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 Gynaecologic 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).
Principles of reconstruction with tissue expanders as immediate reconstruction after mastectomy for breast cancer

M. Friedrich, S. Krämer, A. Terjung - Krefeld, Germany

The aims of reconstruction with expanders are to restore the breast shape and volume as close as possible to the contralateral breast and to reconstruct the inframammary fold with adequate ptosis.

Fertility drugs and breast cancer risk

G. Lo Russo, F. Tomao, G.P. Spinelli, A.A. Prete, V. Stati, P. Benedetti Panici, A. Papa, S. Tomao - Latina, Italy

Nerve sparing radical hysterectomy is a suitable technique for gynaecologist-oncologist familiar with the method in early-stage cervical cancer.

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

Cyclin E expression is related to clear cell histology and to decreased survival in patients with endometrial cancer.

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 - Tangshan, China

SAHA promotes SiHa apoptosis in chemotherapy through up-regulation of mRNA and protein of p21 and Bax, which leads to cell cycle arrest in G0/G1 phase. Low dose of SAHA promotes SiHa apoptosis and inhibits cell repair in radiotherapy through Bax up-regulation and Ku70 down-regulation.

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

Estrogen receptor beta expression in melanomas and sentinel lymph nodes during metastatic process is altered.

Isolated axillary nodal swelling and cancer of unknown primary

S. Bertozzi, A.P. Londero, R. Petri, S. Bernardi - Udine, Italy

Occult breast cancers behave very similarly to Stage IV overt breast cancers, and should be treated accordingly.

S100P is a useful marker for differentiation of ovarian mucinous tumors

Y. Umezaki, M. Ito, M. Nakashima, Y. Mihara, Y. Naruke, H. Kurohama, N. Yatsunami, I. Yasuhi - Nagasaki, Japan

S100P as a histological marker for differentiating among benign, borderline, and malignant ovarian mucinous tumors.
Pelvic exenteration – our initial experience in 15 cases
M.E. Căpîlna, B. Moldovan, B. Szabo - Braşov, ROMANIA
Patient selection, indications, surgical technique, and complication of pelvic exenteration are analyzed.

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
In endometrial carcinoma and in endometrial hyperplasia, the macrophage migration leukemia inhibitory factors are significantly higher than in normal endometrium.

Clinical significance of ASCUS and ASC-H cytological abnormalities: a six-year experience at a single center
G.S. Demirtas, L. Akman, O. Demirtas, B.S. Hursitoglu, M.C. Terek, O. Zekioglu, H. Yilmaz, A.A. Ozsaran - Izmir, TURKEY
The colposcopic biopsy results in patients with cervical cytology abnormalities are evaluated.

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
Long-term quality of life of disease-free patients after ovarian cancer treatment is examined also in relation to chemotherapy administration.

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
The management of 66 cases of primary fallopian tube carcinoma is retrospectively analyzed.

Chemotherapy-induced thrombocytopenia and clinical bleeding in patients with gynecologic malignancy
Estimating risk factor of clinical bleeding seems to be important for safe management of chemotherapy-induced thrombocytopenia.

Metabolomics analysis of cervical cancer, cervical intraepithelial neoplasia and chronic cervicitis by 1H NMR spectroscopy
N. Ye, C. Liu, P. Shi - Beijing, CHINA
The potential biomarkers for good discrimination between cancer and non-cancer groups can be identified by Hydrogen-1 nuclear magnetic resonance and partial least-squares discriminant analysis.

Expression of PKCa, PKCe, and P-gp in epithelial ovarian carcinoma and the clinical significance
X. Lili, T. Xiaoyu - Beijing, CHINA
The presence of PKC alfa, PKC epsilon, and P-gp in the tissue of epithelial ovarian cancer indicates that they play an important role in the generation of chemoresistant drugs.

A diagnostic dilemma for solid ovarian masses: the clinical and radiological aspects with differential diagnosis of 23 cases
M. Genç, A. Solak, B. Genç, O.N. Sivrikoz, S. Kurtulmuş, A. Turan, N. Şahin, E.B. Gür - Izmir, TURKEY
Clinical characteristics, differential diagnosis, and diagnosis features of ovarian solid masses are analyzed.

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
The Human Papillomavirus mRNA test APTIMA is useful for the detection of high-risk HPV in cervical cytology specimen.

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
Melatonin could enhance the sensitivity of tumors to adriamycin in vivo and improve patient's quality life.
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
A large ovarian leiomyoma, with a problem of differential diagnosis of a malignant solid tumor in an elderly woman is described.

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
A case of ovarian combined tumours is described.

Angioleiomyoma of the uterus: report of a distinctive benign leiomyoma variant
A. Zizi-Sermpetzoglou, D. Myoteri, E. Arkoumani, K. Koulia, A. Tsavari, E. Alamanou, E. Moustou - Piraeus, GREECE
A case of angioleiomyoma of the uterus that poses a problem for preoperative differential diagnosis with angiosarcoma is presented.

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
A case of a 52-year-old female with a positive familial history of Muir-Torre syndrome is presented.

Three synchronous primary pelvic cancers – a case report
M.E. Căpîlna, S.C. Rusu, C. Laczko, B. Szabo, C. Marian - Târgu-Mureș, ROMANIA
The management of three simultaneous pelvic cancers in a menopausal woman is discussed.

Metastases of renal clear cell carcinoma to ovary - case report and review of the literature
M. Kostrzewa, M. Żyła, J. Władziński, T. Stetkiewicz, G. Stachowiak, J.R. Wilczyński - Lodz, POLAND
A rare case of ovarian metastases from renal clear cell carcinoma is reported together with literature review.

Spindle-cell epithelioma of the vagina diagnosed during pregnancy - a case report
S. Pantovic, A. Stefanovic, J. Stojnic, K. Jeremic, R. Sparic, S. Kadija, S. Milenkovic - Belgrade, SERBIA
An unusual and intriguing case of vaginal spindle-cell epithelioma diagnosed during pregnancy is reported.

Rectal carcinoma in pregnancy – a case report
G. Karakus, A. Vicente, J. Gameiro, A. Luis, M. Nogueira, J. Matias - Santarém, PORTUGAL
The management of a case of a pregnant woman suffering from colorectal cancer is discussed.

