 200–250 g, the same dose level used in the present study.

A high concentration of cell proliferation was found in the mammary gland of the female rats in permanent estrus in the control group. This finding may be due to the continuous influx of estrogens and higher prolactin release in these rats in persistent estrus, and is in agreement with data reported by Jacobsohn and Norgren [21]. On the other hand, the raloxifene-induced reduction in proliferation in the mammary epithelium of these animals is in agreement with the few studies published in the literature on the effect of SERMS, more precisely raloxifene, on the mammary gland of female rats in persistent estrus [9, 10].

Raloxifene is a benzothiophene SERM that is chemically distinct from tamoxifen and whose mechanism of action has yet to be completely clarified. Nevertheless, the antagonistic effect of SERMS on breast tissue occurs principally through their high affinity for the estrogen receptors and their ability to bind competitively to these receptors, thus blocking estradiol binding. They also act by inhibiting the binding of coactivator proteins to transcriptional activation function 2 (TAF-2) in the ligand-binding domain. A final action involves inducing recruitment of corepressor proteins to the estrogen receptor, resulting in repression of the transcription gene [22]. Therefore, estrogen function does not occur.

Raloxifene may exert a total or partial estrogenic effect, depending on the target tissue and the species under study [23]. Therefore, the effect of the drug in the tissues varies and this may also be explained by the differences both in the number of estrogen receptors and in the proportion between the alpha (α) and beta (β) subtypes [24]. In the mammary epithelium of female rats, ERβ is more abundant than ERα; however, in humans, ERα is predominantly expressed in reproductive tissues such as the uterus and breast [10, 24, 25]. In the present study, raloxifene exerts an anti-estrogenic effect on the mammary epithelium of female rats in persistent estrus, a finding previously reported in the mammary tissue both in animal models and in humans [7, 8, 10].
Therefore, the reduction in cell proliferation in the mammary lobules of female rats in persistent estrus in which an increase in lobular duct tissue was found following raloxifene treatment in the present study reflects the anti-estrogenic effect of the drug on the breast tissue of these animals in which ERβ is predominant [25, 26]. Nevertheless, despite the limitations regarding the ability to extrapolate these results to humans, it is possible that when the risk of breast cancer is high, such as following the use of hormone therapy or when mammography shows increased breast density, raloxifene will reduce cell proliferation and, consequently, the risk of cancer.

References


How to improve the preoperative staging of presumed early-stage endometrioid endometrial cancer?

G. Bleu1, E. Arsène1, B. Merlot1, O. Kerdraon2, J. Bigot3, L. Boulanger1, B. Dedet1, D. Vinatier1, P. Collinet1,4

1 Gynaecology Department, Hospital Jeanne de Flandre, University Hospital of Lille, Lille
2 Anatomopathology Department, University Hospital of Lille, Lille
3 Radiology Department, Hospital Jeanne de Flandre, University Hospital of Lille, Lille; 4 University of Lille-Nord-de-France, Lille (France)

Summary

Purpose of investigation: Accurate preoperative staging of early-stage endometrioid endometrial cancer (EEC) is necessary to avoid under or over surgical treatment. The objective is to determine the rate of understaging and to evaluate the accuracy of different methods: hysteroscopy-curettage versus endometrial biopsy in predicting the final stage. Materials and Methods: This retrospective single-centre study led from 2000 to 2010, included women with EEC preoperatively assessed at low- or intermediate-risk. Understaging was defined as a postoperative FIGO Stage >1 or a determination of high risk after the final histopathologic diagnosis. Results: The study included 101 women (75 low-risk and 26 intermediate-risk). Final diagnosis was upstaged for 26 of them, more frequently in the presumed intermediate-risk group (57.7% vs 14.7%, p < 0.001). The rate of preoperative understaging was higher in the women with endometrial biopsies than those with curettage (34.5% vs 15.2%, p = 0.04). Conclusions: Hysteroscopy-curettage combined with magnetic resonance imaging (MRI) may improve preoperative staging of early-stage EEC, especially for presumed intermediate-risk disease.

Key words: Endometrial cancer; Understaging; Early-stage; Hysteroscopy-curettage; Endometrial biopsy.

Introduction

Endometrial cancer is one of the most common gynaecological malignancies in developed countries, and its incidence is rising. Patients commonly present at an early stage, when the tumour is confined to the body of the uterus, and their prognosis is excellent: the five-year relative survival rate for Stage 1 disease is superior to 95% [1]. Surgical management of endometrial cancer is nonetheless a challenge. Until recently, many hospitals used extensive surgery with pelvic lymph node resection for all women. Two recent multicentre randomized controlled trials found that this procedure provided no benefits in terms of overall or disease-specific and recurrence-free survival in women with early-stage endometrioid endometrial cancer (EEC) [2, 3]. It is thus widely accepted now that pelvic lymphadenectomy should not be performed for low- or intermediate-risk disease; instead, the risks and benefits of each surgical option must be balanced to avoid both over- and under-treatment. Changes in practices including fewer pelvic lymphadenectomies, consistent with for example the publication of new guidelines in Europe by the European Society for Medical Oncology (ESMO) [4]. At the same time, the number of reoperations has increased because of discrepancy between preoperative and final histology results.

In this case, preoperative staging must be highly accurate, for the lack of lymph node resection may increase the risk of recurrence and necessitate further surgery, while unnecessary lymph node resection may increase the risk of surgical complications. The challenge of pre-therapeutic assessment is to reduce staging errors and thus reduce re-interventions.

Guidelines by ESMO [4] and by French institutions [1] classify women with early-stage endometrial cancer in three groups: low-risk (FIGO [5] Stage IA, grade 1 or 2 histology, endometrioid type); intermediate-risk (all Stage IA and grade 3, endometrioid type, or FIGO IB, grade 1 or 2, endometrioid type) and high-risk (Stage IB and grade 3, endometrioid type, or Stage 1A-B non-endometrioid type, or Stage 1 with lymphovascular invasion). Because recurrence is unlikely in women in the low- and intermediate-risk groups, lymph node resection is not recommended for them. Preoperative staging has thus become the cornerstone of optimal management.

The aim of the present study is, first, to determine the rates of understaging in women whose preoperative risk was assessed as low or intermediate. The authors also compared the accuracy of hysteroscopy-curettage with that of endometrial biopsies (both performed with magnetic resonance imaging, MRI) in predicting the final pathology stage.
Materials and Methods

After approval by a national ethic committee, the authors used computerized medical records to identify all women who underwent surgery for EEC assessed preoperatively at low- or intermediate-risk at Lille University Hospital from January 2000 to December 2010 and conducted a retrospective analysis of the data retrieved from their hospital charts. The study excluded women with type 2 histology, high-risk, or a FIGO Stage >1 and those for whom surgery was contraindicated due to severe comorbidities. Preoperative staging was based on MRI and either endometrial biopsy or hysteroscopy-curettage. The study period preceded the change in practices. Accordingly, many women underwent systematic pelvic lymphadenectomy. Pre- and postoperative risk levels (low, intermediate, or high) were attributed a posteriori according to the 2010 Institut National du Cancer (INCa) guidelines [1]. The preoperative low/intermediate-risk assessment was compared with the final risk level based on the final pathology diagnosis of the resected uterus as the reference. The preoperative assessment was considered understaged if the postoperative FIGO stage was more than 1 or the patient was classified at high risk.

The authors first evaluated the overall understaging rate, that is, the percentage of women who would have required reoperation had the current recommendations been in effect then. Then they compared it according to the sampling method used (endometrial biopsy or curettage) and the preoperative risk evaluation had the current recommendations been in effect then.

Results

In all, 130 women were consecutively treated for EEC at the present institution between January 1, 2000, and December 31, 2010. The authors excluded 29 women: 21 at FIGO Stage >1, two high-risk cases based on preoperative staging, and six patients for whom surgery was contraindicated. The analysis included 101 women: 75 assessed preoperatively at low-risk and 26 at intermediate-risk. Table 1 summarizes the women’s characteristics and surgical parameters.

Final histopathology diagnoses were: endometrioid adenocarcinoma in 88 cases, four no residual tumour, five mixed adenocarcinomas, two serous adenocarcinomas, and two clear-cell adenocarcinoma. Table 2 shows discrepancies between the preoperative risk group and final histology results. Eighty-nine pelvic lymphadenectomy were performed (88.1%) and the results showed a lymph node invasion in seven cases (7/89, 7.9%).

Of the 75 women assessed preoperatively at low risk, 11 (14.7%) were upstaged to high risk or FIGO >1: two mixed adenocarcinomas included one with positive pelvic lymph node, two clear-cell adenocarcinoma, one serous adenocarcinoma, four tumour upstaged only because of lymphovascular invasion, one tumour with serosal invasion, and one pelvic lymph nodes metastasis.

Of the 26 women initially considered at intermediate risk, 15 (57.7%) were finally at high risk or FIGO >1: five tumour upstaged only due to lymphovascular invasion, three mixed adenocarcinomas included one with positive pelvic lymphadenectomy, one serous adenocarcinoma with pelvic lymph node metastases, three tumours with a postoperative histological grade of 3, and three with pelvic lymph node metastases.

Finally, 26 patients were upstaged to high-risk or FIGO >1. Thus, the rate of discrepancies between the preoperative risk group and the final histology diagnosis was 25.7% (26/101). The understaging rate was significantly higher in...
the preoperative intermediate- compared to low-risk group (57.7% vs 14.7%, p < 0.001).

The authors also studied these results according to the type of preoperative sample taken for histological examination: endometrial biopsy or curettage. For 55 (54.5%) women, preoperative staging was based on an endometrial biopsy and MRI, and in 46 (45.5%) patients, hysteroscopy-curettage and MRI. The two groups were comparable for the demographic and medical characteristics (Table 3).

The curettage was performed for various reasons. In 27 (58.7%) of these cases, the women also had an endometrial biopsy, at least an attempted biopsy, with a curettage afterwards (13 failure of biopsy, 14 hyperplasia). In ten cases (21.7%), the hysteroscopy was planned for presumed benign pathologies without previous biopsy, and the cancer diagnosis was unexpected and for nine patients (19.6%) hysterectomy was performed without attempted endometrial biopsy for a suspicion of endometrial cancer.

The understaging rates are presented by groups in Table 4. In the endometrial biopsy group, 19 (34.5%) were upstaged at the final histological diagnosis, including nine with histological upgrading (three mixed adenocarcinomas included one with positive pelvic lymphadenectomy, three with a histological grade of 3, one serous adenocarcinoma with pelvic lymph node metastases, and two clear-cell adenocarcinoma). In the hysteroscopy-curettage group, seven women (15.2%) were upstaged at the final diagnosis, three with histological upgrading (two mixed adenocarcinoma and one serous adenocarcinoma). The rate of preoperative understaging was significantly higher in the endometrial biopsy group than in the hysteroscopy-curettage group (34.5% vs 15.2%, p = 0.04).

### Discussion

In the present study, the final pathology staging was upgraded in 26 of 101 patients (25.7%) preoperatively assessed at low- or intermediate-risk. Under the new practice according to ESMO and French guidelines [1, 4], these patients would not have had pelvic lymphadenectomy during the first procedure and another intervention would have been necessary. These data are similar to those in other studies. Preoperative histology samples from endometrial biopsy or curettage often differ from the final pathology finding. In a study of 291 endometrial cancer patients, Goudge et al. found that 18% of tumours were upstaged [6]. However, the comparison of the sampling methods (endometrial biopsy and hysteroscopy-curettage) remains controversial and unclear. Here the present authors evaluated these different preoperative methods to determine which combination (MRI and endometrial biopsy versus MRI and hysteroscopy-curettage) was most accurate. The understaging rate of endometrial biopsy was significantly higher than that for hysteroscopy-curettage: 34.5% vs 15.2% (p = 0.04). Larson et al. found that endometrial biopsy correctly identified the tumour grade in 58% of women, compared with 77% with curettage; again, this difference was significant (p = 0.024), with accompanying risks of understaging of 26% and 10%, respectively [7]. When looking at data in the literature for early-stage EEC only, the present findings – better match between the preoperative and postoperative data with hysteroscopy - are similar to those of Frumovitz et al. [8]. In a prospective study of 156 patients, Ortoft et al. analysed the accuracy of endometrial biopsy versus hysteroscopy and reported that the combination of hysteroscopy and pelvic MRI had the lowest rate of discrepancies [9]. The present study and available literature [6–10] confirm that curettage is more accurate in comparison with endometrial biopsy, because of various degrees of differentiation of endometrial malignant tumours in different parts of the uterus.

Now, preoperative staging for endometrial cancer determines the choice of surgical approach and treatment. An understaged disease may require the patient to undergo a second procedure. The problem of performing surgery twice is the risk of increased complications related to anaesthesia and surgical procedures, particularly for this type of patients (frequent obesity and cardiovascular comorbidities). In intermediate cases, where the risk of erroneous evaluation is most frequent, systematic hysteroscopy-curettage might help to provide the most accurate assessment of the disease and choose the right surgical approach from the beginning, even if the practice of hysteroscopy means an anaesthesia and a minimal risk of complications. The complication rates associated with diagnostic or operative hysteroscopy range from 1.2% to 3.8% [11, 12], including uterine perforations, bleeding or infections. The risk of peritoneal dissemination of cancer cells associated with hysteroscopy has been debated, as the survival of these

<table>
<thead>
<tr>
<th>Understaging</th>
<th>Hysteroscopy-curettage n = 46</th>
<th>Endometrial biopsy n = 55</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No understaging</td>
<td>39 (84.8%)</td>
<td>36 (65.5%)</td>
<td></td>
</tr>
<tr>
<td>Other final histology</td>
<td>2 mixed, 1 serous adenocarcinoma</td>
<td>3 mixed, 1 serous, 2 clear cells adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>FIGO Stage &gt; 1</td>
<td>1 stage 3A</td>
<td>4 stages 3C1</td>
<td></td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
cells after peritoneal implantation. Many studies suggest that hysteroscopy does not increase the risk of extrauterine spread in endometrial cancer [13–15]. In 2011, a literature review covering 2,944 women [16] found no evidence to support an association between preoperative hysteroscopic examination and a worse prognosis and no reason to avoid diagnostic hysteroscopy before surgery in patients with endometrial cancer, especially in early stage. Moreover, several recommendations for good practice allowed hysteroscopy when an endometrial cancer is suspected [12, 17] and confirmed that this procedure is minimally invasive and can be used with a high degree of safety. Furthermore, preoperative staging includes an imaging examination. To determine the tumour’s risk, it is also necessary to assess the initial FIGO stage (according to the 2009 classification) [5], as well as the histologic type and the histologic grade (which is prognostic). Most authors agree that the reference examination is a pelvic and para-aortic MRI. The radiologist must attentively examine, in particular, invasion of the myometrium, cervix, and lymph nodes.

According to the meta-analysis published by Frei et al. in 2000 [18], a positive MRI result significantly increases the probability of deep myometrial invasion, regardless of the grade. Similarly, in a meta-analysis of 18 studies, Selman et al. found MRI to be more accurate than CT or transvaginal ultrasound, especially for the diagnosis of lymph node invasion [19].

In the present series, the understaging in five cases was only due to an error in assessing the extent of disease on imaging. The authors noted that lymph node extension was found on histopathologic analysis of the surgical specimen in four patients in the endometrial biopsy group (21.1% of the understaged cases in this group) and deep myometrial invasion into the serosa in one woman in the hysteroscopy-curettage group (14.3% of the understaged cases). Lymphovascular invasion cannot be predicted by either MRI or by histological analysis in preoperative sample. The present authors chose to include these patients in the endometrial biopsy and hysteroscopy subgroups because they wanted to assess the combination of the histology and imaging examinations, and not only the histology examination in the pre-treatment assessment of these cancers. That is, despite the absence of a relation between the type of preoperative pathology examination and the underestimation of the local/regional extent of the cancer, it seems to be difficult to dissociate the two examinations. The underassessment or understaging may thus be mixed. In everyday practice, women have both imaging and pathology examinations to realize the staging, and it is therefore necessary to take into account the potential errors associated with imaging. In the present study the proportion of understaging associated only with imaging was approximately the same in the two groups and thus did not affect their comparison (14.3% vs 21.1%).

Because of discrepancy between preoperative assessment and final disease, omitting lymphadenectomy carries a risk of inadequate staging, leading to secondary lymphadenectomy or systematic adjuvant radiotherapy for these patients or under-treatment [20]. According to the present study, hysteroscopy is one option to improve the preoperative staging. The lack of consensus regarding the preoperative and the surgical staging lead to different algorithms according surgical teams (e.g., using sentinel-lymph-node biopsy, ultra-staging of the disease [21–23], nomogram [22] or molecular marker [24]). Nevertheless, other studies need to evaluate the cost-effectiveness of these procedures and its feasibility in a large population. In an attempt to minimize under- and over-treatment, others have assessed intraoperative parameters to identify patients having an extremely low probability of lymphatic dissemination [25]. Finally these methods could be complementary to improve the present evaluation of the clinical outcome of early-stage EEC.

One of the limitations of the present study is its retrospective nature, with data predating the current guidelines. Nonetheless, the present histology sampling practices have not changed since 2010. The understaging rate is therefore certainly similar since the new guidelines went into effect. Prospective studies may also be necessary to assess the current rate of understaging, and determine if the surgical re-operation that appears to be indicated actually is effectively performed.

Hysteroscopy-curettage combined with MRI improves preoperative staging of early-stage endometrial cancer, especially for women at intermediate-risk, despite the fact that preoperative staging cannot detected lymphovascular invasion, and MRI can be faulted to evaluate the extension of the disease. Nevertheless, it seems that we can improve the preoperative histological sample. It would then be necessary to study whether women who have a hysteroscopy-curettage, actually have fewer surgical revisions. If they do, it may be appropriate to add this examination to the preoperative work-up of endometrial cancer for women at intermediate- or even low-risk disease.

References


Address reprint requests to:
G. BLEU, M.D.
Jeanne de Flandre Hospital
University of Lille Nord de France
Avenue Eugène Avinée,
59037 Lille Cedex (France)
e-mail: geraldine.bleu@gmail.com
Introduction

Cervical carcinoma is one of the most common malignancies of the female reproductive tract, of which squamous cell carcinoma accounts for 90-95% [1]. Surgery remains the first-line treatment for these patients, but even the best surgical technique is associated with release of tumor cells to blood and lymph [2-4]. Many patients may already shelter preexisting micrometastases and scattered tumor cells at the time of surgery [3]. Consequently, local recurrence or metastatic disease frequently occurs and ultimately may prove lethal. Whether the minimal residual disease results in clinical metastases depends primarily on the balance between the body’s immune activity and the tumor’s ability to proliferate, vascularize, and colonize a new site [4].

The idea that surgery per se depresses immunity and promotes local cancer recurrence and distant metastasis is not novel. The immunosuppressive effects of surgery have been well documented in both humans and animals [5-7]. A number of theories explain how the surgical procedure itself may promote cancer recurrence after excision; most notably that the adverse impact of surgical stress on the body’s innate tumor defense mechanisms [4, 8]. Interestingly, there is growing recognition of the potential for anesthetic technique to influence long-term outcome in cancer patients by modulating the neuroendocrine stress response and via interactions with the immune system. Compared with general anesthesia, regional anesthesia, including epidural and spinal block, can effectively reduce the excessive stress response after surgery [9], and thereby attenuate surgery-induced immunosuppression [10, 11]. Clinical observation has indicated the association between regional anesthesia and reduced tumor recurrence and improved survival in patients with breast cancer [12], ovarian cancer [13], colon cancer [14], prostate cancer [15], and melanoma [16]. However, the possible influence of epidural anesthesia on postoperative immune function in cervical carcinoma patients undergoing radical resection is still largely unknown.

It is common knowledge among tumor immunologists that natural killer (NK) cells play a crucial role in the powerful elimination of tumor cells [17]. They are the primary defense against cancer cells [15]. Multiple studies show an inverse relationship between NK cell activity at the time of surgery and the development of metastatic disease. Patients...
with a low level of NK cell activity have been reported to have a higher incidence of cancer [18]. Animal studies have shown that stress-induced reduction in NK cell activity can cause enhanced tumor development [19]. On the other hand, the body’s immune system is also modulated by a complex, integrated network of cytokines including interleukins (ILs) and interferons. Certain cytokines (such as IL-2 and IFN-γ) are antitumorigenic and thus promote and stimulate the immune system’s antitumor capability, whereas others (such as IL-1β, IL-6, and IL-8) are protumorigenic and thus inhibit an effective immune response and permit tumor growth [20]. Therefore, the present authors designed a prospective randomized study aimed at evaluating the influence of epidural anesthesia on the early immunological changes, including NK cell activity, protumorigenic cytokines, and antitumorigenic cytokines, in cervical carcinoma patients undergoing radical resection. They tested whether combined general/epidural anesthesia, compared to general anesthesia alone, may help to preserve the body’s defenses against tumor progression.

Materials and Methods

Patients

Between January 2010 and December 2012, all pathologically diagnosed cervical carcinoma patients (range 25–70 years) with an American Society of Anesthesiologists (ASA) physical status of I-II scheduled to receive radical resection were enrolled in this study. The exclusion criteria were: emergency surgery, severe cardiac insufficiency, use of analgesic medication the week before surgery, acute medical illness within 16 weeks of current study, history of substance abuse or cognitive dysfunction, ongoing therapies with immune-regulatory drugs, and any contraindication to the epidural catheter placement. The ethical committees of the present hospital approved this study, and informed consent was obtained from all patients.

Protocol

Patients were randomly assigned to combined general/epidural anesthesia (study group) or general anesthesia alone (control group). For radical resection of cervical carcinoma, extensive hysterectomy and pelvic lymphadenectomy are sometimes necessary. Taking into account the possibly of wide operation scope and prolonged operation time, there was no group given epidural anesthesia alone in the present study. Surgical team and procedures and intraoperative and postoperative management were similar in both groups with the only difference in the intraoperative analgesia.

All patients received intramuscular injection of phenobarbital 100 mg and atropine 0.5 mg 30 minutes before the induction of anesthesia. In the study group, an epidural catheter was placed via the L1–L2 interspaces and 10 ml of 0.75% ropivacaine was administered. General anesthesia was induced using 0.05-1 mg/kg midazolam, 2.2–2.5 μg/kg fentanyl, 1.5–2.5 mg/kg propofol, and 0.25-0.5 mg/kg atracurium intravenously, and maintained with continuous intravenous infusion of propofol 4-6 mg /kg/h, atracurium 0.3 mg /kg/h, and remifentanil 4-5 μg /kg/h. The patients received five ml of 0.75% ropivacaine via epidural catheter during operation every 1.5 hours. General anaesthesia was induced using the same method in the control group as in the study group, but maintained with intravenous infusion of propofol 5-10 mg /kg/h, atracurium 0.5 mg /kg/h, and remifentanil 5-10 μg /kg/h.

Table 1. — Patients characteristics (values are expressed as mean ± standard deviation).

<table>
<thead>
<tr>
<th>Variable</th>
<th>General anesthesia</th>
<th>Combined general/ epidural anesthesia</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61 ± 7.8</td>
<td>59 ± 6.3</td>
<td>0.595</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64 ± 8.1</td>
<td>66 ± 7.5</td>
<td>0.634</td>
</tr>
<tr>
<td>Operative time (min)</td>
<td>136 ± 28.8</td>
<td>145 ± 31.3</td>
<td>0.328</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>285 ± 42</td>
<td>298 ± 38</td>
<td>0.459</td>
</tr>
<tr>
<td>ASA risk score</td>
<td>2.3 ± 0.2</td>
<td>2.1 ± 0.3</td>
<td>0.225</td>
</tr>
</tbody>
</table>

Results

From January 2010 to December 2012, a total of 85 patients were entered into this study. Forty-three patients formed the study group while 42 patients formed the control group. The two groups were homogeneous for age, weight, ASA risk score, blood loss, and length of surgical procedure (Table 1). None of the patients experienced complications due to the epidural catheter.

No difference was observed in basal NK cell activity (T1 NK cell activity) between study and control groups. In both of the two groups, NK cell activity was lower at T2 (33.1 ±
General epidural anesthesia in combination preserves NK cell activity and affects cytokine response in cervical carcinoma patients etc.

4.6% and 25.2 ± 4.0%) and T3 (30.2 ± 5.4% and 19.0 ± 4.6%) than at T1 (38.1 ± 9.3% and 36.2 ± 8.3%). Furthermore, levels of NK cell activity were significantly higher in study group than in control group at both T2 (p = 0.008) and T3 (p < 0.001) (Figure 1).

Enzyme-linked immunosorbent assays were performed to detect concentrations of cytokines at different time points. At T1, there were no differences in IL-1β, IL-2, IL-6, IL-8, and IFN-γ concentrations between the study and control groups. Importantly, the study group showed significantly lower IL-1β, IL-6, and IL-8 concentrations and higher IL-2 and IFN-γ concentrations at both T2 and T3 compared to the control group (Figure 2).

Discussion

It is believed that the postoperative period is the most vulnerable period for potential metastasis after surgery [22, 23]. This vulnerability is mostly attributed to immunosuppression [24]. Possible effects of anesthesia on the immune system have been discussed from the early 20th century, and the protective function of regional anesthesia on immunity is increasingly acknowledged. Use of epidural anesthesia may help to preserve the body’s defenses against tumor progression [11], and consequently, decrease tumor recurrence and improve patients’ survival [25]. In the present study, the authors detected NK cell activity and five cytokines in cervical carcinoma patients receiving different anesthesia regimens. They found that patients who received combined general/epidural anesthesia exhibited less suppression of NK cell activity, higher levels of antitumor cytokines IL-2 and IFN-γ, and lower levels of tumorigenic cytokines IL-1β, IL-6, and IL-8 in the early stage after operation, compared with those who received general anesthesia alone.

NK cells are a distinct subpopulation of lymphoid lineage that can “naturally” kill certain tumor cells and virus-infected cells without prior sensitization or MHC restriction. Animal studies have indicated that suppression of NK cell activity can promote the development of some types of tumor, specifically during the metastatic process [19, 26], and human studies have shown associations between low perioperative NK cell activity and higher rates of cancer recurrence or mortality [27]. Surgery induced depression in cell mediated immunity, in-
cluding NK cell functions, has been well documented. The present study showed better NK cell activity in cervical carcinoma patients when epidural anesthesia combined with general anesthesia. This finding is in agreement with some previous studies. For example, similar preservation of NK-cell activity following epidural use has been shown in epithelial ovarian cancer patients [11]. In Koltun et al.’s study, patients receiving general anesthesia had a significant reduction in NK cell cytotoxicity perioperatively, while patients receiving epidural anesthesia had no significant change in NK cell cytotoxicity [28]. Collectively, these observations suggest that epidural anesthesia would attenuate postoperative suppression of NK-cell function and help preserve effective defenses against tumor progression.