Spontaneous intrauterine pregnancy following abdominal radical trachelectomy - a case report
M.E. Căpîlna, S.C. Rusu, C.I. Puia, A. Daniilidis, B. Szabo - Târgu-Mureș, ROMANIA
Abdominal radical trachelectomy offers a safe outcome in early-stage cervical cancer and spares fertility as in this case.

Uterine extra gastrointestinal stromal tumor presenting as intramural leiomyoma
T. Oge, D. Arik, E. Uysal, O.T. Yalçın, S. Kabukcuoglu, S. Ozalp - Eskişehir, TURKEY
A unique case of gastrointestinal stromal tumor mimicking a uterine intramural leiomyoma is described.
Introduction

Tissue expanders are regarded as a simple method for immediate breast reconstruction following mastectomy [1, 2]. However, to achieve a satisfying cosmetic result and avoid complications associated with the procedure, several technical aspects and a careful selection of patients are required [3-6].

After the completion of mastectomy, a tissue expander is inserted under a muscular pocket and then inflated to expand the dermo-muscular layer. When the expansion is completed, the device is changed to a permanent implant.

Today textured surface anatomical breast-shaped expanders are used or permanent expander implants (adjustable saline filled inner volume, silicone gel outer volume), which avoid the need of an exchange of the expander to a permanent implant [7-9].

Indication

Immediate reconstruction with expanders is recommended in patients: with small or moderate-sized breast with minimal or no ptosis; who prefer minimal scarring and no additional donor-site morbidity; with no prior radiation/ no postoperative radiation planned; who are not worried about a silicone implant; who are unwilling or unfit to undergo autologous tissue reconstruction; and undergoing bilateral reconstruction (and meet the criteria mentioned above).

In most patients a contralateral mastopexy / reduction is required for symmetry.

Contraindication

Reconstruction with expanders is not recommended in patients: with poor quality of the soft tissue coverage (skin and muscle) does not allow expansion; with large/ptotic breasts; with prior radiation or radiation planned after surgery; that are obese/with large chest wall diameters; with low patient compliance for the expansion process; with unrealistic cosmetic expectations; that are young (relative contraindication for more re-operations may be required during life-span).

Materials and Methods

Preoperative drawings

The type mastectomy and the amount of skin to be excised should be determined prior to surgery. Skin-sparing techniques are preferred as they leave a skin envelope which aids in reconstruction. Skin very close to or infiltrated by the tumor is excised and narrow skin bridges should be avoided to ensure adequate blood supply of the skin flaps.

The central midline, the vertical breast axis, and both inframammary folds are outlined with an additional line on the mastectomy side at one cm lower than the existing fold. The shape and contour of the new breast is outlined in accordance to the contralateral breast. (Fig-
ure 1). Base width and height of the contralateral breast are measured and transferred to the tumor side (the markings may also be drawn using manufacturer’s templates).

**Surgery**

After completion of the (skin-sparing) mastectomy, the viability of the skin flaps and the integrity of the pectoralis major muscle are assessed. (Figure 2A). Nonviable skin is excised and lesions in the muscle are fixed before insertion of the expander.

**Insertion of the expander**

The lateral border of the pectoralis major muscle is incised (Figure 2B) and a subpectoral pocket is created with the pectoralis major muscle released medially from the third intercostal space down and inferiorly (Figure 2C). The inferior part of the dissection is either subcutaneous or includes the anterior rectus sheath which is then elevated in continuity with the pectoralis major muscle. When total muscular coverage is planned, the serratus anterior muscle is elevated from the chest wall to provide lateral coverage (Figure 2D, 2E).

The size and type of expander used depend on the width and height of the contralateral breast and the volume may be estimated by the weight of the mastectomy specimen. The size of the expander is smaller when a contralateral breast reduction is planned or larger in case of a large or ptotic breast.

The expander is completely evacuated of air using a butterfly needle (Figure 2F). It is then partially inflated with sterile saline (may contain methylene blue to assure puncturing the expansion chamber during the expansion process) to assure that there is no leakage. About 50 cc of saline are left within the expander and this aids in implant insertion. The prosthesis is brought in the submuscular pocket and oriented with the assistance of markers on the implant surface (Figure 2G).

Drains are placed in the submuscular pocket and subcutaneously. The submuscular pocket is closed suturing the serratus and pectoralis muscle with interrupted vicryl 3-0 sutures, which are pre-inserted before placement of the prosthesis to minimize risk of perforation of the implant by the needle (Figure 2H). Interrupted vicryl 4-0 sutures can be used for adapting the subcutaneous tissues and monocryl 4-0 for non-interrupted intracutaneous sutures. A bandage may then be used for three weeks to keep the expander in place.

**Expander inflation**

The expander may be inflated immediately with saline (100 – 300 cc) depending on the quality of the soft tissue coverage. Expansion begins two to three weeks following surgery but depends on the skin flap viability and wound healing. The expander is gradually inflated with saline (100 – 300 cc) depending on the quality of the soft tissue coverage. Expansion begins two to three weeks following surgery but depends on the skin flap viability and wound healing.
expanded using magnetic port locators with 50 – 100 cc of saline every two to three weeks until the desired volume is reached (Figures 3A–C). Usually the expander is slightly overexpanded to gain more tissue for creating a more natural ptosis.

**Expander to implant exchange**

Expansion is maintained for two to six months. In case that a permanent expander implant has been used, the volume is adjusted according to the contralateral breast by aspirating saline. The fill tube is removed later under local anaesthesia.

In case of a temporary expander, the patient is placed in a sitting position and saline is aspirated from the expander until symmetry to the contralateral breast is reached (Figure 4A). The expander is removed through the previous incision (Figure 4B) and a capsulotomy (either circumferential or only in the inferior pole, with or without radial incisions) is performed to release tension and enlarge the pocket (Figure 4C). The volume of the permanent implant is chosen according to the expander volume after symmetry has been reached or may be tested with sizers. Whether to choose an anatomic or a round shaped implant depends on the shape and the upper pole fullness of the contralateral breast (Figure 4D).