The role of cytokines in cancer immunity and carcinogenesis in general has been well established. Certain cytokines are protumorigenic, whereas others are antitumorigenic [20]. Studies have shown that IL-1 influences tumor growth and metastases either directly by proliferative effects or indirectly by enhancing proinflammatory and proangiogenic pathways in host cells [29]. The up-regulation of IL-1β in breast cancer is considered a poor prognostic indicator [30]. Serum IL-6 concentration was also found significantly higher in cancer patients compared with healthy controls [31], and associated with tumor metastases and patient survival [32]. Another tumorigenic cytokine, IL-8, is critical to tumor neovascularization and progression [33]. On the other hand, both IL-2 and IFN-γ are well known antitumor cytokines. The study by Bobe et al. indicated that decreased levels of IL-2 are associated with an increased risk of colorectal adenoma recurrence [34]. Furthermore, a number of positive clinical results have been observed after IL-2 treatment in a variety of solid tumours [20]. In terms of IFN-γ, it has important effects in the tumor microenvironment, including the inhibition of cell proliferation and angiogenesis [35]. Previous investigations have proven the effect of regional anesthesia on perioperative cytokine response and tumor metastasis. For example, in Deegan et al.’s study, patients receiving regional anesthesia showed a decrease in tumorigenic cytokines IL-1β/IL-8 and an increase in antitumor cytokine IL-10 [30]. The present results also showed higher levels of antitumorigenic IL-2 and IFN-γ, and lower levels of protumorigenic IL-1β, IL-6, and IL-8 in the early postoperative period in patients who received combined general/epidural anesthesia. These data may help to explain why epidural anesthesia could defend the body’s immune function and has been associated with decreased recurrence rates and prolonged survival in patients with malignancies.

In conclusion, this prospective trial indicates difference in NK cell activity and cytokine response between cervical carcinoma patients receiving combined general/epidural anesthesia and general anesthesia alone. Combined general/epidural anesthesia seems to positively influence NK cell activity and cytokines pattern. Further investigation is required to determine the significance of these observations.

References

General/epidural anesthesia in combination preserves NK cell activity and affects cytokine response in cervical carcinoma patients etc.


Address reprint requests to:
J.M. LI, M.D.
Department of anesthesiology
The First Affiliated Hospital of Kunming Medical University
No. 295 XiChang Road
Kunming 650032 (China)
e-mail: manulijm@163.com
Introduction

Placental site trophoblastic tumor (PSTT) is a rare form gestational trophoblastic disease (GTD). It was first described in 1976 as trophoblastic pseudotumor, a form of benign, exaggerated placental site reaction [1]. In 1981, Twiggs et al. reported a fatal case associated with widespread metastatic disease [2]. In view of this new evidence of the potential malignant behavior of the disease, it was renamed PSTT [3]. Pathologically the tumor consists predominantly of mononuclear intermediate trophoblastic cells arising from the placental implantation site that infiltrate between smooth muscle fibers of the myometrium. Vascular invasion may also occur. Immunohistochemical staining shows expression of human placental lactogen (hPL) and human chorionic gonadotropin (hCG).

Patients most often present with amenorrhea or irregular vaginal bleeding, months or possibly years after any type of pregnancy including normal delivery, abortion, miscarriage, or hydatidiform mole. Clinically, PSTT tends to be localized within the uterus at diagnosis, grows slowly, and disseminates late. The behavior of PSTT has not been well predicted by the traditional prognostic scoring system of Bagshawe for GTD and needs to be considered separately [4]. Since PSTT is relatively resistant to chemotherapy, surgery (hysterectomy) has been the mainstay of treatment.

Current knowledge of PSTT is based on the experience of authors who reported small series or singular cases. Thus, the understanding of the variable biologic behavior and treatment alternatives of PSTT is still limited. To investigate the diagnosis, treatment, and risk factors of PSTT, the authors analyzed the clinical and pathological characteristics of eight cases of PSTT presenting to Istanbul Medical Faculty Hospital between 1988 and 2010.

Materials and Methods

Review of 23 years of clinical experience at Istanbul Medical University Hospital between 1988-2010 revealed eight cases of PSTT. Pathology specimens were reviewed by three pathologists who are specialized in gyneco-pathology. The pathologic specimens were examined and stained for hCG and hPL, mitotic counts per ten high-power fields (HPF) were also determined.

Patients’ records including pathology, operative and radiology reports, and laboratory results were reviewed. Data collected included patient age, presenting symptom, hCG level, type of and time from antecedent pregnancy, sex of the fetus/infant from antecedent pregnancy, extent of disease at diagnosis, mitotic count and immunohistochemical expression of the tumor, treatment, response to treatment, length of survival, and current disease status. Data was obtained mainly from patient records but some information was also obtained by calling the patients.

Metastatic workup included chest X ray, hepatic function tests, head computed tomography (CT), ultrasound and CT scan of the...
abdomen and pelvis. Serum hCG levels were used as the primary indicator of response to treatment and during follow-up to determine recurrence. Tumours were staged according to the revised International Federation of Gynecology and Obstetrics (FIGO) criteria for gestational trophoblastic neoplasia [5].

Results

The clinical characteristics of the eight patients are outlined in Table 1. The mean age of patients was 31 years (range, 20-43 years). The antecedent pregnancy was full-term delivery in six cases (75%), molar pregnancy in one case (12.5%), and spontaneous abortion in one case (12.5%). The mean interval from the antecedent pregnancy to diagnosis was 35 months (range, six to 192). The sex of the fetus/infant from antecedent pregnancy was male in four cases (57%, 4/7), female in three cases (43%, 3/7), and in one patient because of early spontaneous abortion the gender could not be determined. The average serum hCG levels at the time of diagnosis was 614 mIU/ml (range, 0.1 - 2,280 mIU/ml).

The most common presentation was vaginal bleeding (7 cases, 87%). Six of these patients presented with irregular, abnormal vaginal bleeding; one patient had post-menopausal bleeding. One patient had no complaint but persisting high hCG level was detected after spontaneous abortion. The diagnosis of PSTT was established by dilatation and curettage in seven patients (86%) and was confirmed by hysterectomy. For preserving fertility, presurgical treatment with single agent methotrexate (alternating with folinic acid) in two patients, and one of them subsequently was treated with two cycles of EMA/CO (etoposide, methotrexate, and actinomycin D, alternating with cyclophosphamide and vincristine), had produced no response and these patients ultimately underwent a hysterectomy. None of the patients received adjuvant chemotherapy. One patient died of an unknown reason, one month after the surgery. The rest of the patients were alive and without evidence of disease after an average of 3.5 years (range, 1-one to 11) of follow-up.

Discussion

PSTT, is such an uncommon form of GTD that only eight cases were treated at Istanbul Medical Faculty Hospital, between 1988 and 2010. They accounted for 6.7% of cases of GTD (n=119) treated at the center during that period. The mean age (31.0 years) of the patients reported herein is like the mean age (~32 years) of patients with PSTT reported in previous studies [6, 7]. PSTT is extremely rare in post-menopausal women. The oldest patient with PSTT, as reported by Nigam et al. [8], was a 63-year-old woman. The present authors have found that the most prevailing presenting symptom is abnormal vaginal bleeding. Less common symptoms reported in the literature were amenorrhea, abdominal pain, and galactorrhea [7, 9]. The finding that PSTT

Table 1. — Clinical features of patients presenting with PSTT.

<table>
<thead>
<tr>
<th>Category</th>
<th>Clinical features</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range) (years)</td>
<td>31.0 (20 - 43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antecedent pregnancy</td>
<td>Normal, term delivery</td>
<td>6</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Molar pregnancy</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>Spontaneous abortion</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>Interval from antecedent pregnancy (months)</td>
<td>35 (6 - 192)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean hCG (range) (mIU/ml)</td>
<td>614 (0.1 - 2,280)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presenting symptom</td>
<td>Vaginal bleeding</td>
<td>7</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Persisting high hCG levels</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Sex of the infant/fetus</td>
<td>Male</td>
<td>4</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3</td>
<td>43</td>
</tr>
</tbody>
</table>

Table 2. — Characteristics of patients presenting with PSTT.

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Age (yrs)</th>
<th>FIGO stage</th>
<th>hCG (mIU/ml)</th>
<th>Antecedent Pregnancy</th>
<th>Interval from pregnancy (mo)</th>
<th>Treatment</th>
<th>Mitotic count (per 10 HPF)</th>
<th>Alive</th>
<th>Survival (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>I</td>
<td>2,015</td>
<td>NSVD</td>
<td>6</td>
<td>Surg</td>
<td>6</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>I</td>
<td>113</td>
<td>MP</td>
<td>12</td>
<td>Chemo/surg</td>
<td>NA</td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>I</td>
<td>2,280</td>
<td>NSVD</td>
<td>192</td>
<td>Surg</td>
<td>5</td>
<td>Yes</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>I</td>
<td>0.1</td>
<td>NSVD</td>
<td>24</td>
<td>Surg</td>
<td>NA</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>I</td>
<td>2.0</td>
<td>NSVD</td>
<td>10</td>
<td>Surg</td>
<td>NA</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>I</td>
<td>129</td>
<td>NSVD</td>
<td>11</td>
<td>Surg</td>
<td>NA</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>I</td>
<td>279</td>
<td>SAB</td>
<td>18</td>
<td>Chemo/surg</td>
<td>7</td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>I</td>
<td>100</td>
<td>NSVD</td>
<td>10</td>
<td>Surg</td>
<td>6</td>
<td>Yes</td>
<td>2</td>
</tr>
</tbody>
</table>

*Patient died of an unknown reason, one month after the surgery. yrs: years; mo: months; SAB: spontaneous abortion; NSVD: normal spontaneous vaginal delivery; MP: molar pregnancy; N/A: not available; surg: surgery; chemo: chemotherapy.
occurred after a full-term pregnancy in six of the eight patients reported herein corroborates previous studies that most PSTTs are detected after a full-term normal delivery [6, 7]. In contrast to the literature [7], the present authors have observed that the commonest sex of the fetus/infant resulting from the antecluent pregnancy is male (57%, 4/7). The interval from antecluent pregnancy in this report was ≤ two years in seven patients and 16 years in one patient. There are only a few cases reported in the literature with interval from antecluent pregnancy longer than 16 years [7, 8, 10]. The serum hCG levels of PSTT at diagnosis are lower than in other types of GTD. The mean serum hCG level (614 mIU/ml) was < 1,000 mIU/ml like the other studies [11]. At diagnosis the disease was localized to the uterus in all of the present cases (FIGO Stage 1). Of 119 patients with PSTT collated from four large series in the literature, 78 (65.5%) presented with disease confined to the uterus (Stage 1), 14 (11.8%) with disease extension to the pelvis (Stage 2), 18 (15.1%) with lung metastases (Stage 3), and nine (7.6%) with metastases in other sites (Stage 4) [6, 7, 9, 12].

Since the clinical characteristics of PSTT are non-specific, the diagnosis is often made retrospectively by a combination of pathology, immunohistochemistry, and radiology. hPL stain is a fairly sensitive method of diagnosing PSTT [11]. hCG and hPL staining were all positive in this study.

Identification of adverse prognostic factors is important for the understanding of the biologic behavior and treatment alternatives of PSTT. In this report, only one patient died of an unknown reason one month after the surgery; all of the patients who presented with disease confined to the uterus have survived with no evidence of disease. This finding corroborates previous studies showing that patients with disease confined to the uterus have excellent outcome with survival rates of about 95%, whereas approximately 70% of patients presenting with disease beyond the uterus have progression of disease and die despite surgery and aggressive multidrug chemotherapy. It is concluded that disease extension beyond the uterus is the most important adverse prognostic factor. Other adverse prognostic factors are interval from antecedent pregnancy > two years, age > 40 years, mitotic count > five, mitotic figures /10 HPF, maximum serum β-hCG level > 1,000 mIU/ml, deep myometrial invasion, extensive coagulation necrosis, and presence of cells with clear cytoplasm within the tumor [6, 7, 9, 12].

Since PSTT is less sensitive to chemotherapy than other forms of GTD, hysterectomy remains the primary mode of therapy in disease confined to the uterus [7, 13]. Ovarian micrometastases from disease apparently confined to the uterus is rare (3%); thus, preservation of grossly normal ovaries at the time of hysterectomy in premenopausal women who wish to preserve ovarian function is reasonable [7, 14]. The role of adjuvant chemotherapy after surgery for disease confined to the uterus has yet not been established and remains controversial [12, 15]. In the present study, surgery alone appeared sufficient for good prognosis in early-stage PSTT.

In conclusion, PSTT is a rare entity. Surgery is the primary method of treatment for non-metastatic PSTT. For patients with Stage 1 disease, chemotherapy remains controversial and hysterectomy alone seems sufficient and can provide long-term survival. Serum hCG levels can be used in following treatment response and for long-term monitoring of disease. Cooperation among large centers is necessary in order to advance therapy through prospective treatment trials.

References


Address reprint requests to: O. KURU, M.D.
Department of Obstetrics and Gynecology
Kanuni Sultan Suleyman Research and Teaching Hospital
Atakent mh. Turgut Ozal cd. no. 1
34303 Altıntaş/Kuşkucuknece İstanbul (Turkey)
e-mail: droguzhankuru@yahoo.com
Total laparoscopic radical hysterectomy: a change in practice for the management of early stage cervical cancer in a U.K. cancer center

G. Angelopoulos¹, A. Etman², D.J. Cruickshank¹, J.P. Twigg¹

¹ Department of Gynaecological Oncology, The James Cook University Hospital, Middlesbrough
² Department of Obstetrics and Gynaecology, The James Cook University Hospital, Middlesbrough (United Kingdom)

Summary

In order to evaluate the safety, surgical, and oncological outcomes of the introduction of a total laparoscopic radical hysterectomy (TLRH) service, the authors conducted a retrospective review of all TLRHs performed in the present centre from the beginning of the service in August 2010. TLRH appears in this series to be safe. Complication rates were comparable to National Institute for Health and Clinical Excellence (NICE) and literature standards. Oncological outcomes, despite the short follow up period, appear acceptable. TLRH is a valuable alternative to open surgery for the treatment of early cervical cancer.

Key words: Laparoscopy; Cervical cancer; Radical hysterectomy.

Introduction

Since it was first described in the early 1990s [1, 2], total laparoscopic radical hysterectomy (TLRH) with pelvic and/or para-aortic lymphadenectomy has been used widely around the world for the management of early stage cervical cancer. Uptake and adoption of the procedure has been slow due to concerns about surgical safety and oncological outcomes. There have been a number of case series published in the literature, mainly from Asia [3-14], but also from the United States [1, 15-18] and Continental Europe [2, 19-29]. They all conclude that TLRH is safe and effective for the management of early stage cervical cancer.

In the United Kingdom, the National Institute for Health and Clinical Excellence (NICE), in May 2010 published Interventional Procedure Guidance 338 [30] which describes the use of TLRH for the management of early stage cervical cancer. To date, there is only one published series in the literature from the UK describing surgical and oncological outcomes in obese women with early stage cancer [19].

In 2010 the authors decided to adopt TLRH as standard practice for the management of their patients diagnosed with early stage cervical cancer who would have been offered open radical hysterectomy.

In this retrospective study the authors describe their initial experience, focusing on the safety, surgical challenges, and initial oncological outcomes. They demonstrate that with the appropriate approach to resource and service delivery, minimal access surgery (MAS) can be delivered in the UK NHS healthcare setting and that it offers outcomes on par with open surgery.

Materials and Methods

Between August 2010 and March 2013, 41 patients underwent TLRH and pelvic lymphadenectomy for the management of cervical cancer. All procedures were performed at the James Cook University Hospital, Middlesbrough, UK Cancer Centre. They were all diagnosed via histopathological analysis of cervical biopsies and staged according to the International Federation of Gynaecology and Obstetrics (FIGO) classification system [7]. They all had cross-sectional imaging (MRI and CT scans) and were discussed in the central multi-disciplinary team (MDT) meeting. All patients with pre-operative Stage 1B1 and appropriate 1B2 disease were offered surgery.

All patients were enrolled on the present authors’ enhanced recovery protocol. A gynaecological oncologist and/or a sub-specialty trainee in gynaecological oncology operated on all patients. In the initial phases of learning, patients were operated on by two gynaecological oncology consultants.

Data were collected retrospectively by review of the patient case notes. Recorded or calculated data included: age, body mass index (BMI), pre-operative FIGO stage and grade of tumour, histological type, duration of the procedure, intraoperative complications, estimated blood loss (EBL), postoperative hospital length of stay, postoperative complications, final histology, final histopathological stage and grade of tumour, nodal count and status, para-cervical and vaginal excision margins, adjuvant treatment, disease-free interval, recurrence rate, and mortality.
Surgical technique

After the induction of general anaesthesia the patient was placed in lithotomy position. An examination under anaesthetic was performed, the bladder was catheterised, and a uterine manipulator was placed in the uterine cavity. A uterine manipulator was used in the majority of the cases. The patient then was placed in the Lloyd-Davies position. Carbon dioxide pneumoperitoneum was achieved via intra-umbilical Veress needle insertion up to 25 mm Hg pressure. A primary ten-mm port was inserted two cm above the umbilicus and laparoscopy was performed with a ten-mm 30° laparoscope. A 30° laparoscope was used throughout the procedure as it provides better views of the pelvic sidewalls and para-cervical spaces. Two five-mm ports were inserted to the right and left iliac fossae respectively and a 12-mm port two cm below the umbilicus, all under direct vision. Intra-abdominal pressure was reduced to 15mm Hg and maintained throughout the procedure. The patient was placed in steep Trendelenburg position to allow the bowel to move away from the operating field.

A standard laparoscopic surgical kit was used, including bipolar energy forceps, monopolar energy scissors and a five-mm Ligasure sealing device. During the present authors’ learning curve, different advanced laparoscopic instruments were trialled (e.g. harmonic scalpel); however the Ligasure device is now preferred due to its superior combined dissection and haemostatic ability.

The procedure commences with the dissection of the lateral pelvic wall peritoneum two cm above the infundibulopelvic (IP) ligament. The incision was extended laterally cranially parallel to the IP and then inferiorly towards the round ligament to expose the pelvic sidewall structures. Sharp and blunt dissection was used on the para-vesical and para-rectal spaces in order to identify the anatomical landmarks and pelvic sidewall structures (ureter, obturator nerve and vessels, iliac vessels, obliterated and uterine arteries) before undertaking a full systematic pelvic sidewall lymphadenectomy. Both common iliac vessel nodes and obturator fossa nodes were harvested. The lymphadenectomy was performed en-bloc and the nodes were removed from the peritoneal cavity with the help of a Bert laparoscopic collection bag. The procedure continued with dissection of the IP ligaments if bilateral salpingo-oophorectomy was to be performed. If the ovaries were conserved (younger patients) then bilateral salpingectomies were performed and the uterine pedicle was dissected preserving the ovarian blood supply. The round ligaments were then incised followed by the vesico-uterine peritoneum and the bladder was mobilised. The uterine artery was ligated at its origin and the ureteric tunnel was prepared. Both ureters were fully mobilised from the para-cervical tissue. The recto-vaginal peritoneum (posterior parametrium) and the lateral parametria could be dissected as medially or laterally as required. The extent of dissection and radicality of the procedure depended on the pre-operative tumour stage. At this point another five-mm port was inserted supra-publically under direct vision to aid with the vaginal dissection. The upper vagina was incised two to three cm from the upper edge of the cervix and then circumscribed maintaining the same length throughout. The specimen was removed vaginally and the vaginal vault was secured with a continuous laparoscopic suture. Both monofilament and braided sutures were used during the learning curve with monofilament suture being the one used more often. Haemostasis was ensured and the procedure was completed with removal of all ports under vision and release of carbon dioxide gas. The skin ports were sutured and local anaesthetic infiltration was used for post-operative analgesia.

No urinary catheter was left at the end of the procedure but attention was paid postoperatively to bladder care. Despite the fact that the patients are not catheterised immediately after the operation, they are put on strict fluid monitoring and bladder residuals are recorded using bladder scanning. If urine residuals are high (>100 ml) then intermittent self-catheterisation (ISC) is taught to the patient as the majority of the patients are discharged home the day following the operation. If the patient does not manage with ISC then a urinary catheter is inserted and the patient is discharged home. Bladder care continues at home under the supervision of the district nurses. If the patient is discharged with a catheter follow up is arranged as a ward attender for a trial without a catheter (TWOCC) seven days postoperatively.

Results

Forty-one patients underwent a TLRH with bilateral pelvic lymphadenectomy with or without bilateral salpingo-oophorectomy between August 2010 and March 2013. Patient and tumour characteristics are summarised in Table 1. Mean age was 41.5 years (range 23-86) and mean BMI was 28 (range 18-37). Median operating time was 260 minutes (range 90-390) and average EBL was 157 ml (range 36-500). No patient required intra-or postoperative blood transfusion. The median inpatient hospital stay was 1.8 days (range 1-6).

There were three major intraoperative complications (7.3%). Two patients had an obturator nerve transection (4.8%). The nerve injury was repaired laparoscopically using 4/0 monofilament suture. One patient suffered a bladder injury (2.4%). Due to the position of the injury, the procedure had to be converted and completed via laparotomy. Six weeks postoperatively the patient developed a vesico-vaginal fistula that was surgically repaired at three months via an open procedure (Table 2).
Two of the present patients (4.8%) had delayed return of bladder function requiring ISC (two and three weeks, respectively). Other postoperative complications included one patient (2.4%) with port site herniation (no re-operation required) and one (2.4%) with lack of sensation on her right thigh area (suspected genitofemoral nerve injury). This resolved spontaneously. Notably five of the present patients (12%) reported “leaking” of fluid per vaginum immediately postoperatively. This fluid was sent for biochemistry that confirmed lymphatic fluid. Imaging showed no injury to the urinary tract and no patient developed a uretero-vaginal or vesico-vaginal fistula. Leaking resolved spontaneously in all the patients within two to three weeks.

Forty (of 41) patients had clear cervical and vaginal excision margins (97.5%). One patient had a borderline (four-mm) parametrical excision margin and was offered adjuvant treatment but she declined adjuvant treatment. To date she has had no recurrence of her disease.

Median node count was 16 (range 7-25) and seven patients received adjuvant chemo-radiation therapy (17%). All the patients who received adjuvant treatment did so because they had positive nodes. There was one death from recurrence (2.4%). This patient had negative lymph nodes and negative surgical excision margins. However she presented with distant recurrence within 18 months and died from disease progression despite a course of rescue chemo-radiotherapy treatment. Median follow up period was 11 months (range: 1-31).

Discussion

NICE Interventional Procedural Guidance (IPG) 338 [30] summarises the reported major intra- and postoperative complications in the literature for TLRH and also provides a comparison with the gold-standard open approach. The publication of the NICE IPG guidance allows NHS service providers to introduce new interventions using a defined clinical governance framework provided by NICE. This study was undertaken to determine whether the new service opened in the present centre met these criteria and supported its on-going provision.

Visceral injuries (bladder, bowel, ureteric) in IPG 338 were comparable to the open procedure [30]. Bladder injuries ranged between 1-10%, bowel 2%, and ureteric injuries 0-4%. In the present series the authors report a bladder injury rate of 2.4% and no bowel or ureteric injuries. Fistula formation is reported between 1-2% across the literature (2.4% in present series). Although this percentage appears high, it is explained by the small number of cases (41). In fact only one patient developed a vesico-vaginal fistula, the same patient that also suffered the bladder injury. Most of the intraoperative complications reported in the literature are managed laparoscopically, with the exception of vascular injuries that require laparotomy conversion. In the largest single institute case series reported, Xue et al. [12] report a 1.3% conversion rate (4/317 cases) and Puntambekar et al. [13] 0% (0/248 cases). TLRH also appears to be superior when comparing the total estimated blood loss, blood transfusions rate, and postoperative hospital stay [22].

One of the major challenges in adopting the laparoscopic approach is the learning curve and extended operating time. Most literature suggests that TLRH is a relatively long procedure with reported ranges from 92 to 420 minutes [17, 29, 31]. The present median operating time was 260 minutes (range 90-390). Standardisation of the technique and use of advanced energy instruments does significantly reduce the operating time [13, 14, 17]. Buddy operating has also been proposed [32] and is a sensible approach, especially during the learning curve. The present centre adopted this approach during the initial phase of the learning curve.

Teaching the procedure to trainees is also a major challenge. Only one publication [16] addresses the issue with favourable outcomes. However given the diversity and trends of training in each country, this issue can only be addressed in depth through training curriculum development at a national level.

Oncological outcomes are also important in determining the acceptability of surgical intervention in cancer surgery. Total nodal count, surgical excision margins, and disease-free and overall survival are recognised parameters that characterise oncological success. Generally total lymph node yield laparoscopically seems to be lower than for open surgery [32]. There is also significant variability in the reported numbers of yielded lymph nodes among the published series. An average of 20 nodes is considered to be the gold standard according to some studies [33-35]. However, one needs to approach this matter with caution as some studies report combined pelvic and para-aortic lymph node counts. Undertaking para-aortic lymphadenectomy for the surgical treatment of early stage cervical cancer is not an accepted approach in many centres and this will also influence total lymph node yields. Furthermore, the total number of nodes retrieved during a complete lymphadenectomy may not accurately represent the number of nodes present in each node harbouring area. Therefore, a low yield of lymph nodes per patient is not a sign of poor surgical technique or inadequate oncological staging, but...
is a factor of different patient characteristics (e.g. BMI, left-side asymmetry [33]) that ultimately influences the final lymph node count.

Surgical excision margins, both parametrial and vaginal, compared favourably to that of the open approach. This is an area that causes anxiety for surgeons comfortable with open surgery for cervical cancer, the ability to obtain adequate surgical margins using a minimal access approach. Spirtos et al. [17] reported a mean parametrial length of 3.3 cm (range 1-5) and vaginal length of 2.15 cm (range 1-3.5) in their first report. More recently Ghezzi et al. [28] in a histopathological comparison between TLRH and open radical hysterectomy, report no significant difference between the two surgical approaches.

Another area of debate between open and minimal access surgeons surrounds recurrence, disease-free, and overall survival data after TLRH. In the majority of published case series, the recurrence and survival figures are either immature or missing [22]. There is also a lack of stratification according to stage and volume of disease, which makes interpretation of available data difficult. Puntambekar et al. [13] in 248 patients of Stage IA2-IB1 cervical cancer surgical procedures’, reported seven recurrences (2.8%) and no deaths after a median follow-up period of 36 months (range: 0-50). Lee et al. [7] reported long-term survival outcomes in 139 patients. In a median follow-up of 92.1 months, the mean cumulative disease-free and overall survival rates were 91.01% (+/- 2.77%) and 92.78% (+/- 3.06%), respectively. However, 60 of 139 patients (43.2%) were Stage IA and 26 patients were Stage IIA1, which would not be indication for TLRH in UK NHS practice. Chen et al. [10] in their prospective analysis of 295 patients reported that recurrences or metastasis occurred in 48 patients (16.3%). Of these patients, 43 (14.6%) died of their disease, and five (1.7%) were alive with disease. The overall disease-free survival was 95.2% for IA, 96.2% for IB, and 84.5% for IIA. The median follow-up was 36.45 months in these studies (range, 8-76). In the present study the follow up period was not as long as other authors. Nevertheless, the authors present their initial data as it demonstrates the feasibility and surgical safety of minimal access surgical techniques in the treatment of cervical cancer in the UK. They feel that other centres contemplating the introduction of such a service will be encouraged by this data demonstrating good initial patient outcomes. The authors believe that ideally a randomized control trial (RCT) would reliably answer the questions regarding comparative surgical and long-term oncological outcomes. Given the relatively small numbers of early-stage cervical cancer diagnosed in the UK annually (primarily due to the success of the national screening programme), an alternative would be the setup of a national registry of cases. The authors would also like to encourage other centres to publish their data for comparison and training purposes.

**Conclusion**

TLRH surgical outcome from this series compare favourably to the figures published by NICE and the international literature. Complication rates were comparable to NICE criteria. Oncological outcomes despite the short follow up period and small size of the patient cohort appear acceptable. Other surgical quality indicators such as in-patient hospital stay and EBL offer the patient an advantage over traditional open surgery.