A drain is placed into the pocket and the incisions are closed. Final symmetry is evaluated in a sitting position (Figure 4E). A bandage may be used for three weeks to avoid cranial displacement of the implant. The reconstruction of the nipple-areola complex is performed three to six months later.
Results

Early complications of tissue expansion are skin necrosis with wound dehiscence and implant extrusion. In case the viability of the skin flaps is in doubt, the expansion process should be delayed and any nonviable tissue should be excised early to allow secondary wound healing. The expansion is commenced no earlier than wound healing is completed and viability of mastectomy flaps is secured.

Conclusions

Complete muscular coverage of the expander reduces the risk for expander extrusion in case of wound infection or wound dehiscence. In case of ptosis of the contralateral breast, overexpansion is needed to achieve an acceptable ptosis. If necessary the mobilisation of the lower part can be extended downwards to the rectus sheath to gain an excess amount of skin which is used to create a submammary fold. When the expansion is finished, the excess amount of skin is fixed to the muscle fascia. Another possibility is to overexpand and exchange the expander to a slightly smaller implant.

In large or ptotic breasts, a skin-reducing mastectomy or a mastectomy by a vertical elliptical incision is done to reduce the amount of skin. Suction drains are left until drainage is less than 20 cc for two consecutive days. This avoids seromas which are related to a higher risk for capsular fibrosis.

Most women require a contralateral mastopexy/reduction for symmetrization. Concomitant chemotherapy may negatively influence the expansions process.

References

Infertility, defined as the inability to conceive, or to get pregnant, within one year of regular sexual activity, with the same partner and without any contraceptive use, affects between 9% and 20% of couples in Western countries [1-3]. Many new drugs and techniques against infertility are now available or under investigation, but their side effects are not still completely known. Many authors suggest the hypothesis of a correlation between these drugs and cancer development [4, 5]. Fertility drugs, new reproductive techniques, and new fertility preservation strategies are increasingly investigated and used in patients with breast cancer, one of the most common tumors in younger women still in reproductive age [6, 7]. It is well known that one of the most important etiological agents for the development of breast cancer is the proliferative activity of endogenous and exogenous female hormones [8, 9]. Furthermore the use of hormone replacement therapy or hormonal contraceptives, could play an important role in the development of breast cancer [10, 11] as well as various other hormonal factors such as younger age at menarche, older age at menopause, postmenopausal obesity, late age at first birth, and nulliparity [12-16]. Endogenous and exogenous hormones drive cell proliferation. Fertility drugs stimulate ovulation and increase endogenous progesterone and estrogen levels acting on ovarian and breast tissues. During proliferation, cells can accumulate random DNA mutations and give rise to cancer [17]. Several studies evaluated the impact of fertility medications and techniques on breast cancer risk [18-21]. The aim of the present work was to clarify the possible link between infertility, exposure to ovarian stimulation drugs, and the occurrence of breast cancer.

Materials and Methods

The authors performed a review of the current literature regarding the possible association between the use of fertility drugs and the enhanced risk of breast cancer. They searched digital databases including Pubmed, EMBASE, and the Cochrane Library. The literature search was performed using various combinations of keywords. They carefully analyzed only the full versions of all relevant studies. Results: Using various combination of keywords, the authors examined 930 papers. They considered only papers written in English. With these criteria they selected the studies that had been discussed in detail on the text. Conclusion: None of the works commented provides an indisputable evidence about a link between ovarian stimulation and breast cancer risk. On the contrary, most of them actually suggest a lack of interaction between them or even a protective role of ovarian stimulation.

Key words: In vitro fertilization; Clomiphene citrate; Fertility drugs; Infertility treatment; Breast cancer risk.
time of follow-up, and on the presence of confounding factors in the studies.

Results

Using various combination of keywords, the authors examined 930 papers. They considered only papers written in English. In this work they did not include all case reports and all the articles with a low sample size. Moreover other works were excluded according to the title and to the content of the abstract. With these criteria they selected the studies that have been discussed.

Clomiphene citrate and other fertility drugs

Clomiphene citrate is a selective estrogen receptor modulator (SERM) that increases the production of gonadotropin releasing hormone (GnRH). It is used to treat infertility in women who have ovulatory disorders. The use of clomiphene citrate and other fertility drugs has been associated with breast cancer risk in some studies. However, the evidence is not consistent across all studies and the results are often confounded by other factors such as age, family history, and other medical conditions.

Table 1. — Breast cancer risk and fertility drugs.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year of publication</th>
<th>Population</th>
<th>Treatments</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bernstein et al. [30]</td>
<td>1995</td>
<td>774 breast cancer cases, 744 controls</td>
<td>hCG</td>
<td>OR: 0.77, 95% [0.50-1.19] DR</td>
</tr>
<tr>
<td>Braga et al. [31]</td>
<td>1996</td>
<td>2,569 breast cancer cases, 2,588 controls</td>
<td>Defined as use of fertility drugs</td>
<td>OR: 1.08 [0.80-1.50] exposed vs unexposed. NIR</td>
</tr>
<tr>
<td>Rossing et al. [32]</td>
<td>1996</td>
<td>3,837 infertile women, 27 breast cancer cases</td>
<td>Clomiphene, hCG</td>
<td>RR= 0.50 [0.20-1.2] exposed vs unexposed DR associated with clomiphene</td>
</tr>
<tr>
<td>Modan et al. [33]</td>
<td>1998</td>
<td>2,496 infertile women, 59 breast cancer cases</td>
<td>Clomiphene, clomiphene-hMG, hMG</td>
<td>SIR in infertile women vs general population: 1.3 [1.00-1.60]. NIR</td>
</tr>
<tr>
<td>Potashnik et al. [34]</td>
<td>1999</td>
<td>1,197 women, 20 breast cancer cases</td>
<td>Defined as use of fertility drugs</td>
<td>SIR exposed 1.65 [0.94-2.68], SIR unexposed 0.80 [0.21-2.04]. NIR</td>
</tr>
<tr>
<td>Ricci et al. [35]</td>
<td>1999</td>
<td>3,415 breast cancer cases, 2,916 controls</td>
<td>Defined as use of fertility drugs</td>
<td>OR: 0.8 [0.50-1.30] exposed vs unexposed NIR</td>
</tr>
<tr>
<td>Doyle et al. [36]</td>
<td>2002</td>
<td>5,556 infertile women, 55 breast cancer cases</td>
<td>Defined as use of fertility drugs</td>
<td>OR: 1.16 [0.84-1.56], SIR unexposed 1.15 [0.57-2.05]. NIR</td>
</tr>
<tr>
<td>Burkman et al. [37]</td>
<td>2003</td>
<td>4,575 breast cancer cases, 4,682 controls</td>
<td>Clomiphene, hCG, hMG, other drugs</td>
<td>Increased RR = 2.7; [1.00-6.9] associated with use of hMG &gt; 6 cycles</td>
</tr>
<tr>
<td>Brinton et al. [38]</td>
<td>2004</td>
<td>12,193 women evaluated for infertility, 292 breast cancer cases</td>
<td>Clomiphene, Gonadotropins</td>
<td>SIR in infertile women 1.29 [1.1-1.4] Clomiphene &gt; 20 years follow-up Increased RR for invasive breast cancer: 1.6 [1.0-2.5]</td>
</tr>
<tr>
<td>Gauthier et al. [39]</td>
<td>2004</td>
<td>92555 women, 6602 treated, 183 breast cancer cases</td>
<td>Clomiphene, chorionic gonadotropin, menotropin</td>
<td>RR = 0.95 [0.82-1.11] exposed vs unexposed. NIR</td>
</tr>
<tr>
<td>Terry et al. [40]</td>
<td>2006</td>
<td>116,671 women responded to a questionnaire about their medical histories, 61 breast cancer cases</td>
<td>Clomiphene</td>
<td>RR = 0.60 [0.42-0.85] exposed vs unexposed. DR associated with clomiphene</td>
</tr>
<tr>
<td>Lerner-Geva et al. [41]</td>
<td>2006</td>
<td>5,788 women, 131 breast cancer cases</td>
<td>Clomiphene, clomiphene-hMG, hMG</td>
<td>RR= 1.11 [0.79-1.57] exposed vs unexposed Increased RR: 1.49; [1.15-1.93] associated with clomiphene exposure</td>
</tr>
<tr>
<td>Jensen et al. [42]</td>
<td>2007</td>
<td>331 breast cancer cases, 1,226 controls</td>
<td>Clomiphene, gonadotropin, hCG, GnRH, progesterone</td>
<td>RR= 1.08 [0.83-1.39] exposed vs unexposed Increased RR = 3.36; [1.3-8.6] associated with progesterone exposure</td>
</tr>
<tr>
<td>Kosopoulos et al. [43]</td>
<td>2008</td>
<td>1380 women with BRCA1-BRCA2 mutation</td>
<td>4% exposed to clomiphene and gonadotropin</td>
<td>OR = 1.21 [0.81-1.82] exposed vs unexposed. NIR</td>
</tr>
<tr>
<td>dos Santos Silva et al. [44]</td>
<td>2009</td>
<td>7,355 women, 174 breast cancer cases</td>
<td>43% ovarian stimulation treatments</td>
<td>SIR exposed 1.26 [1.03-1.53], SIR unexposed 0.99 [0.78-1.25]. NIR</td>
</tr>
<tr>
<td>Calderon-Margalit et al. [45]</td>
<td>2009</td>
<td>15,030 parous women, 530 breast cancer cases</td>
<td>Clomiphene, hMG, other fertility drugs</td>
<td>Increased RR = 1.65 [1.15-2.36] exposed vs unexposed</td>
</tr>
<tr>
<td>Orgéas et al. [46]</td>
<td>2009</td>
<td>1,135 infertile women, 54 breast cancer cases</td>
<td>Clomiphene, hCG, hMG, FSH</td>
<td>SIR = 3.00 [1.35-6.67] for women with non ovulatory infertility who received &gt; 4 cycles of clomiphene</td>
</tr>
<tr>
<td>Fei et al. [47]</td>
<td>2012</td>
<td>1,422 women with breast cancer + 1,669 women breast cancer free; 288 women treated with fertility drugs</td>
<td>Clomiphene, FSH</td>
<td>Overall: non-statistically significantly DR of breast cancer, OR= 0.82; [0.63 - 1.08]</td>
</tr>
<tr>
<td>Lerner-Geva et al. [48]</td>
<td>2012</td>
<td>2431 women treated for infertility, 153 breast cancer cases, 30 years of follow-up</td>
<td>Gonadotropins</td>
<td>SIR = 1.16 [0.98-1.36]. NIR associated with exposure to gonadotropins</td>
</tr>
</tbody>
</table>

Abbreviations: hCG: human chorionic gonadotropin; OR: odds ratio; DR: decreased risk; NIR: not increased risk; RR relative risk; hMG: human menopausal gonadotropin; SIR standardized incidence ratio; GnRH gonadotropin-releasing hormone; FSH: follicle-stimulating hormone.
nadotropins by inhibiting negative feedback on the hypothalamus [22]. This drug is in use since the 1960s and is still considered one of the most important starting treatment for the majority of women with infertility [23]. Gonadotropins are commonly used drugs in female infertility treatment and several associations among these different agents have been tested. For example, gonadotropin-releasing hormone (GnRH) analogues/agonists, progesterone or human chorionic gonadotrophin (hCG) are used as single agents or in combination with clomiphene citrate [24-29]. In recent years many studies have been published with the aim to investigate the relationship between breast cancer, infertility, and ovarian stimulation procedure made with these drugs [30-48]. The present authors will discuss more in detail some of these studies in the text. All studies are also described in detail in Table 1.