**References**

Total laparoscopic radical hysterectomy: a change in practice for the management of early stage cervical cancer in a U.K. cancer center


Address reprint requests to:
G. ANGELOPOULOS, M.D.
Department of Gynaecological Oncology
The James Cook University Hospital,
Marton Road, Middlesbrough, TS4 3BW
(United Kingdom)
e-mail: g.angeloopoulos@nhs.net
Increased microRNA-206 and its function in cervical cancer

S. Ling1, M. Ruiqin2, Z. Guohong3, S. Bing1, C. Yanshan1
1 Department of Obstetrics and Gynecology, The First Affiliated Hospital of Shantou University Medical College, Shantou
2 Department of Laboratory Medicine, The First Affiliated Hospital of Shantou University Medical College, Shantou
3 Department of Pathology, Shantou University Medical College, Shantou (China)

Summary
Objective: MicroRNA-206 plays important roles in tumorigenesis and tumor progression of various human malignancies. However, its involvement in cervical cancer has remained unclear. Objective: The aim of this study was to examine the expression patterns and clinical implications of miR-206 in cervical cancer. Materials and Methods: Quantitative RT-PCR was performed to evaluate the expression levels of miR-206 in cervical cancer cell lines and primary tumor tissues. The clinicopathologic significance and the prognostic value of miR-206 expression were further determined. Finally, the effects of miR-206 on HeLa cell proliferation, apoptosis, invasion, and migration were investigated. Results: MiR-206 expression was significantly downregulated in cervical cancer samples when compared with normal adjacent tissues. Low level of miR-206 was associated with advanced FIGO stage (p < 0.001), positive lymph node metastasis (p < 0.001), poor differentiation (p = 0.016), and human papillomavirus infection (p = 0.007). Multivariate Cox regression analysis revealed that decreased miR-206 expression was an independent unfavorable prognostic factor for overall survival. In addition, transfection of miR-206 mimics in HeLa cells was able to reduce cell proliferation, promote cell apoptosis, and inhibit cell invasion and migration. Conclusions: miR-206 may act not only as a novel diagnostic and prognostic marker, but also as a potential target for molecular therapy of cervical cancer.

Key words: MicroRNA-206; Cervical cancer; Prognosis; Proliferation; Apoptosis; Invasion.

Introduction
Cervical cancer is the second most common malignancy in women worldwide, with an estimated global incidence of 500,000 new cases and approximately 233,000 deaths per year [1]. Despite recent advances in surgery, radiation, and chemotherapy, clinical outcomes vary significantly between patients and can be difficult to predict. It is now clear that cervical cancer is a complex disease involving the abnormal expression of many oncogenes and tumor suppressor genes. However, the detailed mechanism of cervical carcinogenesis and progression remains largely unknown. Identification of new candidate molecules in cervical cancer development may lead to new preventive and therapeutic approaches to this disease, and improve patient survival.

MicroRNA (miRNA) is a group of short (~22nt) and non-coding RNAs which can control gene expression by inhibiting mRNA translation or by inducing mRNA degradation [2, 3]. Beyond the involvement in diverse biological processes, including cell growth, apoptosis, development, differentiation, and endocrine homeostasis [4], emerging evidence strongly suggests that the deregulation or dysfunction of miRNAs contributes to human carcinogenesis and cancer progression [5-7]. miRNAs can function as either oncogenes or tumor suppressors according to the roles of their target genes. In terms of cervical cancer, in vitro functional assays showed that both miR-100 and miR-125b suppress the proliferation and promote apoptosis of cervical cancer cells [8, 9]. Clinical analysis demonstrated that increased miR-224 expression in cervical cancer tissues were associated with advanced clinical stage and poor prognosis [10]. Furthermore, upregulation of miR-126 sensitizes SiHa cervical cancer cells to bleomycin [11]. MiR-181a confers resistance of cervical cancer to radiation therapy through targeting the pro-apoptotic PRKCD gene [12]. These findings suggest an important role of miRNAs in the diagnosis, biological target therapy, and prognosis prediction in patients suffering cervical cancer.

Located on chromosome 6p12.2, miR-206 has been identified as a tumor-suppressive miRNA that is downregulated in several human malignancies, including glioma [13], laryngeal cancer [14], breast cancer [15], lung cancer [16], gastric cancer [17], colorectal cancer [18], renal cell carcinoma [19], rhabdomyosarcoma [20], and osteosarcoma [21]. Serum miR-206 expression levels has significant diagnostic value for rhabdomyosarcoma (sensitivity of 1.0 and specificity of 0.913) [22]. Decreased miR-206 expression also correlates with tumor progression and poor survival [17, 21, 23, 24]. However, currently, very little is known about the expression and function of miR-206 in cervical cancer. In the current study, the authors analyzed the association of miR-206 expression with clinicopathologic features and prognosis in cervical cancer patients. In addition, the effects of miR-206 on biological behaviors of cervical cancer cells were elucidated.
Table 1. — Association between miR-206 expression and different clinicopathological features of human cervical cancers.

<table>
<thead>
<tr>
<th>Clinicopathological features</th>
<th>No. of cases</th>
<th>miR-206 expression</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td>Low (n, %)</td>
<td>High (n, %)</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>60</td>
<td>27 (45.0%)</td>
<td>33 (55.0%)</td>
</tr>
<tr>
<td>≥ 50</td>
<td>75</td>
<td>41 (54.7%)</td>
<td>34 (45.3%)</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4.0</td>
<td>61</td>
<td>30 (49.2%)</td>
<td>31 (50.8%)</td>
</tr>
<tr>
<td>≥ 4.0</td>
<td>74</td>
<td>38 (51.4%)</td>
<td>36 (48.6%)</td>
</tr>
<tr>
<td>HPV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>49</td>
<td>17 (34.7%)</td>
<td>32 (65.3%)</td>
</tr>
<tr>
<td>Positive</td>
<td>86</td>
<td>51 (59.3%)</td>
<td>35 (40.7%)</td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well- moderate</td>
<td>62</td>
<td>24 (38.7%)</td>
<td>38 (61.3%)</td>
</tr>
<tr>
<td>Poor</td>
<td>73</td>
<td>44 (60.3%)</td>
<td>29 (39.7%)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>51</td>
<td>37 (72.5%)</td>
<td>14 (27.5%)</td>
</tr>
<tr>
<td>Negative</td>
<td>84</td>
<td>31 (36.9%)</td>
<td>53 (63.1%)</td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ib-IIa</td>
<td>69</td>
<td>22 (31.9%)</td>
<td>47 (68.1%)</td>
</tr>
<tr>
<td>IIb-IIIa</td>
<td>66</td>
<td>46 (69.7%)</td>
<td>20 (30.3%)</td>
</tr>
</tbody>
</table>

Materials and Methods

Patients and tissue samples

This study was approved by the Research Ethics Committee of The First Affiliated Hospital of Shantou University Medical College. Written informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to the ethical and legal standards.

Paired cervical cancer and matched adjacent normal tissue specimens (at least three cm away from the tumor node) were collected from 135 patients who underwent surgery between March 2006 and December 2008 in The First Affiliated Hospital of Shantou University Medical College. The fresh tissue specimens were immediately frozen in liquid nitrogen until use. No patients had preoperative chemotherapy or radiotherapy history or other inflammatory diseases. Clinicopathological information is shown in Table 1. The clinical stage was classified according to the International Federation of Gynecology and Obstetrics (FIGO) criteria. All of the patients received follow-up periodically. Follow-up was performed every three months for the first two years, then every six months for the next three years, and then every year thereafter by telephone visit and questionnaire letters. Overall survival (OS) was defined as the time from primary surgery to death of the patient or, for living patients, the date of last follow-up.

Cell culture and transfection

Four human cervical cancer cell lines (HeLa, SiHa, CaSKi, and C33A) were obtained from a biotechnology company of Shanghai and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were incubated at 37°C in a 5% CO2 atmosphere. Normal cervical squamous cells (NCSC) were obtained from fresh normal cervical epithelium of the hysteromyoma patients treated with surgery, and used as controls.

For RNA transfection, the cells were seeded into each well of 24-well plate and incubated overnight, then transfected with mature miR-206 mimics or negative control (NC) using Lipofectamine 2000 in accordance with the manufacturer’s procedure.

RNA extraction and quantitative real-time PCR

RNA isolation from cells or tissue samples was performed with the mirVana miRNA Isolation Kit according to the manufacturer’s protocol. A total of five ug of small RNA was reverse transcribed to cDNA using M-MLV reverse transcriptase with either the miR-206-RT or U6-RT primers. The cDNA was used as a template for amplification of miR-206 and the endogenous control miRNA, U6, by real-time PCR. The real-time PCR was performed and analyzed on the 7300 real-time PCR system, and the following PCR cycles were used: initial denaturation at 94°C for four minutes followed by 40 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 40 seconds. The RT primers were 5'- GCCCGTGACGAGCTGAGAAATTAAACACCACGGCCG -3' for miR-206 and 5'-TGTTGTGTCGTGGAGTCTG -3' for U6. The PCR primers for mature miR-206 or U6 were designed as follows: miR-206 sense, 5'- GGAATGTAAGAAGTGTTG -3' and reverse, 5'- GACGAGGCTGGAGAA -3'; U6 sense, 5'- TTCGCTTCGCAACACGACGGCAGACACGGCCGCG -3'; U6 antisense, 5'- TTCGCTTCGCAACACGACGGCAGACACGGCCGCG -3'. The relative amount of miR-206 to U6 was calculated using the equation 2−ΔΔCt, where ΔCT = (CTmiR-206 - CTU6).

Cell proliferation assay

The cells were seeded into 96-well plates (5,000 cells/well in 200 ul medium) and incubated at 37°C in 5% CO2 after transfection. After incubation for one to five days, 20 ul of MTT solution (five mg/ml) was added to the media of each well, and the plates were further incubated for another four hours. Then, the media was replaced with 150 ul of dimethyl sulfoxide (DMSO), and the absorbance was measured at 490 nm using a microplate reader.

Detection of apoptosis by flow cytometry

Flow cytometry was used to analyze cell apoptosis. Staining of apoptotic cells was achieved by incubating the cells with propidium iodide (ten ug/ml) and Annexin V-FITC ug/ml, BD) in the dark for 15 min at room temperature. Data were acquired on a flowcytometer.

Transwell invasion assay

After transfection of miR-206 mimics or NC, the cells were collected and 5 × 104 cells suspended in 200 ul of serum-free DMEM medium were placed in the upper chambers of each insert coated with 40 ul of 2 mg/ml Matrigel (eight-μm pore size), and 600 ul of DMEM with 20% FBS was added to the lower chamber. After incubation for 24 hours, cells on the upper surface of the membrane were scrubbed off, and the invaded cells were fixed with 95% ethanol, stained with 0.1% crystal violet, and counted under a light microscope.

Scratch migration assay

Scratch migration assay was also performed to confirm the effect of miR-206 on cervical cancer cell migration. When the cells were transfected with miR-206 mimics or NC, the cells were grown to confluence, a scratch in the cell monolayer was made with a cell scratch spatula. After the cells were incubated under standard conditions for 24 hours, the plates were washed twice with fresh medium and pictures were taken.

Statistics

All computations were carried out using the software of SPSS version 13.0 for Windows. Data are expressed as mean ± SD. The differences between groups were analyzed using the Student’s t-test.
Decreased expression of miR-206 in cervical cancer cell lines and primary tumor samples

The expression levels of miR-206 in cervical cancer cell lines and primary tumor samples were detected by qRT-PCR and normalized to U6 small nuclear RNA. As in Figure 1A, the results showed that miR-206 expression levels were significantly downregulated in cervical cancer tissues (mean ± SD: 8.2 ± 1.9) than those in corresponding non-cancerous tissues (mean ± SD: 19.1 ± 4.3; p < 0.001). The present authors also observed decreased miR-206 expression in cervical cancer cell lines, compared to normal cervical squamous cells (Figure 1B, p < 0.001). The HeLa cell line, which possessed the lowest levels of miR-206 expression among all tested cell lines, was selected for miR-206 mimics or NC transfection and further studies.

Association of miR-206 expression with clinicopathologic features and prognosis in patients with cervical cancer

Table 1 summarizes the association between miR-206 expression and clinicopathological parameters in cervical cancers. Using the median miR-206 expression as a cutoff, the patients were divided into high miR-206 expression group and low miR-206 expression group. The present authors found that miR-206 expression was significantly lower in the patients with advanced FIGO stage (p < 0.001), positive lymph node metastasis (p < 0.001), poor differentiation (p = 0.016), and human papillomavirus (HPV) infection (p = 0.007). No significant association was observed between miR-206 expression and patients’ age and tumor size.

The present authors further evaluated whether miR-206 expression had prognostic potential for OS of patients with cervical cancer. As shown in Figure 2, cervical cancer patients with low miR-206 expression tend to have shorter OS than those with high miR-206 expression (p < 0.001). The survival benefits were also found in those with negative lymph node metastasis (p = 0.005) and...
S. Ling, M. Ruqin, Z. Guohong, S. Bing, C. Yanshan

Multivariate Cox regression analysis enrolling the aforementioned significant parameters revealed that miR-206 expression (relative risk [RR] 6.6; \( p = 0.015 \)), the status of lymph node metastasis (RR 5.8; \( p = 0.02 \)), and FIGO stage (RR 9.4; \( p = 0.006 \)) were independent prognostic markers for OS of patients (Table 2).

### Table 2. — Univariate and multivariate analysis of overall survival in 135 patients with cervical cancer.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate log-rank test ((p))</th>
<th>Cox multivariable analysis ((p))</th>
<th>Relative risk (RR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50 vs. (\geq 50)</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4.0 vs. (\geq 4.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative vs. positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well- moderate vs. poor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>0.005</td>
<td>0.02</td>
<td>5.8</td>
</tr>
<tr>
<td>Negative vs. positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIGO Stage (Ib-IIa) vs. (IIb-IIIa)</td>
<td>(&lt; 0.001)</td>
<td>0.006</td>
<td>9.4</td>
</tr>
<tr>
<td>MiR-206 expression</td>
<td>(&lt; 0.001)</td>
<td>0.015</td>
<td>6.6</td>
</tr>
<tr>
<td>High vs. low</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Finally, the present authors assessed the biological role of miR-206 in cervical cancer. qRT-PCR analysis confirmed increased miR-206 expression after miR-206 mimics transfection in HeLa cells (Figure 3A). MTT assay showed that cell proliferation was significantly impaired after miR-206 mimics transfection (Figure 3B), and flow cytometry indicated that upregulation of miR-206 promoted HeLa cell apoptosis (Figure 4).

Cell invasion is a significant aspect of cancer progression, and involves the migration of tumor cells into contiguous tissues and the dissolution of extracellular matrix proteins. Transwell invasion assay was performed to investigate whether miR-206 had a direct influence on HeLa cell invasion. As shown in Figure 5, upregulation of miR-206 impeded the invasion of HeLa cells compared with control. Scratch migration assay also confirmed the inhibitory effect of miR-206 on HeLa cell migration (Figure 6).

### Discussion

Cervical cancer is a malignant tumor that seriously threatens women’s health. It is of great significance to investigate molecular and cellular mechanisms of cervical cancer, and to identify novel genetic or protein markers for...
Decreased microRNA-206 and its function in cervical cancer

Early diagnosis and prediction of prognosis. In the current study, the authors firstly observed that miR-206 was downregulated in cervical cancer compared with adjacent noncancerous tissues. Then, decreased miR-206 expression was significantly correlated with advanced FIGO stage, positive lymph node metastasis, poor differentiation, HPV infection, and shorter OS. Finally, in vitro functional assays demonstrated that upregulation of miR-206 in HeLa cells was able to reduce cell proliferation, promote cell apoptosis, and inhibit cell invasion and migration. To the authors’ knowledge, this is the first study to investigate the expression and clinical significance of miR-206 in a large number of cervical cancer patients.

Recent studies have proven dysregulated miR-206 expression and its tumor suppressive function in many human malignancies. In vitro, upregulation of miR-206 reduces cell growth and induces apoptosis in glioma [13], breast cancer [25], lung cancer [16], gastric cancer [17], colon cancer [26], and rhabdomyosarcoma [20]. Ectopic miR-206 expression also inhibits cell invasion and migration in breast cancer [27], lung cancer [16], and rhabdomyosarcoma [20]. In vivo, Li et al. revealed decreased miR-206 expression and its correlation with nodal metastasis and clinical stage in breast cancer [28]. Yang et al. reported that miR-206 downregulation occurred more frequently in gastric cancer patients with lymph node metastasis, along with the presence of venous invasion and hematogenous recurrence, and advanced tumor stage [23]. Bao et al. also showed association between low miR-206 expression and poor histological differentiation and later stage of osteosarcoma [21]. Moreover, lower expression of miR-206 indicated shorter overall survival in patients suffering breast cancer [28], gastric cancer [23], and colorectal carcinoma [24]. In xenotransplanted models, miR-206-treated nude mice showed smaller tumor sizes and lower tumor weights in compared with the control group [17, 29]. Collectively, these findings suggest that miR-206 might play an important role not only in tumour initiation and progression but also in the molecular targeted therapy of human malignancies.

It is now clear that miRNAs execute their oncogenic or tumor suppressor functions by regulating the expression of target genes [30]. With regards to miR-206, several targets have been reported in recent research, such as CyclinD2 [17], Otx2 [13], MET [29], Pax7 [31], NOTCH3 [32], estrogen receptor alpha [33], and VEGF [14]; however, there is no ‘one-to-one’ connection between miRNAs and target miRNAs. An average miRNA can have more than 100 targets [34]. Conversely, several miRNAs can converge on a single transcript target [35]. Thus, the potential regulatory circuitry afforded by miR-206 may be enormous, and identification of the complex molecular network involved in its function remains an important subject for future investigation.
In conclusion, the present results revealed that miRNA-206 was downregulated in cervical cancer cell lines and clinical samples. Decreased miRNA-206 expression was associated with tumor progression and adverse prognosis. Regulation of miR-206 expression would affect biological behavior of HeLa cells. These findings demonstrate that miRNA-206 could not only be useful as a novel biomarker but also serve as a potential target for gene therapy of cervical cancer.

Reference


[7] Takahashi M., Cuatrecasas M., Balaguer F., Zhao J.: “MicroRNA-206 is associated with tumor progression and adverse prognosis. Regulation of miR-206 expression would affect biological behavior of HeLa cells. These findings demonstrate that miRNA-206 could not only be useful as a novel biomarker but also serve as a potential target for gene therapy of cervical cancer.”

[8] MI. Ling, Z. Guoshong, S. Bing, C. Yanshan, No. 57 Changping Road, Shantou University Medical College, e-mail: docchenys@163.com
The 16, 18, and 45 HPV infection in high grade squamous cervical lesions in primary hr-HPV test screening program


UOC of Pathology, Department of Medical-Surgical Sciences and Bio-Technologies, Sapienza University of Rome, Polo Pontino, I.C.O.T, Latina (Italy)

Summary
Infection with high-risk human papillomavirus (hr-HPV) 16, 18, and 45 causes 94% of cervical carcinoma. In the present screening center the authors perform the hr-HPV test followed by Pap test to women aged 35-64 years if they result hr-HPV+. The authors’ aimed to provide data regarding the genotyping test and eventually to propose this test as alternative to triage cytology. They used a genotyping test to identify HPV 16, 18, and 45 in 22 women with histological diagnosis of CIN2+, 22 women with histological diagnosis of CINI and 22 women hr-HPV+/Pap-. The group of CIN2+ showed the higher positivity to the test and the higher positivity to HPV 16 than other groups. Analyzing the clinical performance of the genotyping test the authors observed that the specificity was 64%. From these data they concluded that the identification of HPV 16 is predictive for high-grade lesions but this test could not be used alternatively to triage cytology.

Key words: hr-HPV; CIN2+; Cervical cancer.

Introduction
Cervical cancer is the second most common cancer in the female population and in Italy it is estimated to affect about 3,500 women/year [1]. This tumor is due to human papillomavirus (HPV) infection and DNA typing has led to the division of HPV in groups at high or low risk [2]. High-risk oncogenic HPV (hr-HPV) is now considered the most important factor involved in cervical cancer oncogenesis [2].

The rationale for the use of hr-HPV test in cervical cancer screening is its greater sensitivity than the screening program based on only Pap test; the patients that are hr-HPV+ are subjected to cytology triage while women that result negative to the hr-HPV test are referred to colposcopy. The reason is that the majority of HPV infections are transient and they are eliminated by immune response; on average, 50% and 90% of HPV infections disappear within eight months and two years, respectively [3-5]. Therefore, sending immediately to colposcopy, all hr-HPV+ women could lead to an indiscriminate increase of unnecessary treatments [6]. Hence, hr-HPV test followed by triage cytology seems to be the most effective strategy because there is an increase of 30% in sensitivity for CIN3+ [6]. Women hr-HPV+/Pap-, who have a lesion not observed by Pap test, will be sent to colposcopy if they result hr-HPV+ once again [7].

Between hr-HPV, HPV 16 and 18 are considered the most important; HPV 16 is responsible for about 60% of cases of cervical cancer, and HPV 18 represents a further 10% of cases, while other HPV subtypes contribute to less than 5% of cases individually [8]. A study carried out by de Sanjose et al. showed that HPV 16, 18, and 45 accounted for 75% of squamous cell carcinomas and that the invasive cervical cancer, regardless of histological type, caused by infection with one of these three types is diagnosed on average four years before compared to those caused by other types of hr-HPV [9, 10]. Moreover, women infected with these three subtypes are at an increased risk of developing high-grade cervical lesions compared to other hr-HPV types [9, 11-14].

In the present screening center in Latina, the authors invited all women aged 35-64 years to perform an hr-HPV test according to GISCi (Italian Association of Cervical Screening Programs) guidelines [6]; in that program hr-HPV test is followed by Pap test only in women with hr-HPV positive test before referring them to colposcopy. Instead, women aged 25-34 were invited to perform only Pap test.

In this study the authors evaluate the clinical performance of genotyping test for HPV 16, 18, and 45 to identify high grade cervical intraepithelial neoplasia (CIN2+) in hr-HPV+ women and they examined the possibility to use the HPV genotyping PS test, which detects HPV subtypes 16, 18, and 45, alternatively to triage cytology.

Materials and Methods

Study population
The Pathology Unit of ICOT Hospital, Department of Medical-Surgical Sciences and Bio-Technologies, Sapienza University of Rome and Screening Unit of Local Health Unit of Latina, have
been running a new organized cervical-screening in Latina district since 2012. Women aged 35-64 years are invited by mail to perform a hr-HPV test during 2012-2013. A double-sampling for hr-HPV test and Pap test were performed on all participating women. The women positive to hr-HPV test were referred to Pap test; diagnosis were reported according to 2001 Bethesda System [15] evaluated by one cytologist and two pathologist. The colposcopy was performed by two gynecologists of the screening unit. Colposcopy biopsies were read by two pathologist and women with diagnosis of CIN2 or more severe were referred to excisional treatment.

Cytology

The cervical cell samples were obtained by using a cytobrush and were put in PreservCyt solution; liquid-based cytology was performed by using the Sure path system. One slide per woman was prepared according to the supplier’s instructions.

HPV genotyping PS test

Exfoliated cervical cells were collected using a cytobrush and eluted in sample transport medium (STM). First of all, cervical specimens were denatured to disrupt the virus and release the target DNA, they were treated with a base solution, equivalent to half of the sample volume, mixed for ten seconds using a multi-specimen tube vortexer, incubated in a 65°C ± 2°C waterbath for 45 ± five minutes and mixed again for ten seconds using MST vortexer. The RNA probes were diluted in a probe diluent and once loaded all the samples, calibrators, controls and reagents, the hybridization phase was begun according to supplier’s instructions. The chemiluminescent reaction was measured by luminometer and the emitted light was measured as relative light unit (RLU). For each reaction three negative controls and three positive controls were used: three negative controls for HPV16, 18, and 45, respectively and three positive controls for HPV16, 18, and 45, respectively. Samples that showed a RLU ≥ two pg/ml were considered positive.

Statistical analysis

The authors used two-way tabulation to calculate Pearson’s X² test and values p < 0.05 were considered statistically significant.

Results

In 2012 the hr-HPV test was introduced as primary test for cervical cancer screening program. From 2012 to 2013 60,288 women aged 35-64 years were enrolled and 20,968 were screened (34.8%). The hr-HPV positive rate was 5.2% (1,092/20,968) and the overall positivity rate at cytology among women who were hr-HPV+ was 22.7% (248/1092); the most frequent diagnostic category was LSIL (64.5%), then ASCUS (23.8%), HSIL (8.5%), ASCH (2.4%) and, only in 2013 the authors found two adenocarcinoma (AC, 0.8%). Hence, the referral rate to colposcopy was 1.2% (248/20,968). Only 25% of woman had a normal cervix while the other women, during colposcopy, were subjected to a small biopsy in the areas that showed exo-endocervical mucosal disorders, in order to histologically confirm the presence and the type of alteration. The histological category most frequently represented was CIN 1 (84.4%) followed by CIN 3 (10%) and CIN 2 (5.6%). The detection rate (DR) for CIN2+ was 1.6% and 1.4% in 2012 and 2013, respectively. The specificity value of hr-HPV test was 96%.

The 22 patients with a histologic diagnosis of CIN2 and CIN3 (CIN2+) were subjected to molecular analysis on STM samples to search three hr-HPV (16, 18, and 45) through the use of HPV genotyping test. Particularly, of the 22 women hr-HPV+/Pap+/CIN2+, 13 had a histological diagnosis of CIN2 and nine of CIN3. As controls the authors used 22 women hr-HPV+/Pap+/CIN1 and 22 women hr-HPV+/Pap-. Among patients with histological diagnosis of CIN2+, five women had a cytological diagnosis of ASCUS, seven of LSIL, one of ASCH, seven of HSIL, one of AGC, and one squamous cell carcinoma, while in the population of 22 women with histological diagnosis of CIN1, four had a cytological diagnosis of ASCUS, 14 of LSIL, and four of HSIL.

The 45% (30/66) resulted positive to genotyping test, particularly 27% (18/66) were positive for HPV 16, 6% (4/66) to HPV 18, and 12% (8/66) to HPV 45, while the remaining 55% were negative for the presence of these three subtypes. None of the tested patients were positive for the presence of more than one subtype. The proportion of positive patients who had a high-grade dysplasia (CIN2+) was found to be 64% (14/22); the positivity was distributed in the different subtypes as follows: 9/14 (64%) HPV 16+, 2/14 (14%) HPV 18+, and 3/14 HPV 45+ (21%). Of eight patients with CIN2+ and resulted negative to the test, three showed a moderate dysplasia (CIN2) and five a severe dysplasia (CIN3). The group of women with diagnosis of CIN2+ showed the highest percentage of positivity to the test (64%) than the group of women with diagnosis of CIN1 and the negative group that both showed a positivity rate of 36%. In addition, the positivity to HPV 16 was observed more higher in the group of women with diagnosis of CIN2+ (41%) than the other two groups (18% and 23%). Instead, the positivity to HPV 18 was the same in the groups of women diagnosed with CIN1 and CIN2+ (9%), while the hr-HPV+/Pap- group showed no positivity for this subtype; the latter group, however, showed a 14% positivity for HPV 45 as well as the group of women with diagnosis of CIN2+ (Table 1).