In the study of Calderon-Margalit et al. [45] an increased risk of breast cancer was observed in women exposed to clomiphene citrate. However, this risk occurred only among women who waited more than 12 months to conceive, and no association was found among primiparous patients. On the contrary in the prospective study of Terry et al. [40] an analysis limited to patient with infertility caused by ovulatory disorders, found a significant reduction in breast cancer incidence with clomiphene citrate use with the greater risk reduction among women who were subjected to treatment with clomiphene citrate for more than ten months. Brinton et al. [38] in a retrospective cohort study enrolled 12,193 women, who had been treated with clomiphene citrate or gonadotropins. This study did not find a significant increase in risk for clomiphene citrate use, although in a very large cohort. A slight increase in risk was documented in case of high dosage gonadotropins. For both drugs, statistically significant risk was found only when follow-up had been prolonged for more than 20 years. In the study of Lerner-Geva et al. [41] an increased risk of breast cancer was reported in a retrospective analysis of 5,788 patients, with an hazard ratio (HR) of 1.49 for developing breast cancer with clomiphene citrate. However, this association was limited to women with very severe infertility, resistant to other treatments. In the study conducted by Fei et al. [47] the authors analyzed women that used clomiphene citrate or follicular stimulating hormone (FSH). The results showed an overall non-statistically significantly decreased risk of breast cancer, [Odds Ratio (OR): 0.82; 95% CI = 0.63 - 1.08]. Women who used fertility drugs and conceived a 10+ week pregnancy showed a statistically significantly increased risk of breast cancer compared with unsuccessfully treated women (OR: 1.82; 95% CI = 1.10 - 3.00), although their risk was not increased compared with women who had not used fertility drugs (OR:1.13; 95% CI = 0.78 - 1.64). In a subgroup analysis of this study, women who used fertility drugs but had not obtained a 10+ week pregnancy showed a statistically significantly decreased risk of breast cancer (OR: 0.62; 95% CI = 0.43 - 0.89). In a cohort study Jensen et al. [42] enrolled 54,362 women divided in five fertility treatment groups (gonadotropins, clomiphene citrate, hCG, GnRH, and progesterone). The authors identified 331 invasive breast cancers during follow-up. The results showed no increased breast cancer risk associated with fertility drugs treatment. However, a four-fold increased risk of breast cancer was found after exposure to progesterone. The use of gonadotropins have a stronger effect on breast cancer risk among nulliparous women and similar risk patterns were present for ductal, lobular, and tumors of other histologies. In the study by Bernstein et al. [30] the results suggest that hCG may be an instrument for reducing breast cancer risk; 744 patients with newly diagnosed breast cancer and 744 controls were enrolled. Forty-five cases and 65 controls reported exposure to hCG. The OR were reduced substantially for both nulliparous and parous women but only the result for nulliparous women was statistically significant (p < 0.05). In their study Burkman et al. [37] compared patients with breast cancer to healthy controls. In general, they did not find an overall increased risk to develop breast cancer in association with the use of ovulation induction drugs. However women using hMG for ≥ six months or for at least six cycles had a relative risk (RR) of breast cancer ranging between 2.7 to 3.8.

The study of Lerner-Geva et al. [48] evaluated the possible risk for cancer development in infertile women over 30 years of follow-up in a cohort of 2,431 women who were treated for infertility at the Sheba Medical Center, in Israel, during the period 1964-1974. Standardized incidence ratios (SIR) were calculated between the observed cancer cases and the expected cancer rates in the general population. For breast cancer, 153 cases were observed as compared to 131.9 expected (SIR = 1.16; 95% CI = 0.98-1.36). No excess risk associated with exposure to gonadotropins was observed. Infertility was found to be associated with a borderline increased risk for breast cancer.

Most of the results from these and other studies were collected from the meta-analysis by Zreik et al. [19]. The populations included women who were treated for infertility with clomiphene citrate and other various fertility agents. Eight case-control studies and fifteen cohort studies were included in the analyses and the authors concluded that the available data do not suggest higher risk of breast cancer in women who receive fertility treatments.

**In vitro fertilization (IVF)**

Another important application of these drugs (clomiphene citrate, gonadotropins, gonadotropin releasing hormones, and other new fertility agents) concerns their association with IVF. IVF is a technique that allows fertilization of eggs by sperm outside the body. Many studies evaluated the association between breast cancer risk and IVF and most of these have not demonstrated changes in the rate of breast cancer [49-61]. All studies are described in detail in Table 2. Only the most relevant studies will be discussed in the text.
In their study Brzezinski et al. [49] demonstrated a two-fold increase in the rate of breast cancer in women who were subjected to IVF compared to the general population.

Venn et al. [50] in a study that enrolled 29,700 patients, observed an increased risk of breast cancer in women who underwent IVF, but this risk was seen only within one year from last treatment. Moreover Pappo et al. [57] in a more recent study in which 3,375 women were enrolled, found that age ≥ 40 years at IVF treatment, hormonal infertility, and ≥ four IVF cycles, were actually connected with an increased risk for breast cancer compared to the general population.

A study conducted by Katz et al. [56], in which 7,162 women were enrolled, documented that age over 30 at the time of first IVF treatment, was the only parameter significantly associated with IR. RR: 1.24 [1.03-1.48] \( p = 0.02 \).

Pappo et al. [57] in 2008, 3,375 treated women, 35 breast cancer cases observed that age ≥ 40 years at IVF treatment, hormonal infertility SIR: 3.1 [0.99–7.22]; and ≥ 4 IVF cycles SIR: 2.0 [1.15–3.27].

Källen et al. [58] 2011 24,058 treated women, 91 breast cancer cases observed that age over 30 at the time of first IVF treatment, was the only parameter significantly associated with IR. RR: 0.76 [0.62-0.94] exposed vs unexposed DR

Stewart et al. [59] 2012 21,025 treated women observed no overall increase in the rate of breast cancer; HR: 1.10 [0.88-1.36]

Yli-Kuha et al. [60] 2012 18,350 patients, 9,175 IVF women, 55 breast cancer in exposed patients, 115 in general population observed that age over 30 at the time of first IVF treatment was the only parameter significantly associated with increased breast cancer risk.

In their study Yli-Kuha et al. [60] enrolled 9,175 women who purchased drugs for IVF, 55 breast cancer were recorded in exposed patient and 115 in general population (cohort sizes: 18,350 patients). This work showed that IVF women had a slightly fewer risk of breast cancer but this difference was not statistically significant.

In a very recent retrospective cohort study, Brinton et al. [61] analyzed 87,403 women who underwent IVF or were treated for infertility: 522 of them developed a breast cancer during the period of observation. No significant relationships between IVF exposure and breast cancer risk was found. Another recent cohort study, conducted by Stewart et al. [59], did not find an overall increase in the rate of breast cancer in women treated with IVF, though an increased rate in women who commenced IVF at a young age was observed. Instead risk was not increased in women who commenced treatment at age 40 and required IVF.

Two studies [55, 58] investigated on the possible protective effect of a pregnancy after IVF on treated women. In the study conducted by Kristiansson et al. [55], pregnancy after IVF really seemed to be linked to a reduction of breast cancer risk, even if this advantage was seen only in regards to in situ breast cancer. Nevertheless, in the study conducted by Kallen et al. [58], 24,058 women were enrolled: an overall reduction of breast cancer risk could be seen in

<table>
<thead>
<tr>
<th>Study</th>
<th>Year of publication</th>
<th>Population</th>
<th>Treatments</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brzezinski et al. [49]</td>
<td>1994</td>
<td>950 women, 16 breast cancer cases</td>
<td>IVF</td>
<td>Twofold increase in the rate of breast cancer in women who were subjected to IVF compared to the general population</td>
</tr>
<tr>
<td>Venn et al. [50]</td>
<td>1995</td>
<td>10,358 women, 5564 exposed, 34 breast cancer cases</td>
<td>IVF</td>
<td>SIR exposed 0.88 [0.55-1.46], SIR unexposed 0.98 [0.62-1.56]. RR: 1.11[0.56-2.20] exposed vs unexposed. NIR</td>
</tr>
<tr>
<td>Venn et al. [52]</td>
<td>1999</td>
<td>29,700 women, 20,656 exposed, 143 breast cancer cases</td>
<td>IVF</td>
<td>SIR exposed 0.91 [0.74-1.13], SIR unexposed 0.95 [0.73-1.23]. IR within one year of last IVF treatment SIR 2.0 [1.20–3.10].</td>
</tr>
<tr>
<td>Lerner-Geva et al. [54]</td>
<td>2003</td>
<td>Retrospective cohort of 1,082 women, 5 breast cancer cases</td>
<td>IVF</td>
<td>SIR exposed 1.02 [0.33–2.39] NIR</td>
</tr>
<tr>
<td>Kristiansson et al. [55]</td>
<td>2007</td>
<td>647,704 women, 24 breast cancer cases exposed</td>
<td>IVF</td>
<td>SIR exposed 4.31 [2.89-6.43], SIR unexposed 4.12 [3.97-4.27]. RR: 0.93 [0.48-1.43] exposed vs unexposed</td>
</tr>
<tr>
<td>Katz et al. [56]</td>
<td>2008</td>
<td>7,162 treated women, 28 breast cancer cases</td>
<td>IVF</td>
<td>Age over 30 at the time of first IVF treatment, was the only parameter significantly associated with IR. RR: 1.24 [1.03-1.48] ( p = 0.02 ).</td>
</tr>
<tr>
<td>Pappo et al. [57]</td>
<td>2008</td>
<td>3,375 treated women, 35 breast cancer cases</td>
<td>IVF</td>
<td>SIR in exposed 1.4 [0.98-1.96]. IR associated with: Age ≥ 40 at IVF treatment SIR: 1.9 [0.97–3.30]; hormonal infertility SIR: 3.1 [0.99–7.22]; and ≥ 4 IVF cycles SIR: 2.0 [1.15–3.27].</td>
</tr>
<tr>
<td>Källen et al. [58]</td>
<td>2011</td>
<td>24,058 treated women, 91 breast cancer cases</td>
<td>IVF</td>
<td>RR: 0.76 [0.62-0.94] exposed vs unexposed DR</td>
</tr>
<tr>
<td>Stewart et al. [59]</td>
<td>2012</td>
<td>21,025 treated women</td>
<td>IVF</td>
<td>No overall increase in the rate of breast cancer; HR: 1.10 [0.88-1.36]</td>
</tr>
<tr>
<td>Yli-Kuha et al. [60]</td>
<td>2012</td>
<td>18,350 patients, 9,175 IVF women, 55 breast cancer in exposed patients, 115 in general population</td>
<td>IVF</td>
<td>IVF women had a slightly fewer risk of breast cancer but this difference was not statistically significant</td>
</tr>
<tr>
<td>Brinton et al. [61]</td>
<td>2013</td>
<td>87,403 women, 522 breast cancer</td>
<td>IVF</td>
<td>NIR of breast cancer</td>
</tr>
</tbody>
</table>

Abbreviations: IVF: in vitro fertilization; SIR standardized incidence ratio; RR relative risk; NIR: not increased risk; IR: increased risk; DR: decreased risk; HR: hazard ratio.
women who had a pregnancy after IVF, but this reduction was stronger if the women experienced a multiple birth ($p = 0.04$). This study found a reduction in breast cancer risk also among women who were 30 years or older at birth.

Regarding this topic, two meta-analysis have been recently published by Sergentanis et al. [20] and by Li et al. [21]. The first of two [20] included eight cohort studies, for a total of 1,554,332 women; 14,961 of these women received diagnosis of breast cancer, among which 576 underwent IVF. This is probably the largest study about this matter. This work found no increase of breast cancer risk in women who received IVF. Although all the limits can be subjective (short follow up periods, not satisfactory adjustment of confounding factors and others), this meta-analysis has a notable statistical strength, and can be considered, in the present authors’ opinion, a model for future studies in this field. The second meta-analysis [21] included eight cohort studies involving 746,455 participants. The overall combined RR for women with IVF treatment were 0.99 (95% CI, 0.74-1.32) for all-site cancer, 1.59 (95% CI, 1.24-2.03) for ovarian cancer, 0.89 (95% CI, 0.79-1.01) for breast cancer, and 1.07 (95% CI, 0.45-2.55) for cervical cancer. A beneficial effect was shown in the subgroup of breast cancer meta-analysis compared with women who gave birth (RR, 0.79; 95% CI, 0.65-0.95). This meta-analysis suggests that there is no significant association between IVF and cancer risk. A possible beneficial effect was shown in the subgroup of breast cancer meta-analysis.