<table>
<thead>
<tr>
<th>HR-HPV Type</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 16</td>
<td>9 (41%)</td>
<td>2 (9%)</td>
<td>11 (50%)</td>
</tr>
<tr>
<td>HPV 18</td>
<td>3 (14%)</td>
<td>3 (14%)</td>
<td>6 (28%)</td>
</tr>
<tr>
<td>HPV 45</td>
<td>14 (64%)</td>
<td>8 (36%)</td>
<td>22</td>
</tr>
<tr>
<td>Others</td>
<td>8 (36%)</td>
<td>14 (64%)</td>
<td>22</td>
</tr>
</tbody>
</table>
| Total      | 22       | 22       | 44 (

---

Table 1. — Distribution of infections from hr-HPV types by histological results.
Within the group of women with a histological diagnosis of high-grade, the authors made a comparison between the patients with a diagnosis of CIN2 and those with a diagnosis of CIN3. They observed that the percentage of test positivity was slightly higher in CIN2 than CIN3 (67% vs 62%). The positivity for HPV 16 was found to be higher among women with diagnosis of CIN3 (46% vs 34%), vice versa the positivity to HPV 18 was found to be lower among women with diagnosis of CIN3 (8% vs 11%), as well as positivity for HPV45 (8% vs 22%) (Table 1).

Through the comparison made between women with diagnosis of CIN2+ and the group of women with diagnosis of CIN1 plus women hr-HPV+/Pap−, the authors observed a percentage of positivity to the genotyping test of 64% and 36%, respectively, and this difference was statistically significant (p < 0.05). They also observed a difference in the rate of positivity for HPV 16 in the two groups (41% vs 20%); instead, regarding the positivity for HPV18 and HPV45, these were found to be higher in the group of women with diagnosis of CIN2+ (9% -14% vs 5% - 11%).

Stratifying women on the basis of cytological diagnosis, the authors observed that the patients with a diagnosis of HSIL showed a higher positivity to the test (64%) and that there was a decrease of positivity in women with diagnosis of LSIL (43%) and ASCUS (33% ). In addition, women with cytological diagnosis of high-grade showed the highest percentage of positivity for HPV 16 (37%) and HPV 18 (18%) than women with cytological diagnosis of LSIL (19%, 10%) and ASCUS (22%, 0%). The percentage of positivity to HPV 45 was higher in women with diagnosis of LSIL (14%) compared to HSIL (9%) and ASCUS (11%). The three patients with cytological diagnosis of ASCH, AGC, and AC were all positive for HPV16 (Table 2).

Finally, through the comparison made between the women with a cytological diagnosis of HSIL+ and the negative control group, the authors observed that the first group showed a greater percentage of positivity to the test than the other group (64% vs 36%). In addition, the group of women with cytological diagnosis of HSIL+ showed a higher percentage of positivity for all three subtypes of HPV compared to the control group. The specificity of the test was 64%.

Table 2. — Distribution of infections from hr-HPV types by cytological results.

<table>
<thead>
<tr>
<th>HPV 16</th>
<th>HPV 18</th>
<th>HPV 45</th>
<th>Total</th>
<th>Others</th>
<th>hr-HPV Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCUS</td>
<td>2 (22%)</td>
<td>0 (0%)</td>
<td>1 (11%)</td>
<td>3 (33%)</td>
<td>6 (67%)</td>
</tr>
<tr>
<td>LSIL</td>
<td>4 (19%)</td>
<td>2 (10%)</td>
<td>3 (14%)</td>
<td>9 (43%)</td>
<td>12 (57%)</td>
</tr>
<tr>
<td>HSIL</td>
<td>4 (19%)</td>
<td>2 (10%)</td>
<td>1 (14%)</td>
<td>7 (64%)</td>
<td>4 (36%)</td>
</tr>
</tbody>
</table>

Within the group of women with a histological diagnosis of high-grade, the authors made a comparison between the patients with a diagnosis of CIN2 and those with a diagnosis of CIN3. They observed that the percentage of test positivity was slightly higher in CIN2 than CIN3 (67% vs 62%). The positivity for HPV 16 was found to be higher among women with diagnosis of CIN3 (46% vs 34%), vice versa the positivity to HPV 18 was found to be lower among women with diagnosis of CIN3 (8% vs 11%), as well as positivity for HPV45 (8% vs 22%) (Table 1).

Through the comparison made between women with diagnosis of CIN2+ and the group of women with diagnosis of CIN1 plus women hr-HPV+/Pap−, the authors observed a percentage of positivity to the genotyping test of 64% and 36%, respectively, and this difference was statistically significant (p < 0.05). They also observed a difference in the rate of positivity for HPV 16 in the two groups (41% vs 20%); instead, regarding the positivity for HPV18 and HPV45, these were found to be higher in the group of women with diagnosis of CIN2+ (9% -14% vs 5% - 11%).

Stratifying women on the basis of cytological diagnosis, the authors observed that the patients with a diagnosis of HSIL showed a higher positivity to the test (64%) and that there was a decrease of positivity in women with diagnosis of LSIL (43%) and ASCUS (33% ). In addition, women with cytological diagnosis of high-grade showed the highest percentage of positivity for HPV 16 (37%) and HPV 18 (18%) than women with cytological diagnosis of LSIL (19%, 10%) and ASCUS (22%, 0%). The percentage of positivity to HPV 45 was higher in women with diagnosis of LSIL (14%) compared to HSIL (9%) and ASCUS (11%). The three patients with cytological diagnosis of ASCH, AGC, and AC were all positive for HPV16 (Table 2).

Finally, through the comparison made between the women with a cytological diagnosis of HSIL+ and the negative control group, the authors observed that the first group showed a greater percentage of positivity to the test than the other group (64% vs 36%). In addition, the group of women with cytological diagnosis of HSIL+ showed a higher percentage of positivity for all three subtypes of HPV compared to the control group. The specificity of the test was 64%.

Discussion

The present data confirm that HPV 16 (27%) is the most represented subtype compared to HPV 18 and 45, and the authors also observed that the majority of CIN2+ lesions (64%) showed the highest positivity to HPV 16 which decreased in the categories of CIN1 and controls as well as reported in the literature [16]. In the present study, the authors did not observe the presence of HPV 18 subtype in the population of women hr-HPV+/Pap- to support the hypothesis that this type of virus is very poorly represented in pre-cancerous lesions due to its early integration in the genome of the host cell and, consequently, to the rapid progression in cancer [9, 17]. This was confirmed when the authors stratified the women according to the cytological diagnosis; indeed, patients with a diagnosis of ASCUS showed no positivity for HPV 18 subtype, while the percentage of positivity for HPV 16 was similar to group of LSIL (22%, 19%) but still lower than the group of women with a cytological diagnosis of high-grade intraepithelial lesion (37%) [9, 17]. The role of HPV 45 to identify high-grade dysplasia seems less relevant; indeed women with diagnosis of CIN2+ showed the same percentage of positivity of the control group (14%), unlike other studies [16]. Although the difference of this data could be determined by the small number of patients subjected to the test, a certain degree of variability in the distribution of HPV subtypes may depend on the local dynamics of the transmission of various subtypes of HPV, because it has been observed that this variability exists between both countries and within the same country [16]. Another factor to consider is the age of the women examined with this test (35-64 years); the present data may also be the result of this parameter because some studies have shown that the proportion of high-grade lesions caused by HPV 16 and 18 are prevalent among young women [18], and also in the present study there were no observed cases of co-infections, which also seem to be common among young women [16]. Nevertheless, taking into account only the group of women with a histological diagnosis of CIN3+, the authors observed that the percentage of positivity for HPV 16, 18, and 45 were very close to those reported in the literature [9]. Moreover, women with diagnosis of HSIL showed an higher percentage of positivity to HPV 16 than other subtypes according to another study [19].

Even in a small selected clinical population of 22 women hr-HPV+/CIN2+, derived from a single year of institutional clinical screening in Latina, the specificity of the test to detect high grade lesions was 64%, although the results of the genotyping tests are generally consistent with those reported in the literature. If this data will be confirmed on a larger population, the present authors could not suggest the genotyping of HPV subtypes (16, 18, and 45) as an alternative method of triage cytology because it showed no adequate clinical performance; currently therefore, the mor-
phological response in women hr-HPV+ is irreplaceable for the early identification of the real precursors of invasive carcinoma. However, the HPV16 positivity is predictive for high-grade lesions.

Acknowledgments

The authors thank “Fondazione Roma” for the precious support in this research.

References


Address reprint requests to:
C. DELLA ROCCA, M.D.
UOC of Pathology,
Department of Medical-Surgical Sciences and Bio-Technologies,
Sapienza University of Rome, Polo Pontino,
I.C.O.T, Latina, Italy
Corso della Repubblica 79
04100 Latina (Italy)
e-mail: carlo.dellarocca@uniroma1.it
BEP for high-risk gestational trophoblastic tumor: results from a cohort of 45 patients

S.Q. Song1*, C. Wang2*, G.N. Zhang1, Y. Shi1, Y. Zhu3, T. Hu1, S.Q. Xu1, Z.R. Yang1

1 Department of Gynecological Oncology, Sichuan Cancer Hospital, Chengdu, Sichuan
2 Chengdu First People’s Hospital, Chengdu, Sichuan
3 Department of Ultrasound, Sichuan Cancer Hospital, Chengdu, Sichuan (China)

Summary

Aim: To evaluate the effectiveness and safety of combination chemotherapy of bleomycin, etoposide, and cisplatin (BEP) regimen in patients with high-risk gestational trophoblastic neoplasia (GTN). Materials and Methods: The authors analyzed the clinical response, toxicity, and the occurrence of secondary tumors of 45 patients with high-risk GTN under BEP. Results: The total complete remission (CR) rate of BEP regimen was 88.89% (40/45). Five patients developed drug-resistance after average 4.8 courses of BEP, and the regimen converted to etoposide, methotrexate, and dactinomycin (EMA)/cyclophosphamide and vincristine sulfate (CO). Ultimately, four cases achieved CR and one case died of cancer. There were no severe anaphylaxis and obvious impairment of cardiac, liver, pulmonary and kidney function, except one patient who developed grade IV bone marrow suppression and worsened pulmonary fibrosis after chemotherapy. None of survival patients developed secondary tumor during the follow-up. Conclusion: For young high-risk GTN patients, BEP may represent a safe and effective regimen.

Key words: High-risk gestational trophoblastic neoplasia; BEP regimen; Adverse reaction of chemotherapy.

Introduction

Gestational trophoblastic neoplasia (GTN) is a highly aggressive gynecological malignancy. Although it is sensitive to chemotherapy and more than 90% patients reach recovery, there are still 15% of GTN patients, especially with high-risk, developed failure to treatment. How to improve the treatment efficacy and prognosis of GTN patients has drawn intensive thinking of gynecological oncologists. The Sichuan Provincial Cancer Hospital has adopted bleomycin, etoposide, and cisplatin (BEP) regimen since January 1997 for treatment of GTN patients and achieved favorable results. Here, the authors report their experience in 45 patients with high-risk GTN.

Materials and Methods

Patients

GTN patient who received BEP regimen chemotherapy in the Sichuan Provincial Cancer Hospital from January 1997 to October 2012 were included for this study. Ethical approval for the project was obtained from the Sichuan Cancer Hospital Research Ethics Committee. Patients were re-diagnosed according to the standard of diagnosis of GTN, staged, and scored for prognosis according to the FIGO 2000 staging and scoring system [1]. Criteria for inclusion in the study were established as follows: (1) Patients who were diagnosed with high-risk GTN (prognosis score ≥ 7); (2) continuous use of ≥ two courses of BEP regimen, the entire follow-up period after chemotherapy was ≥ three months.

The total of 45 patients followed the inclusion criteria, average age 30.5 years (20 - 49). The serum β-hCG level was 6,756 ~ 22,124 U/L (average 12,523 U/L) before chemotherapy. According to the GTN diagnostic criteria, ten patients were diagnosed with post-mole GTN, 35 cases with non-postmolar GTN, including ten choriocarcinoma and two invasive mole, which were evaluated by experienced pathologists. The prognostic scores of 45 patients were 7 to 18 points (average 11.4 points); eight in Stage II, 32 in Stage III, five in Stage IV. There were two patients with brain metastases, 37 with lung metastases, and 24 with reproductive system metastases. One case each with dorsum scapulae, liver, bowel, and omentum metastases, respectively.

Treatment

BEP regimen. All of 45 patients in this study underwent BEP chemotherapy. The specific usage was: bleomycin 15 mg + saline 20 ml, intravenous injection in the first to third days; etoposide 100 mg/m² + saline 300 ml, intravenous infusion, first to fifth days; cisplatin 20 mg / m² + saline 250 ml, intravenous infusion, first to fifth days, every three weeks. To prevent the bleomycin induced pulmonary toxicity, the treatment was stopped when the cumulative dose of 360 mg was reached. Chemotherapy was continued until the serum β-hCG level was reduced to below 22,124 U/L. For young high-risk GTN patients, BEP may represent a safe and effective regimen.
Table 1. — Clinical characteristics and treatment of the five cases with high-risk GTN who developed BEP regimen resistance

<table>
<thead>
<tr>
<th>Number</th>
<th>FIGO Stage</th>
<th>FIGO Score</th>
<th>Surgical approach before chemotherapy</th>
<th>Courses of BEP</th>
<th>Concurrent regimen radiotherapy</th>
<th>Therapy after resistance</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>III</td>
<td>13</td>
<td>None</td>
<td>5</td>
<td>None</td>
<td>EMA/CO 7 courses</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>III</td>
<td>10</td>
<td>Uterine repair</td>
<td>4</td>
<td>Whole lung</td>
<td>EMA/CO 10 courses</td>
<td>CR</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>15</td>
<td>Hysterectomy</td>
<td>7</td>
<td>None</td>
<td>EMA/CO 3 courses + whole lung irradiation</td>
<td>CR</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>18</td>
<td>Resection of intracranial lesions</td>
<td>5*</td>
<td>None</td>
<td>EMA/CO 6 courses + whole lung irradiation + lobectomy</td>
<td>CR</td>
</tr>
<tr>
<td>5</td>
<td>IV</td>
<td>16</td>
<td>Hysterectomy</td>
<td>3</td>
<td>None</td>
<td>EMA/CO 16 + 5-fluorouracil + cisplatin + dactinomycin + and so on</td>
<td>Death</td>
</tr>
</tbody>
</table>

* represents concurrent intrathecal methotrexate injection while BEP chemotherapy was given.

their serum β-hCG normal or near normal levels, pelvic surgery or resection of metastases surgery was conducted. Ten patients underwent emergency surgery before BEP chemotherapy due to vaginal bleeding or intracranial hemorrhage, or postoperatively diagnosed as high-risk GTN after surgery in other departments.

Radiotherapy. For serious brain, lung metastasis, lesions with no obvious subside after three to five cycles after BEP chemotherapy treatment, concurrent radiotherapy was conducted in addition to chemotherapy. For patients with resistant lesions in the brain or the lungs, chemotherapy regimen was adjusted and radiotherapy was given when necessary.

The diagnostic criteria for drug resistance and relapse

Drug resistance. Patients whose serum β-hCG level was not decreased logarithmically after two to three cycles of chemotherapy, or showed a platform-like level, or even rise; or imaging examination suggest metastases does not shrink or grow, or even the emerge new metastatic lesions [2].

Recurrence. After treatment, the detection of serum β-hCG level remained normal for three weeks, all tests showed (including physical examination, imaging studies) that the lesions had disappeared, clinical symptoms disappeared. Up to three months after the above criteria, the serum β-hCG levels increased (except for re-pregnancy) or other examination revealed new lesions [2].

The evaluation criteria of efficacy and side effects

Effects and side effects. Clinical complete remission (CR): serum β-hCG was monitored once a week and remained normal in three consecutive tests; clinical symptoms disappeared; all metastatic lesions disappear. Afterwards, two or three cycles of chemotherapy regimen was given. Then the patients were followed-up for three months without recurrence. Partial response (PR): serum β-hCG levels decreased > 50%, and metastases was reduced. The side effects of chemotherapy were evaluated according to the WHO antineoplastic drugs acute and subacute indexing criteria.

The follow-up after the end of treatment. After patients discharged when CR standard was met, regular monitoring of serum β-hCG level was required. In this study, all patients were followed up until death or the cut-off in February 2013. The duration of follow-up was from three months to 15 years.

Results

Efficacy

Forty-five high-risk GTN patients received three to eight courses of BEP (average 6.1 courses), including the consolidation of two to three courses of chemotherapy. The CR rate of BEP regimen was 88.89% (40/45). Among the 40 CR patients, ten cases were postmolar GTN and 30 non-postmolar GTN. There were eight Stage II patients, 29 Stage III, and three Stage IV. These patients received five to eight courses of BEP chemotherapy (average 6.3 cycles). The serum β-hCG level of these patients decreased to normal after receiving average 3.65 courses (three to six courses) and achieved CR after average 6.25 courses. Thirty-four of 40 cases were treated with BEP only. Four cases followed with surgery, three patients were diagnosed as choriocarcinoma, and one patient was cancer free. Two cases were given concurrent radiotherapy on the basis of the BEP chemotherapy (one case of brain radiotherapy, one case of pulmonary radiotherapy).

Five patients, including three in Stage III and two in Stage IV, which were non-postmolar GTN, developed drug-resistance after average 4.8 courses (three to seven courses) of BEP. Four of them achieved PR after two to six cycles of chemotherapy. Unfortunately, due to personal or family reasons, three patients delayed the next cycle or ceased consolidation chemotherapy. One patient (49-years-old) failed to take regular monitoring of blood leading to a serious bone marrow suppression, therefore this patient was unable to receive the next course of chemotherapy and ultimately developed resistance. The regimen of these four patients converted to etoposide, methotrexate, and dactinomycin (EMA) / cyclophosphamide and vincristine sulfate (CO), and all of them ultimately obtained CR. The other one case was in Stage IV, with the prognosis score of 16 points, had hysterectomy in another hospital due to vaginal and intra-abdominal bleeding before received BEP chemotherapy. The pathological diagnosis was choriocarcinoma. For family reasons, the patient was not able to receive regular chemotherapy, which led to rapid recurrence after three cycles of BEP chemotherapy. Then the regimen switched to EMA/CO and other kinds for 16 cycles. However, the patient died of brain metastasis ultimately, and survived for 14 months after pathogenesis (Table 1).
Table 2. — Side effects of 45 cases with high-risk GTN received 273 cycles of BEP chemotherapy.

<table>
<thead>
<tr>
<th>Types (degree)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>In total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea, vomiting</td>
<td>92</td>
<td>87</td>
<td>17</td>
<td>0</td>
<td>196</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>101</td>
<td>68</td>
<td>16</td>
<td>1</td>
<td>186</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>33</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>Hemoglobinopenia</td>
<td>12</td>
<td>18</td>
<td>2</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>Alopeia</td>
<td>28</td>
<td>61</td>
<td>180</td>
<td>0</td>
<td>269</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>51</td>
<td>69</td>
<td>0</td>
<td>0</td>
<td>120</td>
</tr>
<tr>
<td>Impaired liver function</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>Pulmonary toxicity</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Decreased heart function</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

BEP chemotherapy side effects

As shown in Table 2, major side effects of BEP chemotherapy were alopecia, gastrointestinal toxicity, and bone marrow suppression, followed by mild liver dysfunction, peripheral neuritis, and occasional mild pulmonary toxicity. The study did not show a severe allergic reaction and obvious heart, liver, lung, and kidney dysfunction in patients. Except for one case in which severe bone marrow suppression occurred leading to chemoresistance, the other patients were not influenced by the side effects in the course of chemotherapy.

The follow-up results

During the follow-up period, one patient died in multiple chemotherapy drug resistance; one patient under BEP regimen obtained CR after four courses, but the serum β-hCG level rose again which implicated recurrence after one year. The patient obtained CR after receiving BEP and EMA/CO regimen and has been followed up for 17 months with no signs of recurrence. Forty-three patients are alive with no signs of recurrence or secondary tumors.

Discussion

Although GTN is a highly malignant gynecological tumors, because of its high sensitivity to chemotherapy and hCG serves as an ideal tumor marker to monitor the disease, its prognosis has become one of the best among all cancers [2]. Currently the cure rate of low-risk GTN is nearly up to 100%; the survival rate of patients with high-risk GTN is about 86% [3].

Currently the treatment principle of high-risk GTN is combinational chemotherapy as the first choice, and on this basis, radiotherapy and (or) other treatments such as surgery are given when appropriate [4]. So far, many chemotherapy regimens have been used for treatment of patients with high-risk GTN, the representative regimens include methotrexate + dactinomycin and cyclophosphamide (MAC), hydroxyurea + cyclophosphamide + dactinomycin + Aminopterin + leucovorin + vincristine + doxorubicin (CHAMOCA), EMA/CO scheme; the response rates were 68%, 71%, and 91% respectively. Internationally, the first choice is the EMA/CO program [5]. Although the efficacy of current treatment GTN has been satisfactory, it is reported that about 20 - 30% of patients with high-risk GTN failed to respond to first line chemotherapy regimens and required remedial chemotherapy [6]. Therefore, more effective chemotherapy regimen need to be developed.

BEP regimen is recognized as the preferred choice in the treatment of malignant ovarian germ cell tumors (including primary ovarian choriocarcinoma). The regimen has been reported in the literature for its good effect in early, advanced and recurrent malignant ovarian germ cell tumors. The adverse reactions are relatively mild and the cure rate of malignant ovarian germ cell tumor could reach over 90%. Following these strategies, most patients will be cured and the vast majority will still be able to give birth [7, 8]. However, there is few research on the effects of BEP regimen in GTN. In 1988, it has been reported, for the first time, that one refractory GTN patient with drug resistance was successfully cured with the BEP regimen [9]. Moreover, one case that developed resistant under etoposide and cisplatin (EP) regimen, obtained CR after adjusting to BEP regimen. It has been demonstrated that most of patients who failed primary treatment with EMA/CO had complete clinical responses to secondary chemotherapy with BEP [10, 11]. However, the other study found that five of six drug-resistant patients with low-risk GTN obtained CR with BEP, and only three of nine high-risk GTN patients obtained CR [12]. Although the prior reports were cases or small studies, it can be inferred that BEP program is often used as first-line remedia tion therapy in GTN patients with failed chemotherapy. The BEP treatment has a secure effect in resistant GTN patients. However, whether the regimen can be used for initial treatment of high-risk GTN as well as its efficacy and side effects has not been reported so far. In the present study, it has been certified that BEP is an effective regimen for high-risk GTN as the same as EMA/CO or fluorouracil-based chemotherapy. Moreover, it has been indicated that, BEP combined with surgery as well as radiotherapy for high-risk GTN patients may be helpful to improve the prognosis.

In the present study, five patients under BEP developed resistance, who were non-postmolar GTN in late clinical stage and high prognosis score (averaging 14.4 points). In addition, for most of them, the chemotherapy regimen was not standardized (chemotherapy was delayed or consolidation therapy was not given after symptoms improved). As a result, resistance occurred. Therefore, the present authors believe that if the management of patient is strengthened, patient confidence to overcome the disease is enhanced, the side effects of chemotherapy are given more attention to personalize treatment, which ensures that patients can schedule regular chemotherapy; hence better efficacy of BEP program is expected which remains to be further studied.

Chemotherapy-related toxicity was in keeping with the present authors’ expectations. Nevertheless, one patient died
of brain metastasis. A retrospective analysis showed that she received non-standard chemotherapy for family and financial reasons. If this case is excluded, acute toxicity was acceptable and severe toxicity was infrequent [8], although grade 1 - III myelosuppression was observed in the majority of patients, specifically neutropenia [13]. Currently, the administration of G-CSF would help to manage such toxicity. In the present study, the oldest patient occurred severe bone marrow suppression (grade IV) after BEP required transfusion therapy, implicating that older patients with BEP were prone to severe bone marrow suppression.

The present authors did not observe any severe otoxicity, or nephrotoxicity, which is consistent with the results of Tangir et al. and Kang et al. BEP trials for GNT [7, 14]. Etoposide may cause secondary tumors, including myeloid leukemia, and colorectal and breast cancers [15]. However, in the present study there were no secondary tumors during the follow-up period.

It is reported that pulmonary toxicity is the major side effects of bleomycin with a rate of 6.8%, and it is positively correlated with the cumulative dose of bleomycin, patient age, poor renal function (creatinine clearance rate in particular) [16]. In severe cases, pulmonary fibrosis may occur, although rare, but it is potentially a deadly threat [17]. Willemsen et al. [9] reported that a drug-resistant and refractory GNT patient (age 53) was successfully cured with BEP, but developed moderate pulmonary toxicity. The present study also showed that the incidence of pulmonary toxicity was 2.56%, lower than the incidence reported in the literature, and to a lesser extent. It may be related to conventional corticosteroid (dexamethasone) treatment, which may inhibit the growth of fibroblasts. However, it still remains to be further studied. The study also found the oldest patients, who experienced severe bone marrow suppression as well as progressed primary pulmonary fibrosis after seven courses of chemotherapy, which implicated that older patients should be careful when receiving BEP.

In summary, BEP is an effective and well-tolerated regimen for high-risk GNT. Combinational surgery and radiotherapy as well as adjuvant therapy can further improve the outcome. Side effects after chemotherapy are tolerable for most patients. However, older patients are prone to experience severe side effects after chemotherapy and should be treated with caution. Therefore, for young patients with high-risk GNT, BEP program is a safe and effective chemotherapy regimen. Since this study was a retrospective study, the number of cases was not enough to draw a definitive result and it remains to be further studied.

References


Identification of cervical cancer markers using cDNA subtraction approach

Y. Liu¹, S.H. Man¹, X. Liu¹, X.Y. Ding¹, W.L. Xiao²

¹ Department of Gynecology, Weifang People’s Hospital, Shandong
² Department of Immunology, Weifang Medical College, Shandong (China)

Summary

Purpose of investigation: Cervical cancer markers are not well known for accurate detection of the disease. Materials and Methods: In the present study, cervical cancer samples were collected from the 42 patients in the Department of Surgery and Medicine of Weifang People’s Hospital and Medical College, Shandong, China. The cDNA subtraction approach was performed to find out the specific transcripts, which are responsible for cervical cancer. The specific differentially expressed transcripts were identified by Basic Local Alignment Search Tool (BLAST) analysis and the results were validated by immunohistochemistry. Results: Following differentially expressed specific transcripts, such as ID-1, Hif 1a, and the Y-box were usefully employed as a marker to accurately detect cervical cancer. Conclusion: The identified markers are promising in the accurate detection of cervical cancer in terms of its molecular basis and management of the disease.

Key words: Cervical cancer; cDNA subtraction; Immunohistochemistry.

Introduction

Worldwide, cervical cancer is the second most common cancer in female populations [1]. More than 90% of cervical cancer is caused by the infection of human papillomavirus (HPV) [2]. HPV detection cannot be used to detect lesions which progress towards invasive carcinoma [3, 4]. Except for Stage I cervical cancer, about 20-30% of patients are predicted to die due to their disease [5]. Identification of early symptoms is not possible, because early stage of cervical cancer is totally asymptomatic [6]. Rather, vaginal bleeding and vaginal mass indicate the presence of malignancy during late stages of cervical cancer. Early detection of cervical cancer is the key to target the disease for treatment. Various prognostic tests were developed up to date, but lack the molecular diagnosis for accurate disease management followed by treatment. One such is the Pap test, which is considered as a cost effective method for the diagnosis of cervical cancer [7]. In addition, it was also reported that Pap test shows 50% false negative results in the cervical cancer patients. Similarly, it was also identified that CA-125 and CA-15-3 were elevated in the blood of cervical cancer patients, and used as serum markers [8]. In contrast, again lack of accurate detection was noticed. The serum marker works only on certain type of cervical cancers with 90% accuracy. Accordingly, SCC is not suitable to detect the cervical cancer as recommended by National Academy of Clinical Biochemistry (NACB). It is important to identify the molecular markers for the accurate detection of cervical cancer. In the present study, by using cDNA subtraction approach, molecular markers for cervical cancer were identified. The identified markers could be used for accurate detection followed by management of cervical cancer.

Materials and Methods

Sample collection and screening of positive cervical cancer tissues

Cervical tissues were collected from the 42 patients in the Department of Surgery and Medicine of Weifang People’s Hospital and Medical College, Shandong, China followed by frozen tissues with liquid nitrogen. The project was approved by the ethical committee of the hospital. In order to screen the positive cervical cancer tissues, the samples were transferred to the Department of Pathology of the above mentioned hospital for careful identification by histological techniques. The positive cervical cancer tissues and control were used for following experiments.

Total RNA isolation and purification of poly A⁺ mRNA

Total RNA was isolated from the positive cervical cancer and control tissues as per the manufacturer protocol and guidelines. Purification of poly A⁺ mRNA from the total RNA were also carried out as per the manufacturer protocol and guidelines. The isolated poly A⁺ mRNA was stored at -70°C deep freezer for further experiments.

cDNA synthesis and identification of cervical cancer markers using cDNA subtraction kit

The purified mRNA from cervical cancer tissues and control were used for first strand cDNA synthesis by reverse transcriptase.
The synthesized cDNA from the cervical cancer tissues, which contains specific (differentially expressed) transcripts act as a tester, and cDNA from the control as a driver. Tester and driver cDNAs were hybridized, and the hybrid sequences were removed as per the protocol. Consequently, the remaining unhybridized cDNAs represent genes that were expressed in the tester yet absent from the driver mRNA were specifically amplified using PCR. The PCR-select cDNA subtraction kit selectively amplifies the differentially expressed sequences, which was expressed specifically in the cervical cancer tissues not in the control.

Cloning and sequencing of differentially expressed genes
The obtained product from the PCR were cloned into the Bluescript vector (pBSK-) and sequenced. In order to clone the PCR amplified differential expressed genes, pBSK vector was restricted digested with SmaI restriction enzyme and depolymerized with shrimp alkaline phosphatase and ligated with the amplified target. The ligated vector was transformed into XL1 Blue bacterial strain and the plated in an IPTG/X-gal coated LB plate with ampicillin. The white colonies (positive clones) were picked and colony PCR was performed using pBSK universal forward and reverse primers. The specific clones were sequenced and the sequence data were analyzed with Basic Local Alignment Search Tool (BLAST) with the nucleotide and EST database by performing the sequence alignment. The differentially expressed genes specific for cervical cancer were identified.

Immunohistochemistry
To visualize the markers in cervical cancer tissues, paraffin-embedded tissue sections (six mm) were de-paraffinized with xylene and hydrated. Endogenous peroxidase was inhibited by incubating the sections for 30 minutes with freshly prepared 10% H2O2 and 10% methanol in 1X PBS. The sections were then treated partially with 0.1% trypsin in 0.1% CaCl2 at 37ºC. Non-served under microscope using a ×40 objective.

1. HIF1A - hypoxia-inducible factor 1-alpha
   - Class of hypoxia inducible factors [31].
2. MGP - matrix Gla protein
   - Calcification inhibitor [32].
3. ID1 - DNA-binding protein inhibitor ID-1
   - Function as cell growth, senescence, and differentiation [33, 34].
4. MUC16 - mucin 16
   - Interact with cytoskeleton by binding members of the ERM protein family [35].
5. COL8A1 - collagen alpha-1(VIII) chain
   - Function as a short chain collagen [36].
6. TP53 - tumor suppressor p53
   - Function as tumor suppressor gene [37, 39].
7. YBX-1 - Y-box binding protein-1
   - Regulate positively or negatively in many numbers of genes such as; multidrug resistance 1, cyclin A, cyclin B1, matrix metalloproteinase 2 and collagen alpha2 (I) [40].
8. FOSL2 - Fos-related antigen 2
   - Regulators of cell proliferation, differentiation and transformation [41].
9. MDK - midkine
   - Induced during oncogenesis, inflammation and tissue repair [42].
10. CDKN2A – p16
    - Function as a tumor suppressor gene [43, 44].

Results

Identification of cervical cancer tissues for cDNA subtraction analysis:
Cervical cancer is the second most dangerous disease in the world. Molecular wise accurate diagnosis of the disease is the problem in the current era. In order to identify the molecular markers for the accurate detection of disease cDNA subtraction approach was performed. In total, 42 cervical cancer tissue samples and normal tissues were collected from the Department of Surgery and Medicine. The collected samples were snap frozen and stored. The samples were carefully observed using histological analysis by the pathologist and specialized scientist. The confounded 42 samples were further processed for the cDNA subtraction analysis.

cDNA subtraction and sequencing of differentially expressed transcripts
The 42 samples along with the control were subjected to cDNA subtraction analysis using the subtraction kit; likewise 42 subtractions were performed. As per the manufacturer’s instruction, the differential expression of specific genes was amplified by PCR and cloned with Bluescript vector. The positive colonies were screened and confirmed by colony PCR and the clones were sequenced. Totally, 2,604 clones were obtained from the 42 samples. The sequenced data were analyzed carefully by BLAST the subtracted cDNA sequences with the EST and Nucleotide database of NCBI. After analyzing the 2,604 cDNA clones, the differentially expressed transcripts, which was common in the 42 samples were taken and shown in Table 1.

Selection of markers for cervical cancer
The ten differentially expressed transcripts (Table 1) were taken into account in the selection of markers for cervical cancer. Based upon the literature survey following three transcripts, such as YBX-1, ID-1, and HIF-1A were focused for the selection of effective markers. Immunohistochemistry experiments were performed to validate the markers.

Validation of cervical cancer markers
In order to validate the markers, the cervical cancer tissues were subjected to immunohistochemistry and stained...
Identification of cervical cancer markers using cDNA subtraction approach

with anti-Y-box, anti-Id1, and anti-Hif-1a antibodies separately, followed by brief staining with hematoxylin. The data (Figure 1) shows the presence of positive cells in the tissues. The data (Figures 1A, 1B) show the expression of Y-box and Hif-1a in the cervical cancer tissues. The localization of Y-box and Hif-1a were found to be in the nucleus, respectively. In contrast, the data (Figure 1C) show the expression of Id-1 positive cells in the cervical cancer tissues, but its localization was found to be in the cytoplasm. Similarly, 42 samples were analyzed for the immunohistochemistry experiments with the same antibodies to validate the markers. Obviously, similar data were obtained to authenticate the markers for the accurate detection of cervical cancer.

Discussion

Cervical cancer is one of the severe diseases among the female populations. Detection and management of the disease is not efficient. Pap test and serum markers such as CA-125 and CA15-3 were used to detect cervical cancer, but the percentage of accuracy is the question. Hence, accurate detection is essential for the treatment and management of the disease. In the present study, a novel technique, namely cDNA subtraction was used to identify the molecular markers for the accurate and effective detection of cervical cancer.

In total, 2,604 clones were obtained from 42 samples. Of these, ten transcripts (Table 1) appeared commonly in all the 42 samples. Putative and partial sequences were also detected in the 2604 clones. Among the ten transcripts, YBX-1, ID-1, and HIF-1A were selected based upon interest, expression rate, and literature survey. To validate the results, immunohistochemistry was performed and the expression of Y-box, HIF-1a and Id-1 were noted in the cervical cancer tissues.

Y-box binding protein-1 (YBX-1) is an oncogenic transcription/translation factor, over-expressed in the following multiple types of cancers: colon [9], lung [10, 11], breast [12, 13], muscle [14], prostate [15], bone [16] and pediatric brain tumors [17]. Interestingly, in the present study over-expression of Y-box was prominent in the cervical cancer tissues, which was confirmed by cDNA subtraction and further validated by immunohistochemistry experiments (Figure 1A).

Hif-1a is one of the hetero-dimeric subunits of hypoxia inducible transcriptional factor. It has the ability to trans-activate above 60 genes, as well as regulate angiogenesis, cell metabolism, and genomic stability [18]. In the present study, the expression of Hif-1a was identified in all the 42 patients. In contrast, it was reported that Hif-1a was not expressed in all the cervical cancer patients [19]. Likewise, Id-1 regulates certain events such as cellular senescence [20-22], cell growth [23, 24], and cellular survival [25], which were frequently deregulated during cancer development. It was also reported that in some types of cancer, the expression of Id-1 was coupled with the activation of angiogenesis [26, 27] and induction of cancer cell invasion [28, 29]. In the present study, Id-1 was expressed in all the 42 cervical cancer samples. Similarly, it was found that during the early stage of cervical cancer, Id-1 was expressed as an independent prognostic marker [30]. Controversially, it was reported that Id-1 expression was found in 80% of cervical cancer patients [30]. To increase the accuracy and to eliminate the false positive detection, a combination of the three markers such as Y-box, Id-1, and Hif-1a could be employed for effective detection of the disease.

Figure 1. — Immunohistochemistry of cervical cancer tissues with Y-box, Id-1 and Hif-1a antibodies. A) Immunohistochemistry data of cervical cancer tissues with anti-Y-box antibody. The arrow indicates the Y-box positive cells stained with anti-Y-box antibody. B) Immunohistochemistry data of cervical cancer tissues with anti-Hif-1a antibody. The arrow indicates the Hif-1a positive cells stained with anti-Hif-1a antibody. C) Immunohistochemistry data of cervical cancer tissues with anti-Id-1 antibody. The arrow indicates the Id-1 positive cells stained with anti-Id-1 antibody. All the sections are counterstained with Elhrich (Specimens A and B) and Meyers (Specimen C) hematoxylin, respectively. The tissue sections are examined under a ×40 microscope objective.
Conclusion

Present study concludes that the three markers namely Y-box, Hif-1a. and Id-1 were expressed strongly in all the 42 cervical cancer specimens. Based on the cDNA subtraction and immunohistochemistry experiments, it was concluded that the three marker expression profiles were essential in the accurate and efficient detection of cervical cancer.

Acknowledgements

The authors are thankful to Weifang People’s Hospital and Medical College, Shandong, China and the ethical committee for the successful completion of this project.

References


Address reprint requests to:
X. LIU, Ph.D.
Department of Gynecology,
Weifang People’s Hospital, No. 151 Guangwen Street, Kuiwen District
Weifang, Shandong 261041 (China)
e-mail: liuxin041@gmail.com
Safety of converting a radical vaginal trachelectomy to a radical hysterectomy during pregnancy

W.A.A. Tjalma

University Multidisciplinary Breast Clinic – Gynecological Oncology Unit, Antwerp University Hospital
University of Antwerp, Edegem, Antwerp (Belgium)

Summary
Almost 1 % of all cervical cancers occur in pregnant women. The recommended management during the first 20 weeks is to sacrifice the pregnancy and to perform standard therapy, which means the loss of future fertility. A trachelectomy during pregnancy could preserve the ongoing pregnancy and future fertility. The author reports a radical vaginal trachelectomy (RVT) during 18 weeks of pregnancy. Definitive pathology of the trachelectomy specimen showed a tumor of 48 millimeters. Subsequently a radical hysterectomy was performed. At present, eight years and six months later the patient is well with no signs of recurrence. RVT is feasible in the first and second trimester of pregnancy. Clinical examination and MRI however are less accurate in the evaluation of stage and the extent of the tumor during pregnancy. Converting a RVT to a radical hysterectomy in a second time is safe in a pregnant woman.

Key words: Cervical cancer; Pregnancy; Trachelectomy; Colposcopy; Magnetic resonance imaging; Safety; HPV.

Introduction
Cervical cancer during pregnancy has a frequency of 1.2 - 10.6 per 10,000 [1]. Cancer detected during pregnancy causes a treatment dilemma. The two standard treatment modalities for cervical cancer are radical hysterectomy and chemoradiation. Both of these modalities will exclude future pregnancy. The first trachelectomies performed during pregnancy were done by an abdominal approach [2]. At present seven radical vaginal trachelectomies (RVTs) in the second trimester of pregnancy have been published [3]. All these report state it is reliable and safe. They have a relative follow up time ranging from nine to 47 months (mean 29 months). The author reports a case of vaginal trachelectomy at 18 weeks of pregnancy followed by a radical hysterectomy after a follow up of 102 months. The goal of present report is to highlight the differences between the used routes and address the pitfalls of staging during pregnancy. Follow-up information is important as it can help the clinician to inform the parents about long-term oncological safety of surgery during pregnancy.

Case Report
A 34-year-old pregnant woman (G2P1) was referred at the gestational age of 16 weeks and four days. Her smear showed a high grade squamous intraepithelial lesion (HSIL) with HPV 16 (2,635 copies/cell) and HPV 68 (0.0680 copies/cell). On colposcopy there was a gray-white lesion with sharp borders and an irregular surface at seven o’clock, highly suspicious for invasive cancer and HSIL (Figure 1A). By Lugol’s staining, no additional lesions were detected (Figure 1B). A biopsy was taken and this confirmed the diagnosis. The fornices and vaginal top were free of abnormalities. The upper border of the transformation zone could not be seen. The maximum estimated size was 10 mm. On rectovaginal examination there was an enlarged uterus in accordance with the gestational age, but no infiltration of parametria. A vaginal ultrasound and pelvic MRI were performed. They showed no infiltration in the parametria, bladder or bowel, and no suspicious pelvic lymph nodes. On MRI, the precise lining of the cervix was not accurate due to the edema of the cervix, but the maximum estimated size of the infiltrating tumor was less then five-mm deep and less then seven-mm wide (Figure 2A, 2B). The clinical stage of FIGO Ib1 was confirmed with the MRI. The four treatment options were discussed with the patient and her partner: (1) standard treatment consisting of radical hysterectomy with lymphadenectomy, (2) trachelectomy with lymphadenectomy, (3) neoadjuvant chemotherapy, (4) delay of active treatment until fetal maturity is reached. At the gestational age of 18 weeks and two days, a RVT was performed together with an abdominal pelvic lymphadenectomy. No tocolytics or progesterone was given. Patient received the standard antibiotics for a laparotomy, consisting of cefazoline and metronidazole. Definitive histology revealed a 48-mm tumor with involvement of both parametria. Extensive lymphovascular space involvement was seen and in the right parametrium a single metastatic involved lymph node was found (Figure 3). All the other removed lymph nodes were tumor free. Further management was discussed with the patient and her partner. The options were (1) radical hysterectomy with para-aortal lymphadenectomy followed...
by chemoradiation, (2) neoadjuvant chemotherapy, (3) delay of further treatment until fetal maturity is reached. On patient’s request, an extended radical hysterectomy (Piver IV) was performed. All surgical margins of the radical hysterectomy specimen were free of tumor for at least 1.9 cm and all para-aortic removed lymph nodes were tumor free. The patient received adjuvant chemoradiation. Currently, eight years and six months after the diagnosis, the patient is well with no signs of recurrence.

Discussion

Once cervical cancer is diagnosed, the management depends on the stage of the disease, the gestational age, and the parent’s wishes for present and future pregnancy. All patients should be carefully consulted by a multidisciplinary team including a gynecologic oncologist, maternal fetal specialist, and neonatologist [1]. It is believed that early-stage cervical cancer treatment can be delayed for 16 – 18 weeks without increasing the risk of advancing cancer [1]. Pregnancies less then 13 weeks, except for Stage Ia1, are generally treated immediately. The treatment in pregnancy older then 25 weeks is generally delayed until fetal maturity is reached. In pregnancies between 13 and 24 weeks, there is a dilemma. One can opt for delay with or without neoadjuvant chemotherapy. Until now there have been five published cases regarding the use of NACT during pregnancy [4]. The short term follow-up revealed that all children developing normally. Unfortunately, at the moment, nothing can be said regarding the long-term effects and safety of the chemotherapy on the children. So far, two of the five mothers have died of their disease.

One of the most revolutionary surgical treatments of the last 15 years is the trachelectomy. The RVT was introduced by Prof. Dargent in 1994 and since he passed away, it is proposed to call this procedure the Dargent operation [5]. Later on the abdominal radical trachelectomy (1997) and the laparoscopic abdominal trachelectomy (2005) were reported.

The first four abdominal trachelectomies during pregnancy were performed during the first trimester (seven, eight, nine, and 13 weeks) and one at the beginning of the second trimester (18 weeks) [2]. The first three were accidental since the surgeons and the patients were not aware of the pregnant status of the patient. Four women had Stage Ib1 and one woman (18 weeks) had an unknown stage. The women who underwent the trachelectomy at nine and 18 weeks had a term delivery of healthy babies at respectively, 38 and 39 weeks. The women with a trachelectomy at seven, eight, and 13 weeks, lost their pregnancy, respec-
Safety of converting a radical vaginal trachelectomy to a radical hysterectomy during pregnancy

At day 1, day 1, and day 16 postoperative. After a median follow-up of 40 months, no recurrences have been detected among these five patients. Current report described the feasibility of a RVT during pregnancy. The major difference between the vaginal and the abdominal route is the uterus manipulation. During a RVT one does not touch the remaining corpus. During pregnancy the corpus of the uterus is very soft and squeezing seems unavoidable when using the abdominal route. It is likely that an increased uterine manipulation will lead to an increased abortion rate. Due to the gravidity, the pregnant uterus gives an increased pressure on the pelvic floor. The abdominal approach will in contrast to the vaginal approach, allow a lifting of the pregnant uterus, facilitating the access to the pelvic side wall. The latter will make the parametrial resection undoubtedly more feasible. The question is of course: “How much of the parametria should be removed?” Present case illustrates the importance of full parametrial resection in case the parametrium is at risk for lymphatic metastases.

Pregnant women are staged according to the same FIGO rules as non-pregnant women. The accurate assessment of the tumor extent can be difficult due to physiological and anatomical changes during the pregnancy. Current case showed how misleading the staging can be during pregnancy. The cervical cytology gave HSIL, colposcopy, and biopsy showed an invasive cervical tumor with an estimated size of ten mm. Rectovaginal examination suggested free parametria. Ultrasound and MRI are no part of the stage but they were done to tailor the therapy. Despite the fact that MRI is considered very accurate, it missed the extent of the disease completely. Instead of the suggested maximum of seven mm width, the tumor appeared to be 48 mm wide. One has to take in consideration of course that due to the pregnancy, no intravenous gadolinium was used. The pregnancy induced enlargement of the cervix clearly masquerades the extension of the infiltration. A preoperative MRI is essential to determine the extent of disease, to exclude obvious metastatic cancers and to plan the location of the cervix transaction [2]. However, clinicians and patients should be aware that due to the pregnancy, there can be a severe underestimation of the extent of the disease.

If cervical cancer occurs in non-pregnant women of childbearing age, a trachelectomy seems a safe uterus sparing procedure which preserves the fertility. In pregnancy, this procedure is also feasible by both routes. Not only can the present pregnancy be saved, but it also will preserve future fertility. From a surgical point of view, a RVT seems more feasible in second trimester then an abdominal trachelectomy. Present report demonstrated the potential role of RVT in early-stage cervical cancer. The outcome for the baby was unfavourable due to the fact that the tumor was more advantaged, which lead to a radical hysterectomy. The long follow-up period of the mother showed that the approach was safe if the correct oncological rules are followed.

References


Address reprint requests to:
W.A.A. Tjalma, M.D., Ph.D.
University Multidisciplinary Breast Clinic
Gynecological Oncology Unit,
Antwerp University Hospital
University of Antwerp, Wilrijkstraat 10,
2650 Edegem, Antwerpen (Belgium)
e-mail: wiebren.tjalma@uza.be
Diagnostic usefulness of FDG-PET/CT in advanced malignant lymphoma of the uterus: report of two cases

T. Okuda¹, S. Ijichi¹, S. Yamashita¹, T. Yoshioka¹, H. Nishigaki², J. Kitawaki³
¹ Department of Obstetrics and Gynecology, Fukuchiyama City Hospital, Kyoto
² Department of Medicine, Fukuchiyama City Hospital, Kyoto
³ Department of Obstetrics and Gynecology, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto (Japan)

Summary
Malignant lymphoma of the uterus is difficult to diagnose because of its rarity and nonspecific symptoms [1, 2]. However, recently, ¹⁸F-fluoro-2-deoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) has become an important non-invasive diagnostic tool for the management of lymphoma patients. The authors report two cases of malignant lymphoma of the uterus, in which FDG-PET/CT was useful for diagnosis. Examination using ultrasonography or magnetic resonance imaging (MRI) demonstrated a normal-sized uterus and normal endometrium, but FDG-PET/CT showed FDG accumulation in the uterine body in both cases. Endometrial biopsy revealed diffuse large B-cell lymphoma, and chemotherapy with rituximab, cyclophosphamide, adriamycin, vincristine, and prednisone (R-CHOP) was initiated immediately. Primary malignant lymphoma of the female genitalia is reported to be rare. The present authors’ experience with FDG-PET/CT suggests that malignant lymphoma of the female genitalia (including metastasis) may not be as rare as previously reported. Uterine malignant lymphoma may be overlooked by the examination of ultrasound, CT, or MRI.

Key words: PET-CT; Diffuse large B-cell lymphoma; Uterus.

Introduction
Malignant lymphoma of the uterus is quite difficult to diagnose because of its rarity and nonspecific symptoms [1, 2]. However, ¹⁸F-fluoro-2-deoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) fusion has recently become an important non-invasive diagnostic tool for the management of patients with lymphoma [3, 4]. The authors report two cases of malignant lymphoma of the uterus (diffuse large B-cell lymphoma [DLBCL]) of the uterus, in which early detection and treatment were possible by using FDG-PET/CT.

Case Report
Case 1
A 73-year-old postmenopausal woman (G3P3) was referred to the present hospital for further evaluation of uterine corpus cancer. Her chief complaint was cough for one month. In the previous hospital, blood test results revealed thrombopenia (29×10³/µl [normal, 140–340×10³/µl]) and marked elevation of the serum lactate dehydrogenase (LDH) level (1,127 IU/L [normal, 115–245 IU/L]). Therefore, hematological malignancy was suspected, and FDG-PET/CT was performed. It showed strong FDG accumulation in the uterine body, both internal iliac nodes and para-aortic nodes, and the right adrenal gland (Figure 1A, B). Therefore, she was suspected of having Stage IV corpus cancer and was referred to the present hospital. She had no complaint of abnormal vaginal bleeding or abdominal pain. No swelling of the superficial lymph node was found. Other examinations, including ultrasonography (US) and pelvic magnetic resonance imaging (MRI), did not reveal malignancy of the uterus (Figure 1C, D). However, endometrial biopsy revealed DLBCL (Figure 2A, B). Moreover, immunohistochemistry analysis revealed atypical lymphocytes that stained positive for CD20. Flow cytometry analysis (FCM) showed no monoclonal antibody. Chromosome examination revealed abnormalities in two out of 50 cells (93XXXX in one cell and 95XXXX in the other); however, clonality was not detected. A bone marrow biopsy was obtained, which showed normocellular bone marrow; however, cancer cells were found. Staging was compatible with Stage IVA for DLBCL of the uterus. According to the patient’s International Prognostic Index (IPI) score, she was in the high-risk group (age > 60 years, Stage III/IV disease, elevated LDH level, > one extranodal site of the disease). According to the patient’s Revised International Prognostic Index (R-IPI) score, she was in the “poor” group (Stage III/IV disease, elevated LDH, > one extranodal site of the disease). Treatment with rituximab, cyclophosphamide, adriamycin, vincristine, and prednisone (R-CHOP) chemotherapy on the seventh day after the initial diagnosis was successful and resulted in marked remission. On follow-up, PET-CT examination was performed after four cycles of R-CHOP chemotherapy. PET-CT images revealed normal findings (Figure 1E, F).

Case 2
A 54-year-old postmenopausal woman (G3P2) was referred to the present hospital. She had a history of Stage I breast cancer five years prior. Her chief complaint was cough and high fever (over 38°C) for one month. At the previous hospital, blood test results had revealed no signs of infection (white blood cell count, 7,000/µl; C-reactive protein level, 2.27 mg/dl), and her chest ra-
diograph revealed no abnormal findings at the initial diagnosis. Her platelet count was $13.5 \times 10^3/\mu l$. Dextromethorphan hydrobromide hydrate was prescribed for her cough. However, her night fever still did not improve, and her cough worsened. She was re-examined because of her high fever. No infection sign or thrombopenia was found (white blood cell count, 7,000/μl; marked elevation of the serum LDH level at 1,079 IU/L). Hematological malignancy was suspected; therefore, she was referred to the present hospital where she underwent FDG-PET/CT. Uterine cancer metastasis at various parts was diagnosed using FDG-PET/CT (Figure 3A, B). On admission to the present hospital, she had no complaint of abnormal vaginal bleeding or abdominal pain, and had no swelling of the superficial lymph nodes, but had a high fever (39.4°C) and had been experiencing sharp pain in the shoulders, elbows, and wrists. Other examinations, including US and pelvic MRI, did not reveal malignancy of the uterus (Figure 3C, D). Her cervical smear and endometrial cytology was negative. However, FCM of the endometrium rapidly demonstrated a neoplastic increase (Figure 4). On the basis of these findings, DLBCL was diagnosed. A few days later, a bone marrow biopsy revealed atypical cells; their nuclear-cytoplasm ratio was high. Chromosome examination revealed abnormalities in four out of 50 cells; however, clonality was not detected. Staging was compatible with Stage IV BE for DLBCL of the uterus. According to the patient’s IPI score, high-risk disease was indicated (Stage III/IV disease, elevated LDH level, Eastern Cooperative Oncology Group performance status ≥ two, and > one extranodal site of the disease). According to her Revised-IPI score, the patient was in the “poor”
stage of the disease (Stage III/IV disease, elevated LDH level, performance status ≥ two, and more than one extranodal site of the disease). On admission, rapid myeloid invasive destruction was suggested because her platelet rapidly decreased to 75×10³/µl, and treatment was immediately started with R-CHOP chemotherapy at the initial diagnosis on the fourth day when the FCM results were obtained. Her high fever and dull pain alleviated soon after treatment. On follow-up, PET-CT examination was performed after four cycles of R-CHOP chemotherapy. Accumulation was still observed only on the right thighbone (Figure 3E, F); therefore, a biopsy was performed, but no malignant tissue was obtained. Sequentially, R-CHOP therapy was administered, and peripheral blood stem cell transplantation was performed.

**Discussion**

With the former diagnostic imaging modalities, the most common presentation of lymphoma of the uterus is diffuse symmetrical enlargement of the cervix and corpus, without mucosa abnormalities [5]. CT is the most commonly used means for staging patients with malignant lymphoma. However, imaging of characteristics of extranodal involvement can be subtle or absent with conventional CT. Furthermore, CT lacks functional information, which impedes identification of disease in a normal sized organ [4]. For these reasons, it is quite difficult to diagnose malignant lymphoma in a normal sized uterus. Therefore, many clinicians often do not consider lymphoma because of its rarity, and non-specific symptoms and characteristics in imaging studies [5]. However, recently, imaging of the tumor metabolism with PET has facilitated the identification of affected extranodal sites, even when CT has demonstrated no lesions. Moreover, hybrid PET-CT has become the standard imaging modality for initial staging, follow up, and treatment response assessment in lymphoma patients, and has been proven to be superior to CT [3, 4]. The first case reported here had been misdiagnosed as adrenal cancer; in this case, operation may have been performed first and chemotherapy may not have been initiated if PET-CT had not been performed.