Discussion

At present infertility is a very important problem, causing a rise in health and social costs. About 45% of the causes of this disorder are to be found in female illness (malformative, infective, endocrine, autoimmune or psychological) [1-3]. Ovarian stimulating drugs use is associated with dramatic and impressive increase in estradiol (E2) levels and the role of endogenous and exogenous female hormones in the development of breast tumors, due to their proliferative and oncogenic activity, is well established [9-11]. In fact it is known that abnormal exposure to estrogens and other ovarian stimulating agents may facilitate malignant activation of cell cycle regulatory proto-oncogenes in breast tissue, suggesting a direct association between infertility drugs and breast cancer onset [12-14]. However, despite the extensive use of fertility drugs and the large number of papers published on the topic, the impact of fertility treatments on breast cancer risk is still under investigation.

It is undeniable that all these works often give contrasting or even opposite results. In the past, some of them suggested an increased breast cancer risk in fertility drugs users [34,3 7]. On the contrary, other authors hypothesized a protective role of fertility medications on breast cancer [32, 40]. It can be due to the fact that many of these studies are affected by bias. In fact, investigations in this field suffer from some methodological limitations. Many studies present very small sample size, so that statistical significance could not be reached. Some other studies were based on imprecise information about clinical data of the patients enrolled. In addition, a lot of studies used SIR as statistical parameter to analyze breast cancer risk in infertile women. SIR is not a reliable parameter because it compares the number of breast cancer observed in infertile women with the number of expected breast cancer cases in general population, without considering all those factors influencing cancer risk probably present in these two groups of patients.

Finally it is not clear if there is a difference between certain subgroups of patients, for example between women who were treated for infertility but remained nulliparous and women who received fertility drugs but became pregnant [62].

In conclusion the present authors can say that, despite the controversies that still remain open, none of the studies analyzed provides an indisputable evidence about a link between ovarian stimulation and breast cancer risk. On the contrary, most of them actually suggest a lack of interaction or even a protective role of ovarian stimulation on breast cancer risk, as underlined in the three recent available meta-analysis [19-21].

Nevertheless this issue deserves further clarifications, not just to give an answer for a mere scientific curiosity, but also in order to give the possibility to all women to have a child, without making them feeling as they had to choose between satisfying this desire and running into an increased risk of breast cancer.

Acknowledgments

The authors want to thank the Director of Distretto ASL 1, Dr. Belardino Rossi for his contribution in the study development.

References


Address reprint requests to:
F. TOMAO M.D.,
Department of Gynaecology and Obstetrics,
University of Rome "Sapienza"
Viale Regina Elena 324, 00161, Rome (Italy)
e-mail: federica.tomao@uniroma1.it
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
Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of Toledo College of Medicine and Life Sciences, Toledo, Ohio (USA)

Summary
Objective: Upregulation of cyclin E and cyclin D1-6 accelerates the transition from G1 to S phase. The objective of this study was to determine if cyclin D1 and E are prognostic indicators in endometrial cancer. Materials and Methods: Surgically-treated patients with endometrial carcinoma had their tumors stained for nuclear expression of cyclin D1 and E. Quantification of staining and measurement of growth phase fraction were performed using image analysis. FIGO stage, grade, and histology were also analyzed. Results: Cyclin D1 and E expression was unrelated to DNA index (p = 0.93). While cyclin D1 expression did not correlate with S+G2M phase fraction (p = 0.69), increased cyclin E expression was directly correlated with increased S+G2M phase fraction (p = 0.002). Cyclin E expression was highest in clear cell carcinomas (p = 0.042) while cyclin D1 expression was highest in adenosquamous carcinomas (p = 0.028). Patients dying from cancer had significantly higher expression of cyclin D1 (p = 0.042) and E (p = 0.02) as compared to patients surviving their disease. Multivariate logistic regression revealed FIGO stage, grade, and lack of cyclin E overexpression to be independent prognostic indicators of survival. Conclusion: Cyclin E expression is related to increased growth fraction, clear cell histology, and decreased survival in patients with endometrial cancer.

Key words: Cyclin D; Cyclin E; Cell cycle; Clear cell carcinoma; Endometrial cancer.

Introduction
Endometrial cancer is the most common gynecologic malignancy in the Western World. It is predicted that in the United States there will be over 42,160 new cases this year and 7,780 resulting deaths [1]. The majority of endometrial cancer is endometrioid type I which is responsible for 70-80% of cases. Type I is observed as often being preceded by hyperplastic endometrium [2], occurring at younger age, and expressing hormone receptors. It is correlated with a favorable prognosis. The five-year survival rate of properly treated patients is nearly 90% [3]. Type II endometrial cancer often arises from the background of atrophic endometrium and usually occurs at in older patients. The patients are five to ten years older than type I [2]. Although both Type I and II can be endometrioid endometrial cancer, Type II usually does not have estrogen and progesterone receptors. Non-endometrioid types are typically more aggressive and present a poorer prognosis.

The purpose of this paper was to determine whether either cyclin D1 or E immunohistochemical overexpression is predictive of changes in survival in women with endometrial cancer and to see if their overexpression is associated with a certain histologic type.

Materials and Methods
The primary tumors from 222 patients treated with primary surgery were stained immunohistochemically for cyclin D1 and cyclin E. All patients underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and bilateral pelvic and paraortic lymphadenectomy.

Frozen specimen of endometrial cancer were obtained from surgical sections and stored at -80°C centigrade. Frozen sections were cut five-μm thick. Slides were immediately fixed in neutral buffered formalin for 30 minutes and then rinsed with tris-HCl buffer pH 7.6. The endogenous peroxidases were then blocked with 1.5% hydrogen peroxide/methanol for three minutes. Staining was performed according to published protocols [4]. Image analysis and measurement of percent positive nuclear area (PPNA) staining of both cyclins was completed according to previously published protocols by the authors [5]. DNA index and cell phase analysis was performed according to previously published methods by the authors [5-7].