Next, with regards to pathological diagnosis, cervical cytology is usually negative [6,7], and the rate of false negative results is high in biopsied specimens [5]. However, the use of FCM to analyze monoclonal antibody-coated single cell suspensions is known to increase the speed and accuracy of
Figure 4. — Flow cytometry of the endometrium. Neoplastic increase is demonstrated (increased κ to λ ratio, and CD19- and CD20-positive cells). Based on these data, diffuse large B-cell lymphoma was diagnosed.
leukemia diagnosis, although its use in solid tumor diagnosis has not been widely reported. Only a small sample is required as the analysis is carried out on a single cell suspension; the method is rapid, and the diagnosis can be made within three hours. FCM provides information on cell lineage and the stage of differentiation. The diagnostic accuracy is good when compared with that of histological results [8]. The second case reported here could not have been diagnosed unless PET-CT and FCM were carried out, and chemotherapy would have been delayed if diagnostic laparotomy had been performed.

Some clinicians consider that the prognosis of uterine lymphoma may be relatively favorable when the disease is treated properly. According to the criteria by Fox et al., the malignant lymphoma of the uterus in the present cases was Stage 1, indicating a good prognosis [9]. On the other hand, other clinicians have reported that uterine lymphoma has a poor prognosis [6, 10]. The present authors suggest that the reason for this inconsistency is the difficulty to acquire a definite diagnosis.

Primary lymphoma of the female genital tract is extremely rare [1, 2]. According to Freeman et al., it accounts for less than 2% of all malignant lymphomas [11], and Harris et al. reported that primary lymphoma of the cervix and vagina was more prevalent than primary lymphoma of the corpus (78%) [7]. Fox et al. proposed the criteria for diagnosing primary extranodal lymphoma [9], which was roughly identical to that of clinical Stage 1 disease by the Ann Arbor system. In the present cases, uterine cancer was diagnosed using PET-CT imaging and isolated tissue. The cases did not meet the criteria proposed by Fox et al.; however, the origin is not important in the treatment of malignant lymphoma. With respect to malignant lymphoma, especially DLBCL, the most important factors influencing therapeutic decisions and prognosis are the histologic subtype and extent of the disease [12]. DLBCL is a heterogeneous group of aggressive B-cell lymphomas rather than a single clinicopathologic entity, and the pathogenesis of DLBCL is complex and heterogeneous [13]. The IPI has been the basis for determining prognosis in patients with aggressive non-Hodgkin lymphoma. Based on the IPI score, both the present patients belonged to the high-risk group, and rapid treatment was needed to improve their symptoms and prognosis. Recently, the R-IPI was proposed to predict the prognosis of DLBCL patients treated with R-CHOP therapy [14].

However, identification of the origin of the malignant lymphoma is difficult in cases such as the present, and using PET-CT and FCM as a diagnostic method for DLBCL of the uterus may allow for early and certain diagnosis, avoiding unnecessary or incomplete operation. Rapid as well as accurate diagnoses of uterine lymphoma are important in the planning of appropriate treatment, and this is possible by using PET-CT and FCM. The diagnosis and treatment of malignant lymphoma of the uterus will dramatically change in the future. Gynecologists should consider malignant lymphoma regardless of its rarity.

Some factors should be considered when performing PET. Most lymphoma subtypes have been shown to have a high affinity for 18F-FDG, and a lower 18F-FDG affinity has been reported for lymphoma subtypes, such as small lymphocytic lymphoma, peripheral T-cell lymphoma, anaplastic large T-cell lymphoma, and extranodal marginal zone lymphoma [8]. Fortunately, most of the uterine lymphomas are DLBCL, which is classified as aggressive and progresses on a monthly basis [1, 2].

Conclusion

The authors reported two cases of advanced malignant lymphoma in patients with normal-sized uterus, in which early detection and treatment was possible using PET-CT.

References


Address reprint requests to:
T. OKUDA, M.D., PhD
Department of Obstetrics and Gynecology
Fukuchiyama City Hospital
231, Atsunaka-cho, Fukuchiyama City
Kyoto 620-8505 (Japan)
e-mail: tomo.rx400h@dg7.so-net.ne.jp
Introduction

Primary ovarian fibrosarcoma is very uncommon neoplasm with poor prognosis [1-3]. It may arise de novo or as a result of malignant change in a pre-existing ovarian fibroma [1]. According to Shakfeh and Woodruff [2], fibrosarcomas arise from the undifferentiated theca externa, submesothelial tissues, and fibrous components in the hilum of the ovary, and differs from the sarcomas arising from the ovarian stroma compatible with fibrosarcoma. Twenty-four months follow-up showed no recurrence of disease. Ovarian fibrosarcoma is a very uncommon neoplasm with a poor prognosis. Despite the efforts of several authors in reporting morphological, histological, and immunohistochemical features of this neoplasm, nowadays, the diagnosis, treatment, and prognosis are unresolved issues. The present case highlights the important role of immunohistochemistry to define histological type and differential diagnosis. As demonstrated by the authors’ experience, they believe that surgery is curative in the early stages with low immunohistochemical positivity for ki67 and that chemotherapy should be reserved in advanced stages with regimens in use for the treatment of sarcomas.

Case Report

A 58-year-old woman presented with intermittent pelvic pain at Gynecology Unit of the Second University of Naples at Sant’Anna and San Sebastiano Hospital of Caserta, Italy. She had no family history of malignancy. She was taking antihypertensive drugs and reported clinical history of chronic autoimmune thyroiditis and restrictive lung disease. On gynecological physical examination, the patient had a demarcated, mobile, and solid lump in the right adnexa. Diagnostic imaging revealed a solid-cystic inhomogeneous mass occupying the right adnexa and the CA125 level was elevated. The patient underwent total hysterectomy with bilateral salpingo-oophorectomy and infracolic omentectomy. Histological findings with immunomarkers led to the final diagnosis of low-grade malignant mesenchymal neoplasm derived from the ovarian stroma compatible with fibrosarcoma. Twenty-four months follow-up showed no recurrence of disease. Ovarian fibrosarcoma is a very uncommon neoplasm with a poor prognosis. Despite the efforts of several authors in reporting morphological, histological, and immunohistochemical features of this neoplasm, nowadays, the diagnosis, treatment, and prognosis are unresolved issues. The present case highlights the important role of immunohistochemistry to define histological type and differential diagnosis. As demonstrated by the authors’ experience, they believe that surgery is curative in the early stages with low immunohistochemical positivity for ki67 and that chemotherapy should be reserved in advanced stages with regimens in use for the treatment of sarcomas.

Key words: Fibrosarcoma; Fibrothecoma; Immunohistochemistry; Mitosis; Ovary.
salpingo-oophorectomy and infracolic omentectomy. Pelvic lymph node dissection was not performed because of the absence of masses detected by diagnostic examination in the pelvic and lombo-aortic regions. Macroscopic examination of the abdominal cavity showed ascites, which were aspirated for cytology. No reproductive lesions were observed in the abdominal cavity as well as in subdiaphragmatic and parieto-colic spaces. Macroscopic examination of the pelvic cavity showed the right adnexa completely transformed into a well-capsulated solid-cystic mass measuring nine cm in diameter. The histopathological diagnosis on frozen section reported malignant mesenchymal neoplasm of the right adnexa to characterize on the definitive sections. Macroscopic examination of the specimen revealed a solid, grayish, and widely congested nodular lesion, measuring 6 × 5 × 3 cm, with mucoid central area, (measuring 2.7 cm) and hemorrhagic areas at cutting (Figure 1). Microscopic examination of the multiple sections showed multinodular proliferation of pleomorphic-spindle shaped and partly epithelioid cells with expansive growth (Figure 2), placed to form irregular bundles with multiple hemorrhagic foci, mixed with fibrous and edematous tissue; numerous mitosis were identified (7-8 for ten HPF), some of these atypical (Figure 3). Immunohistochemical stainings for CD34, CD3, cytokeratin pan, cytokeratin7, and EMA were negative; immunohistochemical stainings was diffusely positive for vimentin; smooth muscle actin (SMA) was focally positive and calretinin was positive. Mitotic activity was observed with a high proliferation index (20%), assessed with Ki67. The histological and immunohistochemical findings established the diagnosis of low-grade malignant mesenchymal neoplasm derived from the ovarian stroma compatible with fibrosarcoma. This diagnosis was confirmed by the U.O.C. Pathology and Cytopathology - IRCCS Pascale – Naples, Italy. There is no standard follow up for this neoplasm, so the authors decided to perform physical examination, blood dosage of CA125, and total body CT every six months. Twenty-four months follow up showed no recurrence of disease (even the CA125 levels returned to the normal range).

Discussion

The diagnostic criteria of Prat and Scully [6] are currently the most widely used in the case of ovarian fibrosarcoma. These authors found that mitotic activity was the most important factor for diagnosis and that nuclear grading with cellular pleomorphism was not so reliable. They concluded that tumors containing 1–3 mitotic figures/HPF should be designated as benign cellular fibromas, others containing 4 mitotic figures/HPF as malign fibrosarcomas [2]. However, subsequent case reports [9-13] suggested that such tumors with a high number of mitotic figures often have favourable outcomes. As a result, the mitotic counts were indicated as not a strict criterion [2, 7, 14]. Although in their study, the lesions selected were pure fibromas showing no evidence of thecomatous differentiation, in a later review, Young and Scully [15] suggested that, in the absence of established criteria for malignancy, the same criteria could be applied to fibrothecomatous lesions. Despite the apparent reliability of mitotic counts in differentiating fibrosarcomas from cel-
lular fibrothecomas, few examples exist in which cellular fibrothecomatosous lesions demonstrating low mitotic activity show an unexpected aggressive behaviour [14]. Tsuji et al. [7] established that the MIB-1 (Ki-67) labeling index (LI) in atypical fibromatoses of the ovary was reflective of the potential aggressiveness of the tumor. MIB-1 LI for cellular fibromas ranged from 0.5 to 4.0 with a median of 2.3, while that for fibrosarcomas ranged from 3.0 to 10.8 with a median of 6.6. MIB-1 LI, was related to the mitotic count, and their results may support a formal mitotic count as a diagnostic tool, especially in cases with a count of three to four mitoses per ten HPF. A recent large study [16] suggested that cellular fibromatous neoplasms of the ovary with weak nuclear atypia should not be classified as fibrosarcomas, even though they showed more than ten mitotic figures per ten HPF. Such tumors with weak nuclear atypia are to be subclassified as either cellular fibromas or mitotically active cellular fibromas, depending on the number of mitotic figures. This is based on the fact that there was no recurrence in 18 patients with mitotically active cellular fibroma, including three patients with ovarian surface adhesions or extravarian involvement. Kaku et al. [17] described the case of a solid mass in the left iliac fossa that microscopically was made of two components, one with 17 mitotic features/10 HPFs, the other one with less than 3. In these cases, mitotic activity assessed with Ki67 was positive in the high cellularity area and negative in the low cellularity area. Therefore, the patients affected by this neoplasms, undergoing unilateral salpingo-oophorectomy, had no evidence of recurrence within one year from surgery. Those were “high mitotic activity cellular fibroma”. According to the study of Garcia-Jiménez et al. [5], fibrosarcoma and “high mitotic activity cellular fibroma” could be the same tumor with different evolution. Furthermore, primary ovarian fibrosarcomas, unlike the “high mitotic activity cellular fibromas”, shows cellular atypia, a higher growth rate, and it presents, commonly, with several adhesions and necrotic-hemorrhagic areas. However, these histological differences are not always assessable and specific as reported by Garcia-Jiménez et al. [5]. They reported a case of neoplasm in which, although there was a low grade of cellular atypia and mitotic activity, the tumor metastasized to the liver 14 months after surgery. The only discrepancy was the high levels of Ki67 associated with a low mitotic activity. 

In a meta-analysis study, Long Huang et al. [18] evaluated 31 cases of ovarian fibrosarcoma with a FIGO Stage from I to IV. The cellular mitotic activity ranged from 1 to 25/10 HPFs. The Ki67 index was much higher in fibrosarcoma compared to the fibroma. Moreover, the expression of vimentin (22/22, 100%) and the absence of CD117 expression (0%, 5/5) were also found. Further, immunohistochemical analysis evaluated the SMA (2/18, 11%), desmin (1/13, 8%), epithelial membrane antigen (EMA) (0/11, 0%), S-100 (1/19, 5%), CD99 (0/6, 0%), CD34 (1/5, 20%), a-inhibin (7/15, 47%), estrogen receptor (ER) (1/6, 17%), and progesterone receptor (PR) (1/6, 17%). The results of retrospective multi center study of Huang et al. [18] showed that most patients experienced a fatal outcome due to early metastasis via the bloodstream and tumor recurrences that usually occurred within two years of diagnosis. Furthermore, there is no universally accepted treatment for ovarian fibrosarcoma as there is for epithelial ovarian cancers. Surgical treatment of ovarian fibrosarcoma ranges from a simple oophorectomy to total hysterectomy with bilateral salpingo-oophorectomy and omentectomy. In all cases, however, adjuvant chemotherapy or radiotherapy is planned [18, 19]. After reviewing several studies, Miles et al. [20] reported that surgery did not prevent the recurrence of this disease regardless of the extent of surgery. Adjuvant chemotherapy and radiation therapy did not influence patient survival [20]. There are few reports indicating that adjuvant chemotherapy may improve patient survival rates, although Huang et al. reported that the use of MAID (mesna, doxorubicin, ifosfamide, and dacarbazine: DTIC) for the treatment of ovarian fibrosarcoma showed potential for prolonging patient survival [21]. Moreover, Celyk et al. reported that a regimen of paclitaxel plus cisplatin could improve the prognosis for patients with advanced stage tumors in some cases [22]. Regarding treatment, Huang et al. [18] found that the overall survival (OS) at two years for patients receiving BAO (total hysterectomy with bilateral adnexectomy and an omentectomy), OR (oophorectomy), or BAO+RT (radiotherapy) was 27.9%, while patients receiving BAO+CT(chemotherapy) had an OS percentage of 100%. For the same categories of patients, the disease-free survival (DFS) at two years was 28.5% and 72.9%, respectively, dividing the patients into two groups: those who received BAO, OR, CT, OR+RT or BAO+RT, and those receiving BAO+CT, 60% of patients in the first group died within five years from this disease, compared to 9.1% of patients in the second group. Therefore, the authors identified the FIGO stage and type of treatment as important prognostic factors for survival. In contrast, patient age, tumor size, mitosis events per ten HPFs, and percentage of Ki-67-positive cells present were not found to be significant independent prognostic factors for survival [18]. Primitive ovarian fibrosarcoma staging is comparable to that of ovarian carcinomas established by FIGO. It is very difficult to assign the FIGO stage, because of the exiguous number of reported cases, a prognostic value, as well as for ovarian epithelial tumors. Huang et al. [18] reported for OS and DFS within two years from treatment a percentage of 77.1% and 36.2% for neoplasms at FIGO Stages IA-IC and 68.4% and 22.9% for neoplasm at FIGO Stages II-IV, respectively. In the case reported by Garcia-Jiménez et al. [5] (FIGO Stage IC), free survival disease after total hysterectomy with bilateral salpingo-oophorectomy, omentectomy, and bilateral iliac and para-aortic lymphadenectomy was 14 months, then a metastatic lump to the liver was de-
tected. It seems that FIGO staging and low immunohistochemical positivity for Ki67 may influence prognosis. The present case corresponds to a well-differentiated FIGO Stage IA with an immunohistochemical positivity for Ki67 of just 20%. The follow-up at 24 months of the present patient shows no recurrence of disease, according to the study of Garcia-Jiménez et al. [5]. Therefore, we can consider Stage IA, showing lower mitotic activity assessed with Ki67, as a stage with good prognosis.

Conclusion

Treatment and prognosis of ovarian fibrosarcoma are unresolved issues because of the absence of standard and approved management. Nowadays, the present authors suggest that the most reliable prognostic factors are FIGO staging and the immunohistochemical positivity for Ki67. Regarding the role of the adjuvant chemotherapy, the present authors believe that surgery is curative in the early stages with low immunohistochemical positivity for Ki67 and that chemotherapy should be reserved in advanced stages with regimens in use for the treatment of sarcomas. The present authors expect that the development of molecular biology technics could offer the possibility to define the molecular profile of the single neoplasia in order to suggest prognosis and response to therapy, than more detailed classification of fibrous tumors of the ovary.

References


Address reprint requests to:
G. BALBI, M.D.
Guido de Ruggiero, 118
80128 Napoli (Italy)
e-mail: giancarlo.balbi@unina2.it
Postradiation carcinosarcoma of the corpus uteri – a case report

A. Zwierczowska, G. Panek, M. Gajewska

1st Department of Obstetrics and Gynecology, Medical University of Warsaw, Warsaw (Poland)

Summary

Introduction: Radiation therapy is a very effective treatment modality, commonly used for numerous gynecological malignancies, e.g. cervical cancer. Unfortunately, ionizing radiation is associated with numerous side effects, including secondary cancer formation. A case of carcinosarcoma of the corpus uteri in a woman with a history of pelvic irradiation for cervical carcinoma is reported. The literature has been reviewed to present the incidence, optimal management, and prognosis in cases of postradiation uterine carcinosarcoma.

Case: A 55-year-old woman with a history of pelvic radiotherapy for cervical cancer five years earlier was diagnosed with a pelvic mass. Endovaginal ultrasound examination revealed a solid and cystic tumor, 12.5 cm in diameter. The patient was scheduled for surgery. Gross examination revealed an enlarged, plain corpus uteri, 12 cm in diameter. Both adnexa were normal. Pelvic and abdominal peritoneum were macroscopically normal and normal on palpation. Iliac and obturatory lymph nodes were enlarged on both sides. Radical hysterectomy, omentectomy, and ilio-obturator lymph node dissection were performed. The pathology report revealed carcinosarcoma of the corpus uteri with lymph nodes metastases - FIGO IIIC1. No adjuvant treatment was given. The patient is still alive and disease-free one year after surgery.

Conclusions: Clinicians should remain conscious of the potential carcinogenic effect of radiation therapy. Uterine carcinosarcoma may occur years after radiotherapy applied for cervical cancer. Therefore, long-term control following pelvic irradiation is always necessary.

Key words: Second primary neoplasms; Radiation induced neoplasms; Carcinosarcoma.

Introduction

Radiation therapy is one of the most commonly used treatment methods in various pelvic malignancies, including cancers of the female reproductive system. It is utilized both as adjuvant therapy, following surgery, or as a sole radical treatment modality.

The progress that has been made in treating tumors with radiotherapy has resulted in an increased number of patients surviving radiosensitive cancers, including malignant tumors of the female reproductive system [1]. However, ionizing radiation is associated with numerous side effects. In patients receiving radiotherapy, both local and systemic post-irradiation reactions are observed. The former include ulcerations, fibrosis, necrosis and fistulae, the latter - bone marrow suppression [2]. Moreover, it appears that previous radiation therapy may promote the development of a secondary malignancy in the irradiated field. The incidence of postradiation cancers occurring after radiation therapy to the pelvis is 0.5% to 1.6%. The most common secondary malignancies following irradiation for cervical cancer include leukemia, tumors of the corpus uteri, urinary bladder, vulva, ovary, bone, connective tissue, and rectum. They may occur up to several dozen years after radiation therapy [1, 3-5]. Pelvic radiotherapy is a risk factor for secondary sarcoma of the female genital tract [6]. Carcinosarcoma of the uterine corpus can be a radiation-induced tumor [4, 7-11]. Between 5% to 22% of carcinosarcomas are related to previous radiation therapy [7-9], and a history of irradiation is associated with poorer prognosis [8, 12].

Carcinosarcoma (formerly, malignant mixed Müllerian tumor - MMMT), is a rare malignant neoplasm composed of both, mesenchymal and epithelial elements. It constitutes approximately 1% to 2% of all tumors of the uterus [13]. It is currently considered to be a metaplastic, aggressive form of uterine corpus carcinoma, whose biology and clinical course are similar to ovarian cancer [14, 15]. The prognosis is poor, with the five-year survival rates observed in 33-39% of the affected individuals [8, 12, 16-18]. Despite the fact that carcinosarcoma does not occur often, it is responsible for up to 16.2% of deaths caused by cancers of the uterus [19].

A case of carcinosarcoma of the corpus uteri in a woman with a history of pelvic irradiation for cervical carcinoma is presented. The literature has been reviewed in order to present the existing data on the incidence, optimal management, and prognosis in cases of postradiation uterine carcinosarcoma.

Case Report

A 55-year-old woman was admitted to the Gynecology Ward of the 1st Clinic of Obstetrics and Gynecology, Medical Univer-
A case of carcinosarcoma of the corpus uteri occurring after pelvic radiation therapy for cervical carcinoma is reported. According to Cahan et al., a tumor may be perceived as an irradiation therapy side effect if the following criteria are fulfilled: it occurs in a region that had previously been irradiated, it is histopathologically different from the primary cancer, and at least five years passed from the treatment for the primary cancer [20].

Radiation therapy plays a significant role in both, radical and adjuvant treatment for various malignancies. The ongoing advancement in radiation techniques and devices has resulted in decreased rates of post-radiation complications. Despite the improvement in the safety of radiotherapy, the carcinogenic effect of ionizing radiation remains a challenging problem. The pathogenesis of radiation-induced malignancies has not yet been fully understood. Even when low doses are used (< 0.1 Gy), base damage and single DNA strand breaks occur. When the affected cells replicate, these changes lead to double strand breaks and, as a result, genetic mutations [21]. When the cells are exposed to higher doses (0.2 Gy), DNA repair mechanisms become impaired [22]. ‘By-stander effect’ is another theory on the origins of radiation-induced cancer. According to this hypothesis, neoplastic transformation is caused by signals transduced between the irradiated and non-irradiated cells by means of cytokines and through gap-junctions [23]. The majority of gynecologic patients undergoing radiation therapy receive high total doses (65-80 Gy). Normal tissues lying near the tumor are also inevitably affected by the radiation. According to Sachs et al., in these cases secondary malignancies are initiated by tissue repopulation by premalignant stem cells [24].

In a study by Boice et al., in which 182,040 women treated with radiation for cervical cancer took part, a slightly increased risk (RR 1.1) was proven with regard to the tumors of the following organs: urinary bladder, rectum, corpus uteri, ovary, small intestine, bone and connec-

Figure 1. — Uterus: carcinosarcoma in the endometrial cavity.
tive tissue, as well as multiple myeloma. The risk increased over time and was 1.2 and 1.8 after 20-24 and 30 years since treatment completion, respectively, for tumors of the corpus uteri. However, it is suggested that the risk is underestimated since 35% of the women had had their uterus removed and that fact was not taken into consideration [4].

The incidence of secondary neoplasms in women treated for cervical carcinoma was also evaluated by Kleinerman et al. In a group of 49,828 women with cervical carcinoma treated with radiation, 3,750 survived over 30 years. There was a twofold increase in the risk of developing a secondary cancer in the irradiated region when compared to the general population of the region. The increased risk referred mainly to the rectum, vagina, vulva, ovary, and urinary bladder [1]. Another study, conducted in Denmark, also revealed a greater risk of secondary malignancy in the irradiated region after cervical cancer therapy. Similarly to what was found by Boice et al., the longer the time that passed since the primary treatment, the greater the risk of a secondary neoplasm. The maximal risk was found to be 30 years post radiotherapy. The most common were tumors of the genital tract - other than cervical cancer (RR 5.8), urinary bladder (RR 5.5), connective tissue (RR 3.3), and rectum (RR 2.4) [3]. Moreover, numerous cases of bone sarcoma occurring as a complication of pelvic radiation therapy for cervical cancer have been described [25]. On the other hand, Lee et al., and Macara et al., who evaluated 1,048 and 3,911 cases, respectively, found no increase in the incidence of second malignancies in the irradiated region in patients treated for cervical cancer when compared to cancer risk in the general population [26, 27].

Since 1980s, an increase in the incidence of sarcomas of the corpus uteri associated with previous radiation therapy has been reported [6]. Carcinosarcoma of the corpus uteri may be a radiation-induced malignancy caused by the treatment of pelvic tumors [4, 7-11]. According to Hoffman et al., carcinosarcoma is the most common type of postradiation sarcoma [28]. In a research study performed by Doss et al., 49 cases of carcinosarcoma of the corpus uteri were described, 11 (22%) of which were associated with previous radiation therapy [9]. The time interval between the occurrence of postradiation carcinosarcoma of the corpus uteri and pelvic irradiation for cervical cancer was five to 17 years [9, 10, 29].

It is possible that the aforementioned carcinosarcoma report was associated with the radiation therapy that the patient had undergone as treatment for cervical cancer. Due to low incidence of this malignancy, no specific risk factors have so far been determined. Apart from age (55 years), no other risk factors of sarcoma were present in the present patient – parous, with normal weight and no history of long-term estrogen therapy. When describing cases of postradiation carcinosarcoma, various authors identify radiation therapy as the most significant risk factor [10, 11]. In the Polish literature, a case of postradiation rhabdomyosarcoma has been described [30].

Surgery is the basic treatment modality for uterine carcinosarcoma. The majority of patients require adjuvant therapy. Depending on the experience of a given unit, both chemo- and radiotherapy are used postoperatively [9, 12, 16, 31, 32]. Surgery is also the principal treatment in postradiation carcinosarcoma [9], as was the case in the aforementioned patient. In a research study by Murray et al., four cases of postradiation carcinosarcoma of the uterine corpus were described. Three of them were treated with surgery alone and one patient, who had the longest survival (two years), received adjuvant postoperative chemotherapy [29].

The prognosis in postradiation carcinosarcoma is unfavorable. In a previously cited study, the five-year survival rate for all cases of carcinosarcoma was approximately 35%, whereas the maximal survival for the three patients with postradiation carcinosarcoma was 15 months [12]. These numbers indicate that prognosis in radiation-induced carcinosarcoma is even worse.

Conclusions

Radiation therapy plays an important role in the management of many malignancies, including cancers of the female genital tract. Clinicians should always remain conscious of the potential carcinogenic effect of this treatment modality. Uterine carcinosarcoma may occur years after radiotherapy conducted for cervical cancer. The risk implies the necessity of regular, long-term control in oncological patients who have been subjected to pelvic irradiation.

Acknowledgements

The authors wish to thank Szymon Kozłowski, M.D., for his assistance and submitting the photo for the manuscript.

References


Postradiation carcinosarcoma of the corpus uteri – a case report
Is gastrointestinal stromal tumor (GIST) originating from the rectovaginal septum GIST or extra-GIST (EGIST)?
A case report with literature review

Y.H. Lee, G.O. Chong, D.G. Hong
Department of Gynecology and Obstetrics, Kyungpook National University Medical Center, Daegu (Republic of Korea)

Summary
Gastrointestinal stromal tumors (GISTs) are rare tumors of the gastrointestinal (GI) tract that arise from primitive mesenchymal cells. Extragastrintestinal stromal tumors (EGISTs) are extremely rare tumors that show the features of GISTs outside the GI tract. The authors report herein a case of a 54-year-old woman with GIST in rectovaginal septum. The patient underwent low anterior resection of the rectum, total abdominal hysterectomy, bilateral salpingo-oophorectomy, and partial resection of the posterior vagina. She received adjuvant therapy with an oral tyrosine-kinase inhibitor. She is presently healthy without any evidence of recurrence at 26 months after surgery. For GISTs arising in the rectovaginal septum, it is difficult to ascertain whether the tumor origin site is the rectum, rectovaginal septum, or vagina. In other words, it is difficult to classify these tumors as GISTs or EGISTs. More consideration for the exact origin should be given to the GIST in the rectovaginal septum for the precise diagnosis (GIST or EGIST) and risk classification in future.

Key words: Gastrointestinal stromal tumor; Rectovaginal septum; Origin.

Introduction
Gastrointestinal stromal tumors (GISTs) are rare tumors of the gastrointestinal (GI) tract that arise from primitive mesenchymal cells. The majority of GISTs are immunohistochemically positive for c-KIT protein (CD117) and CD34. Discovered on GIST-1 (DOG-1), a novel diagnostic marker for GISTs, has a higher sensitivity than CD117, particularly for CD117-negative GISTs with suspicious morphology [1].

GISTs occur throughout the GI tract but are usually located in the stomach and small intestine. Apart from the GI tract, they have been reported in the omentum, mesentery, bladder, and female reproductive organs [2-4]. Occurrence of GISTs in the female genital tract is rare, and have been reported as cases in the literature.

The majority of the published cases report the occurrence of rectovaginal GISTs. Fifteen cases of rectovaginal GISTs have been reported in the English literature including this case. However, the sites of tumor origin have been controversial in these cases. There are no methods to ascertain whether the GISTs originated from the rectum, rectovaginal septum, or vagina. This study reports a case of a rectovaginal GIST in a Korean woman and presents a review of the literature.

Case Report
A 54-year-old woman, gravida 4, para 2, abortion 2, visited the outpatient clinic complaining of a growing vaginal mass. Pelvic examination revealed a baseball-sized hard mass beneath the posterior vaginal wall. The inferior border of the tumor was two cm above the vaginal orifice and three cm below the uterine cervix. The vaginal mucosa on the mass was intact and did not show any erosion or bleeding. On rectal examination, the mass was palpable just above the anal verge. Magnetic resonance imaging (MRI) showed a 6 × 6 × 7 cm-sized mass located between the anterior rectal wall and posterior vaginal wall (rectovaginal space). MRI showed no demonstrable rectal invasion. No local spread and regional lymph node metastasis were detected. Colon fibroscopy revealed narrow intraluminal space by pushing of outside mass lesion. The mucosa was intact and showed no erosion or bleeding (Figure 1).

No abnormal results were obtained on routine laboratory tests. The patient underwent low anterior resection of the rectum, total abdominal hysterectomy, bilateral salpingo-oophorectomy, and partial resection of the posterior vagina.

Initially, the vagina was dissected longitudinally. The tumor was firmly adherent to the vagina. Partial resection of the posterior vagina was performed for clear resection of the tumor. The tumor was also firmly adherent to the anterior rectal wall. The mass was detached from the rectum along with the anterior muscle layer of the rectum. Although there was no rectal mucosal injury, a large defect was observed in the anterior rectal muscular layer. To prevent rectal stricture or leakage after primary suture, low anterior resection of the rectum and end-to-end anastomosis were performed.

Revised manuscript accepted for publication December 10, 2014

Eur. J. Gynaecol. Oncol. · issn: 0392-2936
XXXVI, n. 6, 2015
doi: 10.12892/ejgo2785.2015
During surgery, a vaginal approach did not provide a sufficient surgical field for tumor resection. Therefore, hysterectomy using an abdominal approach was performed for a better surgical field. Tumor rupture occurred during the surgery. Grossly, the tumor measured 7.5 × 5.5 × 5.5 cm. The cut surface of the tumor revealed a light brown-yellow, soft solid mass with necrosis and hemorrhage. Most of the tumor was encapsulated, but the capsule was partially ruptured and was not removed initially because of severe adhesion to the rectum and vagina. After the removal of the main tumor bulk, the remaining capsule was removed. The removed capsule showed GIST lesions.

Microscopically, the tumor consisted of spindle cells with high cellular density and necrosis, and the mitotic count was four to five mitoses per 50 high-power fields (HPF). Diffuse and strong immunohistochemical reactions for CD117 (c-KIT) were observed throughout the tumor. The tumor was partially positive for S-100 and negative for smooth muscle actin. The Ki-labeling index was 4% (Figure 2). Mutational analysis of KIT (exons 9, 11, 13, and 17) in...
Is gastrointestinal stromal tumor (GIST) originating from the rectovaginal septum GIST or extra-GIST (EGIST)? A case report etc.

The primary tumor showed mutations (c.1689A>G [p.Ile563Met]) at exon 11 and (c.1924A>G [p.Lys642Glu]) at exon 13. The mutation at exon 11 is an unclassified mutation that has not been reported to date. The mutation at exon 13 has been reported previously. There were no mutations at exons 9 and 17.

The patient received adjuvant therapy with an oral tyrosine-kinase inhibitor (imatinib) (400 mg/day). A postoperative rectovaginal fistula occurred in the patient, but she is presently healthy without any evidence of recurrence at 26 months after surgery.

Discussion

Previous studies on the prognostic factors of GISTs show that tumor size and mitotic count are the most important of all prognostic factors [5, 6]. Recently, tumor origin site has been considered an important prognostic factor. The relationship between tumor origin site and prognosis of GISTs remains controversial; however, previous studies have described a risk classification system that employs tumor origin site as a prognostic factor.

The National Institute of Health (NIH) and the Armed Forces Institute of Pathology (AFIP) have proposed prognostic criteria for the risk stratification of GISTs [5-9]. Mitotic activity and tumor size are considered as prognostic variables in both criteria, but tumor origin site is considered as a prognostic variable in the AFIP criteria but not in the NIH risk criteria.

Accurate prognostication of GISTs is essential, not only to guide the clinician with regards to the frequency and intensity of postoperative surveillance but also, and more importantly, to enable better selection of tumors for potential adjuvant treatment.

Although GIST recurrence is higher in the small intestine than in the stomach [10], disease prognosis of stomach GISTs varies depending on which area of the stomach they occur in. GIST recurrence is higher in the gastric fundus or gastroesophageal junction/cardia than in the antrum. This difference is considered to be caused by different cell growth and proliferation rates in each part of the stomach [7]. According to previous study, when they have same or similar tumor size and mitotic count, stomach GISTs have a better prognosis than GISTs occurring at other sites [11]. GISTs rarely occur at sites other than the GI tract.

There is a lack of data regarding the prognosis of EGISTs. Several studies show that the prognosis of EGISTs is poorer than that of GISTs. Qi et al. reported that GISTs with different origin sites had significantly different NIH
risk levels and prognoses. Further analysis showed that based on the tumor origin site, the prognoses worsened and the NIH risk levels increased in GISTs arising from the following sites: esophagus, stomach, duodenum, small intestine, and colorectum from lowest to highest. The NIH risk levels and prognoses of GISTs originating from the colorectum are worse than those of GISTs originating from other sites [12]. The majority of the published cases of EGISTs report the occurrence of those arising from the rectovaginal septum. Since 2004, 14 cases of rectovaginal GISTs have been reported in the English literature.

Fregnani et al. suggested that the tumor origin site should be considered for better management and prognosis of rectovaginal GISTs [13].

For GISTs arising in the rectovaginal septum, it is difficult to ascertain whether the tumor origin site is the rectum, rectovaginal septum, or vagina. In other words, it is difficult to classify these tumors as GISTs or EGISTs.

Of 15 reported cases of GISTs including present case, in the rectovaginal septum, only three cases were considered to originate from the rectovaginal septum itself [14-16]. The tumors in other three cases originated from the rectum [17-19]. The tumors in the other three cases from the vagina [20, 21]. In the six remaining cases, tumor origin could not be determined because of the lack of surgical and pathological data and anatomical changes such as severe adhesion [14, 21-23].

In the present case, the authors could not determine the exact tumor origin site by using immunohistochemical analysis because the tumor was firmly adhered to the rectum and vagina. Rectovaginal GISTs are usually large and show severe adhesion and anatomical changes. These anatomical changes make it difficult to determine the origin site. Until now, the trend has been considering the GIST in rectovaginal septum as EGIST rather than GIST. Although a previous study reported that the prognosis of rectal GISTs is worse than that of EGISTs, there is no significant difference between the two disease entities. The survival rates in the two diseases drop sharply compared with GIST in other sites of GI tract [12].

The treatment of choice for GISTs or EGISTs is complete resection and adjuvant chemotherapy with imatinib, a tyrosine-kinase inhibitor. Patients with c-KIT mutation at exon 11 show better response to imatinib than those with mutations at exon 9. Patients with mutations at exon 11 and those with mutations at exon 13 show similar sensitivities to imatinib [24].

Of 14 patients who underwent surgery, eight patients (57%) showed definite tumor rupture or a margin positive for GIST cells (Table 1). These tumor rupture and margin positivity can increase GIST recurrence after surgery.

When soft tissue lesions in the rectovaginal septum appear suspicious, preoperative transvaginal biopsy, immunohistochemical analysis, and histologic confirmation are recommended. After disease confirmation, preparation for complete resection should be undertaken, and the treatment plan should be determined [17].

In conclusion, many of GISTs reported in rectovaginal septum are considered as rectal GIST or none-EGIST. More consideration for the exact origin should be given to the GIST in the rectovaginal septum for the exact diagnosis (GIST or EGIST) and risk classification in future.

References

Is gastrointestinal stromal tumor (GIST) originating from the rectovaginal septum GIST or extra-GIST (EGIST)? A case report etc.


Address reprint requests to:
D.G. HONG. M.D., Ph.D.
Department of Gynecology and Obstetrics,
Kyungpook National University Medical Center,
474 Hakjeongdong, Buk-gu,
Daegu 702-210 (Republic of Korea)
e mail: chssa0220@hanmail.net
Primary melanoma of the vagina: a case report and review of literature

A. Stefanović1,3, J. Jeremić2,3, K. Jeremić1,3, I. Likić1,3, M. Mitrović1,3, J. Stojnić1,3

1 Clinic for Obstetrics and Gynecology, Clinical Center of Serbia, Belgrade
2 Clinic for Burns, Plastic and Reconstructive Surgery, Clinical Center Serbia, Belgrade
3 School of Medicine, Belgrade University, Belgrade (Serbia)

Summary
Primary melanoma of the vagina is a rare and very aggressive tumor with an incidence of only 0.46 per one million women per year and less than 250 cases reported in literature [1]. About 1.6% melanomas appear on the genitals, and 0.3% - 0.8% on the vagina [2-4]. Vaginal melanoma typically appears in the sixth and seventh decades of life and is mostly located in the lower third and anterior wall of the vagina [4]. Melanoma is the second common neoplasm of the vagina, and represents less than 3% of all neoplasms in this region [5,6]. Lesions are usually multifocal and in only 2% cases they presented as amelanotic melanoma [7]. The prognosis of vaginal melanoma is usually very poor due to diagnosing of melanoma in a late stage. The five-year survival rate ranges from 5% - 25% with the prognosis being in correlation with the tumor stage [6]. Currently, because melanoma of the vagina is rare entity, there is a lack of consensus regarding treatment of primary vaginal melanoma [8].

Key words: Melanoma; Vagina; Malignancy.

Introduction
Malignant melanoma of the vagina is a rare and very aggressive tumor with an incidence of 0.46 cases per million women per year and less than 250 cases reported in the literature. Here the authors present a case of a 60-year-old woman, gravida 5, para 5, post-menopausal by 28 years, admitted to the Clinic for Obstetrics and Gynecology, with recurrent vaginal bleeding for the last year and with the complaint of a palpable tumor near the vaginal introitus. The preoperative biopsy revealed melanoma. CT scan did not prove she had distant metastasis. The patient was treated surgically, with wide local excision of a measured lesion and safety margins of two cm. Bilateral inguinal lymphadenectomy was performed. Follow-up five months after initial diagnosis, revealed no evidence of local recurrence or distant metastasis.

Case Report
A 60-year-old woman, gravida 5, para 5, post-menopausal by 28 years, was admitted to the Clinic for Obstetrics and Gynecology with recurrent vaginal bleeding for the last year and complaint of a palpable tumor mass near the vaginal introitus close to distal part of urethra.

Vaginal examination revealed a dark-brown raised, ulcerated lesion sized four x five cm in the lower third of anterior vaginal wall. Bilateral parametria were free and rectum was normal, with no inguinal lymphadenopathy (Figure 1).

The preliminary biopsy of the tumor with immunohistochemical analysis positive for Melan A, HMB45, and S-100, revealed melanoma.

The patient suffered from chronic cardiomyopathy, hypertension, and diabetes mellitus. The patient had noticed weight loss over the past year, but without urinary or bowel complaints. The patient showed no skin lesion suspicious of melanoma.

Preoperative thoracic, pelvic and abdominal CT scan, sonography of abdomen, chest radiography, and urethrocystoscopy were normal. CT scan did not prove she had distant metastasis.

The patient was treated surgically, with wide local excision of four x five cm measured lesion and safety margins of two cm. Bilateral inguinal lymphadenectomy was performed. Follow-up five months after initial diagnosis, revealed no evidence of local recurrence or distant metastasis.

Discussion
Melanomas can arise in any part of the urogenital tract, including vulva and vagina, uterine cervix, urethra, and urinary bladder. Female genital tract accounts for 18% of all mucosal melanomas [9]. Among female genital tract, the
Malignant melanoma is marked by early local recurrence, usually multifocal, fragile, and easily bleed on touch [2]. Depending on the stage of the disease, melanoma has a high rate of recurrence and poor long term survival, especially in neglected cases [2]. Unlike melanoma of the skin that is staged according to American Joint Committee of Cancer (AJCC) classification, the vaginal melanoma can be staged according to the International Federation of Gynecology and Obstetrics (FIGO) criteria. The FIGO staging system is probably not the optimal, because it does not include tumor size, and the study of the lymph glands [13]. Breslow method valid for cutaneous melanomas could be used for early stages. Clark levels is due to the absence of dermal and subcutaneous structures not applicable [14].

The optimal treatment of vaginal melanoma is a subject of debate. Treatment modalities include wide local excision, radical surgery (total vaginectomy with or without vulvectomy and exenteration), and non-surgical treatment (primary radiation therapy, chemotherapy, and immunotherapy) [6, 12, 13]. Surgery is the mainstay of treatment, with more conservative approach, since radical surgery did not show advantage for survival [6]. Due to difficult anatomic location of the tumor, wide local excision is usually considered up to two cm circumferential. There is no effective systemic therapy for aggressive melanoma of the vagina. Melanoma is known to be a radioresistant tumor, and many reports favor surgery over primary radiotherapy. Radiotherapy can provide better local control of the disease, but did not show better overall survival rate. Surgery also provides better clinical outcomes compared to chemotherapy.

Independent of the treatment for vaginal melanoma, overall survival of five years is 0 - 21% [2, 12, 15]. Buchen et al., reported the overall survival rate between 13% - 19% [17]. The size of the tumor is important prognostic factor; tumors more than three cm in diameter localized in the lower third of the vagina is associated with better prognosis and survival around 41 months [12]. In tumors more than three cm in diameter, tumor-free margin surgery is difficult, and survival is around 12 months [17]. Patients with positive lymph nodes have lower median survival compared with negative lymph nodes (7.8 vs. 30 months) [16]. Local excision, radical surgery, radiotherapy, chemotherapy and immune therapy, and combination of these modalities, have been described by different authors, but in neglected cases have a poor outcome. The local recurrence following surgery is reported around 40%, with high risk for distant metastas [12]. Because of rareness of vaginal melanoma, and insufficient knowledge about their pathogenesis and risk factors, there are no well established protocols for staging and treatment of the disease. As there is no consensus regarding treatment, further reports in the literature would be of a great importance.

References


Address reprint requests to:
K. VJEREMIĆ, M.D., PhD
Clinic for Obstetrics and Gynecology
Clinical Center of Serbia,
Višegradska 26, Belgrade (Serbia)
e-mail: jeremick@hotmail.com
REVIEW ARTICLE
Nerve sparing radical hysterectomy in early stage cervical cancer: Latest developments and review of the literature

A. Kavallaris, D. Zygouris, A. Dafopoulos, I. Kalogiannidis, E. Terzakis - Thessaloniki, GREECE

ORIGINAL ARTICLES
Sentinel node mapping with radiotracer alone in vulvar cancer: a five year single-centre experience and literature review

- S. Bogliolo, P. Marchiole, P. Sala, E. Giardina, G. Villa, E. Fulcheri, M. Valenzano Menada - Genoa, ITALY

MicroRNA signatures of platinum-resistance in ovarian cancer

- H-C Ying, H-Y Xu, J. Lv, T-S Ying, Q. Yang - Shenyang, CHINA

Prevalence of endometriosis in epithelial ovarian cancer. Analysis of the associated clinical features and study on molecular mechanisms involved in the possible causality


Laparoscopic ovarian transposition in young women with cervical squamous cell carcinoma treated by primary pelvic irradiation


The prognostic significance of pretreatment [18F]FDG-PET/CT imaging in patients with uterine cervical cancer: preliminary results


The role of mTOR signaling pathway in premalignant and malignant cervical lesions


Effect of lentivirus mediated cyclooxygenase-2 gene shorthairpinRNA on invasiveness of endometrial carcinoma

- Y.T. Xiao, L.M. Luo, R. Zhang - Shanghai, CHINA

Primary peritoneal cancer: study of 14 cases and comparison with epithelial ovarian cancer


Combination therapy of liposomal paclitaxel and cisplatin as neoadjuvant chemotherapy in locally advanced cervical cancer

- Y. Li, X. Wang, J. Li, W. Ding - Guangzhou, CHINA

Pyometra in elderly post-menopausal women: a sign of malignity


Expressions of survivin, P16INK4a, COX-2, and Ki-67 in cervical cancer progression reveal the potential clinical application


Epidemiology of ovarian cancer in North Sardinia, Italy, during the period 1992-2010


Prevalence of human papillomavirus and the correlation of HPV infection with cervical disease in Weihai, China

- L. Yang, S.Z. He, X.Y. Huang, H.N. Liu, J.Y. Tao - Weihai, CHINA

Correlation analysis of hormone receptors and the expressions of HER-2 and Ki-67 in breast cancer


CASE REPORTS
Leiomyosarcoma: a rare malignant transformation of a uterine leiomyoma

Small cell carcinoma of the ovary of the hypercalcemic type (SCCOHT) – case report - J. Lubin, M. Pawalowska, A. Markowska, A. Bielas - Poznań, POLAND


No. 2, March-April

DISTINGUISHED EXPERT SERIES
Principles of reconstruction with tissue expanders as immediate reconstruction after mastectomy for breast cancer - M. Friedrich, S. Krämer, A. Terjung - Krefeld, GERMANY

REVIEW ARTICLE

ORIGINAL ARTICLES
Cyclin E is overexpressed by clear cell carcinomas of the endometrium and is a prognostic indicator of survival - K. Zapiecki, K.J. Manahan, G.A. Miller, J.P. Geisler - Toledo, Ohio, USA

Sensitization of suberoylanilide hydroxamic acid (SAHA) on chemoradiation for human cervical cancer cells and its mechanism - J. Xing, H. Wang, S. Xu, P. Han, D.M. Xin, J.L. Zhou - Shijiazhuang, CHINA

Expression of estrogen receptors in melanoma and sentinel lymph nodes: a “female” clinical entity or a possible treatment modality? - C. Spyropoulos, M. Melachrinou, P. Vasilakos, E. Tzorakoleftherakis - Rion, GREECE

Isolated axillary nodal swelling and cancer of unknown primary - S. Bertozzi, A.P. Londero, R. Petri, S. Bernardi - Udine, ITALY

S100P is a useful marker for differentiation of ovarian mucinous tumors - A. Papa, S. Tomao - Latina, ITALY

Pelvic exenteration – our initial experience in 15 cases - M.E. Căpilna, B. Moldovan, B. Szabo - Braşov, ROMANIA

Correlations of leukemia inhibitory factor and macrophage migration inhibitory factor with endometrial carcinoma - W. Xiao, O. Jin, S. Han, R. Nie, L. Zhu, X. Gao, L. Li - Harbin, CHINA


Disease-free ovarian cancer patients report severe pain and fatigue over time: prospective quality of life assessment in a consecutive series - S. Shinde, T. Wanger, P. Novotny, M. Grudem, A. Jatoi - Rochester, Minnesota, USA

Primary fallopian tube carcinoma - a retrospective analysis of 66 cases - L. Liu, X. Xu, L. Jia, M. Wei, B. Qian, Y. Wu, Y. Shen, X. Wang, H. Pei, X. Chen - Nanjing, CHINA


Metabolomics analysis of cervical cancer: cervical intraepithelial neoplasia and chronic cervicitis by 1H NMR spectroscopy - N. Ye, C. Liu, P. Shi - Beijing, CHINA

Expression of P53A, P53C, and P-gp in epithelial ovarian carcinoma and the clinical significance - X. Lili, T. Xiaoyu - Beijing, CHINA


Evaluation of the Human Papillomavirus mRNA Test for the detection of cervical lesions in Japan - Y. Nakayama, M. Yamada, A. Kurata, H. Kiseki, K. Isaka, M. Kuroda - Tokyo, JAPAN

EXPERIMENTAL RESEARCH
Protective and sensitive effects of melatonin combined with adriamycin on ER+ (estrogen receptor) breast cancer - C. Ma, L.X. Li, Y. Zhang, C. Xiang, T. Ma, Z.Q. Ma, Z.P. Zhang - Shijiazhuang, CHINA

CASE REPORTS
A large ovarian leiomyoma discovered incidentally in a 76-year-old woman: case report - S. Ichigo, H. Takagi, K. Matsunami, T. Murase, T. Ikeda, A. Imai - Gifu, JAPAN
Coexistence of mature cystic teratoma and adenocarcinoma in situ within atypical proliferative mucinous tumour of ovary – a case report of 35-year-old woman - A. Wincewicz, P. Lewitowicz, O. Adamczyk-Gruszka, S. Sulkowski, L. Kanczuga-Koda, M. Koda - Kielce, POLAND ................................................................. 206

Angioleiomyoma of the uterus: report of a distinctive benign leiomyoma variant - A. Zizi-Sermetzoglou, D. Myoteri, E. Arkoumani, K. Koulia, A. Tsavari, E. Alamanou, E. Moustou - Piraeus, GREECE ................................................................. 210

Adenocarcinoma of the cervix associated with a neuroendocrine small cell carcinoma of the cervix in the spectrum of Muir-Torre syndrome - P. Donati, G. Paolino, M. Donati, C. Panetta - Rome, ITALY ......................................................................................... 213


Rectal carcinoma in pregnancy – a case report - G. Karakus, A. Vicente, J. Gameiro, A. Luis, M. Nogueira, J. Mattas - Santarém, PORTUGAL .................................................................................................................. 226


Uterine extra gastrointestinal stromal tumour presenting as intramural leiomyoma - T. Oge, D. Arik, E. Uysal, O.T. Yalçin, S. Kabukcuoglu, S. Ozalp - Eskisehir, TURKEY ........................................................................................................... 231

No. 3, May-June

ORIGINAL ARTICLES

Human papillomavirus combined with cytology and margin status identifies patients at risk for recurrence after conization for high-grade cervical intraepithelial neoplasia - Y. Ruano, M. Torrelos, F.J. Ferrer - Oviedo city, Asturias, SPAIN ........................................................................................................... 245

30 years of preventive studies of uterine cervical cancer 1982–2012 - J.L. Garrido - Panama City, REPUBLIC OF PANAMA ..................................................................................................................................................... 252


Correlation between transcription factor activator protein-2β (TFAP-2β) and endometrial carcinoma - P. Cui, K. Shi, H.X. Cui, L.Y. Hao, Y. Su, P.L. Li - Harbin, CHINA ............................................................................................................... 268

Evaluation of residual tumor locations in advanced ovarian cancer patients after incomplete primary cytoreduction - J.P. Grabowski, M. Mardas, A. Markowska, J. Markowska - Poznan, POLAND ........................................................................................................... 274

A comparative study of intensity-modulated radiotherapy and standard radiation field with concurrent chemotherapy for local advanced cervical cancer - C. Yu, W. Zhu, Y. Ji, J. Guo, P. Pan, J. Han, X. Zhou - Hua’ian, CHINA ........................................................................................................... 278

Identification of potential targets for ovarian cancer treatment by systematic bioinformatics analysis - Q. Ye, L. Lei, A.X. Aili - Shanghai, CHINA ........................................................................................................... 283


The differences of phyllodes and acoustic attenuation in breast lesions diagnosed with Breast Imaging-Reporting and Data System for Ultrasonography (BI-RADS-US) category 4C - Y.Y. Li, C. Liu, J. Geng, J.G. Li, F. Jin, X.M. Wang - Shenyang, CHINA ........................................................................................................... 294

Cancer testis antigen OY-TES-1: analysis of protein expression in ovarian cancer with tissue microarrays - R. Fan, W. Huang, B. Luo, Q.M. Zhang, S.W. Xiao, X.X. Xie - Nanning, Guangxi Zhuang Autonomous Region, CHINA ........................................................................................................... 298

Comparison of nine morphological scoring systems to detect ovarian malignancy - Erhan Aktürk, Murat Dede, Müfit C. Yenen, Y. Kemal Koçyiğit, Ali Ergün - Ankara, TURKEY ........................................................................................................... 304

Cervical squamous cancer mRNA profiles reveal the key genes of metastasis and invasion - Yuan Cheng, Ding Ma, Youyi Zhang, Zijian Li, Li Geng - Beijing, CHINA ........................................................................................................... 309

Comparative study of phosphorylated histone H2AX expressions in the cervical cancer patients of pre- and post-neoadjuvant chemotherapy - J. Zhao, Q. Wang, J. Li, T.B. Si, S.Y. Pei, Z. Guo, C. Jiang - Lanzhou, CHINA ........................................................................................................... 318
CASE REPORTS

Rare case of coexistence of primary ovarian carcinoid in mature teratoma with primary serous carcinoma in second ovary – a case report - E. Mieczkowska, A. Marciniak, I. Szydlowska, A. Brodowska, A. Starczewski - Szczecin, POLAND ........................................ 330

An unusual case of mammary gland-like carcinoma of vulva: case report and review of literature - C. Baykal, I. Dündar, I.Ç. Turkmen, E. Özyar - Istanbul, TURKEY ........................................ 333

Endoscopic surgery combining chemotherapy for vaginal yolk-sac tumor: a case report - X. Fang, W. Du, Q. Wang, X. Zhao - Hangzhou, CHINA ........................................ 335

Leiomyosarcoma of the vagina in pregnancy - B. Bassaw, H. Fletcher, J. Chinnia - Trinidad, WEST INDIES ........................................ 339

Addition of bevacizumab to neoadjuvant chemotherapy for Stage IV ovarian serous adenocarcinoma with multiple lymph node metastases: a case report - H. Liu, Y. Shì, G.N. Zhang, S.Q. Song, T. Hu - Chengdu, CHINA ........................................ 341

Carcinosarcoma in endometrial polyp. Diagnosis and treatment – case report - M. Zamurovic, M. Prorocic - Belgrade, SERBIA ........................................ 346


Late recurrence of ovarian cancer: a literature review and description of two cases - N. Izyczka, J. Lubin, A. Markowska, J. Markowska - Poznan, POLAND ........................................ 351

Granulosa cell tumor presenting with ovarian torsion and de novo borderline mucinous ovarian tumor in the contralateral ovary - S. Ates, O. Sevket, S. Sudolmus, F.C. Sonmez, R. Dansuk - Istanbul, TURKEY ........................................ 354

Rare case of concurrent severe chylous ascites after radical surgery for cervical cancer - Z. Qi, Y. Zhang - Wuxi, CHINA ........................................ 356

DISTINGUISHED EXPERT SERIES

Abridged republication of FIGO’s staging classification for cancer of the ovary, fallopian tube, and peritoneum - J. Prat for the FIGO Committee on Gynecologic Oncology - Barcelona, SPAIN ........................................ 367

ORIGINAL ARTICLES

Correlation of progression-free and post-progression survival with overall survival in phase III trials of first-line chemotherapy for advanced epithelial ovarian cancer - M. Shimokawa, M. Ohki, T. Kaku - Fukuoka, JAPAN ........................................ 370

Prevention, diagnosis and treatment of cervical cancer precursor lesions at the Xingu Indigenous Park, Brazil - E. Ribeiro Pereira, N.M. de Gois Speck, D.A. Rodrigues, V. Grisolia de Freitas, J.C. Lascasas Ribalta - São Paulo, BRAZIL ........................................ 376


Anti-Hsp20 antibody concentrations inversely correlated with tumor progression in ovarian cancer - Yanhui Zhu, Qingchao Tian, Naian Qiao, Yin Cheng, Haiying Li - Jinan, CHINA ........................................ 394


Serum human epididymis protein 4 can be a useful tumor marker in the differential diagnosis of adnexal masses during pregnancy: a pilot study - F. Gucer, G. Kiran, E. Canaz, M. Kilinc, H.C. Ekerbicer, F. Avci, H. Kiran, A. Coskun, D.C. Arikari - Kahramanmaras, TURKEY ........................................ 406
Prevalence and predictors of abnormal Papanicolaou smears in HIV-infected women - M. Vide Tavares, F. Coutinho Nunes, S. Saleiro, F. Mota, I. Torgal - Coimbra, PORTUGAL ................................................................. 410


Biological and pathological features in pregnancy-associated breast cancer: a matched case-control study - S. Baulies, M. Cusió, F. Tresserra, F. Fargas, I. Rodríguez, B. Úbeda, C. Ara, R. Fábregas - Barcelona, SPAIN .................................................. 420


Upregulation of microRNA-224 sensitizes human cervical cells SiHa to paclitaxel - F. Lin, P. Wang, Y. Shen, X. Xie - Hangzhou, CHINA .......................................................... 432


An evaluation comparing Californium252 neutron brachytherapy with neoadjuvant intra-arterial embolism chemotherapy assisted surgery effect for treating advanced cervical carcinoma patients - H. Fei, P. Ke, N. Wang, H. Shen, J. Huang, J. Tan, L. Liang, X. Song - Guangzhou, CHINA .......................................................... 442

Diagnostic accuracy of 1.5 Tesla breast magnetic resonance imaging in the pre-operative assessment of axillary lymph nodes - C. de Felice, V. Cipolla, A. Stagnitti, L.M. Porfliri, D. Guerrieri, A. Musella, D. Santucci, M.L. Meggiorini - Rome, ITALY .......................................................... 447

Calcitriol does not significantly enhance the efficacy of radiation of human cervical tumors in mice - F. Zhang, Y. Yu, S. Song, M. Wang, Y. Ma, L. Xing - Harbin, CHINA .......................................................... 452

Diagnostic performances of CA125, HE4, and ROMA index in ovarian cancer - Z.G. Dikmen, A. Colak, P. Dogan, S. Tuncer, F. Akbiyik - Ankara, TURKEY .......................................................... 457

Expression and prognostic significance of microRNA-451 in human epithelial ovarian cancer - S. Ling, M. Ruiqin, Z. Guohong, W. Ying - Shantou, CHINA .................................................. 463

Effects of cyclopamine on the biological characteristics of human breast cancer MCF-7 cell line and its mechanism - D.M. Zhu, W.L. Xue, W. Tao, J.C. Li - Jinzhou, CHINA .................................................. 469

CASE REPORTS


Guide wire surgery in breast cancer and why to avoid scissors - A. Lafaille, W.A.A. Tjalma - Edegem, BELGIUM ........................................................................ 477

Bilateral salpingo-oophorectomy and adhesiolysis with single port access laparoscopy and use of diode laser in a BRCA carrier - S. Angioni, A. Pontis, F. Sorrentino, L. Nappi - Cagliari, ITALY .......................................................... 479


Paraneoplastic neurological syndromes (PNS) caused by occult breast cancer and metastatic carcinoma of the lymph node - Jingjing Gong, Yan Zhang, Yonghua Huang, Weimin Yin, Weiwei Zhang, Jun Feng, Shijie Wang, Xinhuai Wu - Beijing, CHINA .................................................. 485

No. 5, September-October

REVIEW ARTICLE


ORIGINAL ARTICLES

Syndecan-1 serves as a marker for the progression of epithelial ovarian carcinoma - Q. Guo, X. Yang, Y. Ma, L. Ma - Guangzhou, CHINA .......................................................... 506

Confluence analysis of multiple omics on platinum resistance of ovarian cancer - Xin Hong Ye - Shanghai, CHINA .......................................................... 514
Factors contributing to the low participation rate of Turkish women to a breast cancer screening program in Antwerp, Belgium - F. Topal, S. Van Roosbroeck, G. Van Hal, Y. Jacquemyn - Edegem, Belgium 

Pathologic characteristics and prognosis of a rare advanced cervical cancer treated with radical surgery and radiotherapy - L. Li, L.Y. Li, F.J. Hu, S.Y. Zeng, Z.Q. Qiao - NanChang, China 


Extracellular matrix metalloproteinase inducer (EMMPRIN) remodels the extracellular matrix through enhancing matrix metalloproteinases (MMPs) and inhibiting tissue inhibitors of MMPs expression in HPV-positive cervical cancer cells - Q. Xu, X. Cao, J. Pan, Y. Ye, Y. Xie, N. Ohara, H. Ji - Fuzhou, China 

Association of EBV and HPV co-infection with the development of cervical cancer in ethnic Uyghur women - A. Abudoukadeer, M. Niyazi, A. Aikula, M. Kamilijiang, X. Sulaiman, A. Mutailipu, A. Abudula - Urumqi, China 

Human papillomavirus effect on the development of endometrial polyps - U. Korucuoglu, I. Guler, H. Dogan, A. Biri - Ankara, Turkey 


Human papillomavirus infection among Uyghur women with cervical intraepithelial neoplasia in Xinjiang area - R. Du, Z.F. Chen, X.H. Li, Y. Ding, Y. Zhang - Changsha, China 


DVP parametric imaging for characterizing ovarian masses in contrast-enhanced ultrasound - H. Sha-sha, H. Li, M. Jie, F. Gui, G. Wen-jun, H. Ming, Z. Yang, Y. Qing - Wuhan, China 

Assessment of primary radical hysterectomy and neoadjuvant chemotherapy followed by radical hysterectomy in Stage IB2, IIA bulky cervical cancer - A. Musaev, A.B. Guzel, G. Khatib, U.K. Gulec, M.A. Vardar, A. Altuntas, D. Gumurdulu - Adana, Turkey 

The presence of advanced lesions and associating risk factors for advanced cervical carcinoma in patients with atypical squamous cells of undetermined significance - L.L. Sun, W. Chen, Y.Y. Fan, M.L. Wang, L.N. Wang - Changchun, China 

The construction of cDNA library and the screening of related antigen of ascitic tumor cells of ovarian cancer - Q. Hou, K. Chen, Z. Shan - Zhengzhou, China 

CASE REPORTS

Do we understand the pathophysiology of endometrial cancer? - V.L. Parker, P. Sanderson, D. Raw, K. Farag - Barnsley, United Kingdom 

Endometrial cancer in unicornuate uterus: a case report - L. Cobellis, M.A. Castaldi, V. Frega, L. Mosca, F. Corvino, S. Cappabianca, N. Colacurci - Naples, Italy 


Granular cells tumor of the vulva: an exceptional entity - I. Brunel, E. Moreno-Palacios, J. De Santiago, I. Zapardiel - Madrid, Spain 

Primary malignant melanoma of the cervix: a case report - V. Mihmmani, G. Toprakci, N. Cetinkaya, A. Kilickaya, G. Kamali - Istanbul, Turkey 

Aggressive angiomyxoma of the pelvis: a series of four cases and literature review - Honglin Wu, Wei Liu, Hai Xu, Dehang Wang, Aimei Ouyang - Changzhou, China 

A successful management of a giant mucinous ovarian tumor with intraoperative controlled fluid aspiration - H. Güraslan, L. Yaşar, M. Ekin, C. Kaya, H. Cengiz, M. Gönenç - İstanbul, Turkey 

Carcinosarcoma of the fallopian tube with disappearance of carcinoma cells by neoadjuvant chemotherapy: case study - Y. Takemoto, T. Ota, Y. Aoki, K. Ogura, D. Ogishima, T. Matsumoto - Tokyo, Japan
ORIGINAL ARTICLES

Decreased expression of SIRT6 promotes tumor cell growth and correlates closely with poor prognosis of ovarian cancer - G. Zhang, Z. Liu, S. Qin, K. Li - Jinan city, CHINA .......................................................... 629

Surgical Stage I high-grade ovarian cancer: is adjuvant chemotherapy warranted? - Y. Segev, N. Ismiil, R. McVey, A. Covens - Toronto, Ontario, CANADA ........................................ 633

Which is the appropriate surgical procedure for Stage I endometrial carcinoma? - L. Sun, X.G. Sheng, L. Wei, F. Gao, X. Li, N.F. Liu, D.P. Li, X. Zhang, T.T. Zhang, P. Wei - Jinan, CHINA ........................................ 637

Intraoperative subserosal approach to label sentinel nodes in intermediate and high-risk endometrial cancer - P. Valha, E. Kucera, P. Sak, O. Stepanek, M. Michal - Budejovice a.s., CZECH REPUBLIC ........................................ 643

HPV16 infection up-regulates Piwi2, which affects cell proliferation and invasion in cervical cancer by regulating MMP-9 via the MAPK pathway - W. Ling, H. Zhigang, H. Tian, Z. Bin, X. Xiaolin, Z. Hongxiu - Lanzhou, CHINA ................. 647

Adjuvant treatment with a dialyzable leukocytes extract contributes to maintain HPV-infected women free of low-grade cervical lesions - A. Rodriguez-Flores, G. Nuñez-Fernandez, I. Estrada-Garcia, M. Aguilar-Santelises, O. Rojas-Espinosa, S. Estrada-Parrà - Mexico City, MEXICO ........................................ 655

Cytoplasmic p21 is responsible for paclitaxel resistance in ovarian cancer A2780 cells - X. Xia, T. Ji, R. Liu, Y. Weng, Y. Fang, Z. Wang, H. Xu - Shenzhen, CHINA ........................................ 662

Endometrial adenocarcinoma in young-aged women: a Turkish population study - T. Gungor, N. Cetinkaya, B. Ozdal, H. Yalcin, S. Erkaya, H.I. Yakut - Ankara, TURKEY ........................................ 667

Comparison of whole-body PET/PET-CT and conventional imaging procedures for distant metastasis staging in patients with breast cancer: a meta-analysis - Zhe Sun, Yu Li Yi, Yu Liu, Jian Ping Xiong, Chao Zhu He - Nanchang, CHINA ........................................ 672

Relationship between smoking, HPV infection, and risk of cervical cancer - E. Mazarico, M.D. Gómez-Roig, L. Guirado, N. Lorente, E. Gonzalez-Bosquet - Barcelona, SPAIN ........................................ 677


Protein kinase D1 inhibits breast cancer cell invasion via regulating matrix metalloproteinase expression - X.J. Qin, Z.G. Gao, J.L. Huan, X.F. Pan, L. Zhu - Shanghai, CHINA ........................................ 690


Decreased microRNA-206 and its function in cervical cancer - S. Ling, M. Ruijin, Z. Guohong, S. Bing, C. Yanshan - Shantou, CHINA ........................................ 716

The 16, 18, and 45 HPV infection in high grade squamous cervical lesions in primary hr-HPV test screening program - C. Chiappetta, E. Lendaro, J. Cacciotti, R. Zaralli, C. Puggioni, G. Migliore, V. Petrozza, C. Della Rocca, C. Di Cristofano - Latina, ITALY ........................................ 722


Identification of cervical cancer markers using cDNA subtraction approach - Y. Liu, S.H. Man, X. Liu, X.Y. Ding, W.L. Xiao - Shandong, CHINA ........................................ 730
CASE REPORTS

Safety of converting a radical vaginal trachelectomy to a radical hysterectomy during pregnancy - W.A.A. Tjalma - Antwerp, BELGIUM ................................................................. 734

Diagnostic usefulness of FDG-PET/CT in advanced malignant lymphoma of the uterus: report of two cases - T. Okuda, S. Iijichi, S. Yamashita, T. Yoshioka, H. Nishigaki, J. Kitawaki - Kyoto, JAPAN ......................... 737


Postradiation carcinosarcoma of the corpus uteri – a case report - A. Zwierzchowska, G. Panek, M. Gajewska - Warsaw, POLAND ................................................................. 746

Is gastrointestinal stromal tumor (GIST) originating from the rectovaginal septum GIST or extra-GIST (EGIST)? A case report with literature review - Y.H. Lee, G.O. Chong, D.G. Hong - Daegu, REPUBLIC OF KOREA ............... 750

Index of Authors in alphabetical order

Abudoukadeer A., 546
Abudula A., 546
Adamczyk-Gruza O., 206
Adonakis G., 91
Aguilar-Santelises M., 655
Aikula A., 546
Aili A.X., 283
Akbari M.R., 681
Akbiyik F., 457
Akman L., 150
Aktürk E., 304
Alamanou E., 210
Arlen A.P., 694
Altintas A., 579
Andrikopoulou M., 36
Androoutsopoulos G., 91
Angelopoulou G., 711
Angioni S., 479
Aoki D., 397, 424
Aoki Y., 618
Ara C., 420
Arik D., 231
Arikan D.C., 406
Arkoumanis E., 210
Arsène E., 698
Ates S., 354
Attamante L., 383
Avci E., 406
Aydin A., 348
Balbi G., 742
Banno K., 397
Bassaw B., 339
Baulies S., 420
Baykal C., 333
Bellardini P., 569
Benedetti Panici P., 107
Bernard S., 131
Bertozi S., 131
Bielas A., 88
Bigot J., 698
Bin Z., 647
Bing S., 716
Biri A., 551
Blažičević V., 482
Bleu G., 698
Bogliolo S., 10
Borges C.S., 694
Bouchardy C., 529
Boulanger L., 698
Boutas I., 36
Browoswa A., 330
Brunel I., 605
Budroni M., 69
Cacciotti J., 569, 722
Caioia A., 742
Canaz E., 406
Cao X., 539
Căpîlna M.E., 142, 216, 229
Capobianco G., 69
Cappabianca S., 599
Carta G., 84
Cascales P., 21
Castaldi M.A., 599
Celli C., 59
Cengiz H., 615
Cesaraccio R., 69
Cetin C., 708
Cetinkaya N., 607, 667
Chamarro Laseasas Ribalta J., 437
Chen K., 414, 590
Chen W., 585
Chen X., 161
Chen Y., 25
Chen Z., 25
Chen Z.F., 564
Cheng Y., 309, 394
Chiappetta C., 569, 722
Chinnia J., 339
Chiofalo B., 495
Chiyoda T., 424
Cho S.H., 30
Choi S.K., 389
Chong G.O., 560, 750
Chung H.W., 30
Ciancimino L., 495
Cipolla V., 447
Cobelis L., 599
Colacureci N., 599
Colak A., 457
Colinett P., 698
Colonese E., 495
Colonese F., 495
Corvino F., 599
Coskun A., 406
Cossu A., 69
Costa-Silva D.R., 694
Coutinho Nunes F., 410
Covens A., 633
Creatas G., 36
Cruckshank D.J., 711
Cui H.X., 268
Cui P., 268
Cusidò M., 420
Cvejić M., 685
D’Alfonso A., 84
da Silva B.B., 694
Dafoopoulos A., 5
Danilidis A., 229
Dansuk R., 354
de Felice C., 447
de Gois Speck N.M., 376
De Santiago J., 605
de Sousa G.V., 694
de Souza Bezerra Sakano C.R., 437
Decavallas G., 91
Dede M., 304
Dedet B., 698
Deligecorgolu E., 36
Della Rocca C., 569, 722
Demirtas G.S., 150
Demirtas O., 150
Deng K.X., 554
Dessole M., 69
Dessole S., 69
Di Cristofano C., 569, 722
Di Luigi G., 84
Di Martino L., 742
Di Stefano L., 84
Dikmen Z.G., 457
Ding W., 54
Ding X.Y., 731
Ding Y., 564
Dogan H., 551
Dogan P., 457
Donati M., 213
Donati P., 213
Du R., 564
Du W., 335
Dünder I., 333
Ekbericier H.C., 406
Ekin M., 615
Ergün A., 304
Erkaya S., 667
Estrada-Garcia L., 655
Estrada-Parra S., 655
Etman A., 711
Fábregas R., 420
Falcone F., 742
Fan L., 260
Fan R., 298
Fan Y.Y., 585
Fang J., 326
Fang X., 335
Fang Y., 662
Farag K., 595
Fargas F., 420
Fei H., 442
Feng J., 485
Ferrer J.F., 245
Fioł G., 21
Fletcher H., 339
Frega V., 599
Friedrich M., 103
Fukuda T., 49, 168
Fulcheri E., 10
Fuslo L., 383, 428
Gadducci A., 428
Gajewska B.B., 746
Gameiro J., 226
Gao F., 637
Gao X., 146
Gao Z.G., 690
Garrido J.L., 252, 323
Geeds Y.P., 402
Geiser J.P., 114
Geng B., 186
Geng M., 186
Gençoğlu Bakbak B.B., 59
Genestic C., 473
Geng J., 294
Geng L., 399
Geropoulou E., 91
Giardina E., 10
Girgin B., 348
Gocmen A., 348
Godiris-Petit G., 473
Gómez-Roig M.D., 677
Gönçer M., 615
Gong J., 485
Gonzalez-Bosquet E., 677
Grabowski J.P., 274
Granese R., 495
Grauso F., 742
Grisolia de Freitas V., 376
Grudem M., 155
Gucer F., 406
Guerrieri D., 447
Gui F., 574
Guirado L., 677
Gulec U.K., 579
Guler I., 551
Gumrudulu D., 579
Gungor T., 667
Guo J., 278
Guo L., 414
Guo Q., 506
Guo Z., 318
Guohong Z., 463, 716
Gür E.B., 186
Güraslan H., 615
Guzel A.B., 579
Guzin K., 348
Hamasaki T., 290
Han J., 278
Han P., 117
Han S., 146
Hao L.Y., 268
Hashiguchi Y., 49, 168
Hashimoto S., 424
He C.Z., 672
He S.Z., 73
Higashino T., 290
Hong D.G., 750
Hong S., 30
Hong Y.O., 389
Hongxu Z., 647
Hou Q., 590
Hou W.J., 62
Hu F.J., 524
Hu T., 341, 726
Huan J.L., 690
Huang J., 442
Huang W., 298
Huang X.B., 554
Huang Y., 73
Huang Y., 485
Huber D., 529
Hursitoglu B.S., 150
Ichigo S., 203
Ichimura T., 49, 168
Iguchi Y., 424
Ijichi S., 737
Ikeda T., 203
Imai A., 203
Imai K., 49
Isaka K., 192
Ishiko O., 168
Ismiil N., 633
Ishoshishi F., 290
Ito M., 138
Iiyozokurt C., 708
Izuka N., 351
Endometrial Cancer: Current Epidemiology, Detection and Management
Samir A. Farghaly

Endometrial cancer is a neoplasia that continues to increase in developed countries with also high socio-economic and healthcare standards. Although it does not have a high mortality rate compared to other female neoplasias, its development nonetheless continues to pose a threat to women’s life. A textbook that discusses this topic can be a practical aid in the correct first diagnosis and approach that will inevitably have an impact on the progression of the neoplasia. The textbook presented by the Author includes all of the knowledge insights of endometrial cancer appropriate to those dedicated to gynaecological oncology. The topics covered are linked with the epidemiology, diagnosis, and management of endometrial cancer. Furthermore, as can be deduced from the contents of each chapter, all the aspects of this neoplasia have been considered and treated with a didactic approach, which can be handy to both the experts and to those beginning their training in gynaecologic oncology.

CONTENTS
Chapter 1: Epidemiology of Endometrial Cancer. Authors: Anjum Memon and Priyamvada Paudyal.
Chapter 4: Endometrial Endometrioid Adenocarcinoma: Histology, Precursors and Molecular Alterations. Authors: Helena Hwang, Kara Duncan and Paulette Mhawech-Fauceglia.
Chapter 5: Hereditary Endometrial Cancer. Authors: Agnieszka Rychlik, Giselle Steinberg, Alicia Hernandez, Maria D. Diestro, Javier De Santiago and Ignacio Zapardiel.
Chapter 6: Chapter 6 - Lynch Syndrome and Endometrial Cancer. Authors: Carmen Guillén-Ponce and Maria-José Molina-Garrido.
Chapter 7: Chapter 7 - Sentinel Lymph Nodes in Endometrial Cancer. Authors: Helena Robova and Lukas Rob.
Chapter 8: Chapter 8 - Lymphatic Mapping for Endometrial Cancer. Author: Valerio Mais.
Chapter 9: Chapter 9 - Detection of Sentinel Lymph Node in Endometrial Cancer. Authors: Sambor Sawicki and Dariusz Wydra.
Chapter 11: Chapter 11 - Surgical Modalities for Treating Patients with Endometrial Cancer. Author: Samir A. Farghaly.
Chapter 13: Clinical Trials Evidence for Efficacy of Postoperative Chemotherapy for Early Endometrial Cancer. Author: Nick Johnson.
Chapter 14: Chapter 14 - Medical Treatment of Endometrial Cancer. Authors: Antonella Venturino, Giuseppe Colloca and Gianfranco Carfagna.
Chapter 15: Chemotherapeutic Agents for Patients with Endometrial Cancer. Authors: Sirewan Tangjitgamol and John Kavanagh.
Chapter 16: Novel Therapeutic Agents for Treatment of Endometrial Cancer. Authors: Kouji Banno, Mihoko Iida, Samir A. Farghaly, Kiyoko Umene, Iori Kisu and Daisuke Aoki.
Chapter 17: Targeted Therapies for Endometrial Cancer. Authors: Selen Dogan, Nasuh Utku Dogan and Kubra Boyukalin.
Chapter 18: ErbB Targeted Therapy in Endometrial Cancer. Authors: Georgios Androustopoulos, Georgios Adonakis and Georgios Decavalas.
Chapter 19: Radiotherapy Treatment for Endometrial Cancer. Authors: G. Eminowicz and M. McCormack.

The present textbook is able to transmit an in-depth knowledge of all the aspects of this tumor and can be appreciated for its contribution in improving women’s health.

European Academy of Gynaecological Cancer, EAGC

Chairman: Péter Bősze (Hungary)

Executive Board:
PIERLUIGI BENEDETTI PANICI (Italy)
CARLOS F. DE OLIVEIRA (Portugal)
GIUSEPPE DE PALO (Italy)
SANTIAGO DEXEUS (Spain)
WILLIAM DUNLOP (UK)
STELIOS FOTIOU (Greece)
GERALD GITSCH (Austria)
A. PETER M. HEINTZ (Netherlands)
MICHAEL HOECKEL (Germany)
JAN JACOBS (UK)
JACQUES LANSAC (France)
TIZIANO MAGGINO (Italy)
HARALD MEDEN (Germany)
JOSEPH MONSONEGO (France)
LASZLO PÁLFALVI (Hungary)
SERGIO PECORELLI (Italy)
DENIS QUELLEU (France)
STELIO RAKAR (Slovenia)
PIERO SISMONDI (Italy)
CLAES TROPÉ (Norway)
LÁSZLÓ UNGÁR (Hungary)
ANDRÉ VAN ASSCHE (Belgium)
RAIMUND WINTER (Austria)

International Advisory Board
Chairman: Antonio Onnis (Italy)
HUGH ALLEN (Canada)
CURT W. BURGER (Netherlands)
ALBERTO COSTA (Italy)
ANDRÉ GORINS (France)
NEVILLE F. HACKER (Australia)
MARIA MARCHETTI (Italy)
STELIOS P. MICHALAS (Greece)
MARIA TERESA OSORIO (Portugal)
ULF ULMSTEN (Sweden)
JAN B. VERMORKEN (Belgium)
GEORGE D. WILBANKS (USA)
JAN ZIELINSKI (Poland)

All questions concerning the Academy may be sent to:
PETER BOSZE, M.D. - P.O. Box 46 - Budapest 1301 (Hungary)
Phone: +36 1 4290317 - Fax: +36 1 2752172 - E-mail: eagc@cme.hu

www.cme.hu

Administrative Office:
1301 Budapest, P.O. Box 46 - Hungary
Fax (36 1) 4290318 - E-mail: eagc@cme.hu
Clinical and Experimental Obstetrics & Gynecology

Subscription Order Card 2016

Founded in 1974 (ISSN 0390-6663) - Vol. XLIII. Issued bimonthly. All subscriptions are entered on a calendar-year basis. Individual rate is not applicable if payment is made through an Institution.

Subscriptions are entered with prepayment only and are accepted per calendar year only but can be backdated depending on availability. If not cancelled by the end of October, they will be tacitly considered as renewed; cancellations will not be refunded.

Discounts: 10% to book sellers and subscription agencies.

Please enter my subscription at the rate I have checked:

**PAPER ISSUE**

- Institutional: 700 USD
- Individual: 500 USD
- Single copy: 150 USD

**ONLINE ISSUE**

- Institutional: 450 USD
- Individual: 270 USD
- Single issue: 100 USD
- Single article: 30 USD

Payment: (USD ONLY)

- for PDF file: online through PayPal (all credit cards)
- for hard copy

Credit Card:  

- Mastercard  
- Visa  
- Diners

Bank transfer:  

Beneficiary: 7847050 Canada Inc. - 4900 Côte St-Luc, #212 - Montréal, Québec, H3W 2H3 Canada - Account number 00001 003402-402245 SWIFT ROYCCAT2

N° __________________________ Exp. Date __________________________

Signature __________________________ Date __________________________

An invoice is issued only after payment is processed; no proforma receipts will be issued. The subscription order form is available through the Montréal office (Fax +1-514-485-4513) or Padua office (Fax +39-049-8752018) or through our website www.irog.net

---

EUROPEAN JOURNAL OF GYNAECOLOGICAL ONCOLOGY

Subscription Order Card 2016

Founded in 1980 (ISSN 0392-2936) - Vol. XXXVII. Issued bimonthly. All subscriptions are entered on a calendar-year basis. Individual rate is not applicable if payment is made through an Institution.

Subscriptions are entered with prepayment only and are accepted per calendar year only but can be backdated depending on availability. If not cancelled by the end of October, they will be tacitly considered as renewed; cancellations will not be refunded.

Discounts: 10% to book sellers and subscription agencies.

Please enter my subscription at the rate I have checked:

**PAPER ISSUE**

- Institutional: 700 USD
- Individual: 500 USD
- Single copy: 150 USD

**ONLINE ISSUE**

- Institutional: 450 USD
- Individual: 270 USD
- Single issue: 100 USD
- Single article: 30 USD

Payment: (USD ONLY)

- for PDF file: online through PayPal (all credit cards)
- for hard copy

Credit Card:  

- Mastercard  
- Visa  
- Diners

Bank transfer:  

Beneficiary: 7847050 Canada Inc. - 4900 Côte St-Luc, #212 - Montréal, Québec, H3W 2H3 Canada - Account number 00001 003402-402245 SWIFT ROYCCAT2

N° __________________________ Exp. Date __________________________

Signature __________________________ Date __________________________

An invoice is issued only after payment is processed; no proforma receipts will be issued. The subscription order form is available through the Montréal office (Fax +1-514-485-4513) or Padua office (Fax +39-049-8752018) or through our website www.irog.net