Statistics were performed using SPSS for Windows version 9.0. Statistical tests included Student’s t-test, log-rank test, multivariate logistic regression, Kaplan-Meier analysis, and χ² test. For Kaplan-Meier analysis, patients who did not die from endometrial cancer were treated as survivors with those dying from other causes being censored observations.
Results

Median follow-up of the 222 patients was 95 months (mean 90 months). Figure 1 depicts the various histologies encompassed by the study’s patient population. The most common was endometrioid (165) with papillary serous next (21). Most patients had FIGO Stage I tumors (144). However, Table 1 demonstrates that the second highest stage of patients was III (54). Table 1 further shows that advanced disease (Stage III and IV tumors) have higher expression of cyclins E and D1 than lower stage tumors.

Tables 2 and 3 depict the relationships among histology and cyclin staining. Cyclin E expression was highest in clear cell carcinomas of the endometrium ($p = 0.042$) while cyclin D1 expression was highest in adenosquamous carcinomas ($p = 0.028$).

Cyclin D1 and E expression was unrelated to DNA index ($p = 0.93$). While cyclin D1 expression did not correlate with S+G2M phase fraction ($p = 0.69$), increased cyclin E expression was directly correlated with increased S+G2M phase fraction (as determined by flow cytometry) ($p = 0.002$).

Table 4 depicts mean PPNA staining for cyclin D1 and E. As shown in the Table, patients dying from endometrial cancer had significantly higher mean expression of cyclin D1 ($p = 0.042$) and E ($p = 0.02$) as compared to patients surviving their disease. Multivariate logistic regression analysis revealed FIGO stage, grade, and cyclin E expression to be independent prognostic indicators of survival (Table 5).

Figure 2 shows Kaplan-Meier analysis of survival by cyclin E staining for all histologies of endometrial cancer. As shown in the figure, patients whose tumors did not overexpress cyclin E have a better percentage survival at 60 months than those patients whose tumors over expressed cyclin E.

Discussion

Type I and Type II endometrial cancers have shown differences in clinical factor (symptoms, age, prognosis) and molecular factors (p53, PTEN) [8]. Reid-Nicholson et al. described the immunophenotypes of endometrial cancer as diverse, suggesting the immunohistochemistry can be used to determine the type of tumor (I or II) [9]. Geisler et al. demonstrated that gene expression differences differentiated clear cell tumor from serous and endometrioid tumors [6]. In the current study, cyclin E expression in clear cell carcinomas was greater than double the nearest other cell type (serous). Yasmeen et al. and Spruck et al. described cyclin E as a critical factor for G1/S transition [10, 11]. The overexpression is associated with proliferation and chromosomal instability may result in a more aggressive type of cancer. Cyclin E is the marker for the cell cycle’s point of no return, the passing from the resting state to the division cycle [12]. The overexpression of cyclin E may show the inability to stop the dividing process. The instability of the cyclin E/Cdk2 kinase activity may be partially responsible for the instability of the karyotype [13]. The unstable, unbalancing of proteins, is described as a initiating event in carcinogenesis [14].

The current study also has shown that the survival for the patients with CCE was very poor indicating the aggressiveness of the tumor. Cyclin E was described as a powerful predictor of the prognosis in early stage breast cancer and also for as a marker for the aggressiveness [15]. All the information above verifies the finding of the poor survival rate in comparison to the other types of the endometrial cancer and Cyclin E’s possible role in it.

In contrast, cyclin D1 overexpression was statistically high in adenosquamous carcinomas; its expression was not an independent prognostic factor. This may be true lack of

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cyclin E mean PPNA*</th>
<th>p value</th>
<th>Cyclin D1 mean PPNA*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n = 144)</td>
<td>6.6</td>
<td>0.012</td>
<td>4.3</td>
<td>0.013</td>
</tr>
<tr>
<td>II (n = 15)</td>
<td>8.4</td>
<td></td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>III (n = 54)</td>
<td>12.9</td>
<td></td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>IV (n = 9)</td>
<td>13.7</td>
<td></td>
<td>17.5</td>
<td></td>
</tr>
</tbody>
</table>

* Univariate analysis; PPNA = percent positive nuclear antigen.

<table>
<thead>
<tr>
<th>Histology</th>
<th>Cyclin D1 PPNA*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Papillary serous</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>3.8</td>
<td>0.028</td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

* Univariate analysis; PPNA = percent positive nuclear area.

<table>
<thead>
<tr>
<th>Histology</th>
<th>Cyclin E (% positive nuclear area)*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>Papillary serous</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>17.8</td>
<td>0.042</td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>6.5</td>
<td></td>
</tr>
</tbody>
</table>

* Univariate analysis.

<table>
<thead>
<tr>
<th>Factor</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO stage</td>
<td>0.0001</td>
</tr>
<tr>
<td>Histologic grade</td>
<td>0.039</td>
</tr>
<tr>
<td>Histology</td>
<td>0.65</td>
</tr>
<tr>
<td>Lymphovascular space invasion</td>
<td>0.18</td>
</tr>
<tr>
<td>Depth of myometrial involvement</td>
<td>0.51</td>
</tr>
<tr>
<td>Cyclin E</td>
<td>0.015</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Cyclin E is overexpressed by clear cell carcinomas of the endometrium and is a prognostic indicator of survival

significance or may be an artifact due to the low number of adenosquamous tumors compared to other histologic types. Cyclin D1 overexpression is described as not sufficient to drive oncogenic transformation but to introduce oncogenic events [14, 16]. Tashiro et al. showed a shorting of the G1 phase with the overexpression of cyclin D1 [17].

Conclusion

As this study has shown, cyclin E expression in clear cell tumors is nearly double the expression seen in any other endometrium cancer cell type. Raised expression of cyclin E is related to increased growth fraction and decreased survival in patients with endometrial cancer. This identification of cyclin E as a prognostic factor may also provide a future therapeutic target provided that a difference between tumor and non-tumor associated cyclin E can be clarified.

References


Address reprint requests to:
J.P. GEISLER, M.D.
University of Toledo College of Medicine and Life Sciences, Division of Gynecologic Oncology
3125 Transverse Drive
Mail Stop 1194
Toledo, OH 43614 (USA)
e-mail: john.geisler@utoledo.edu
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
Department of Gynecology and Obstetrics, Affiliated Hospital of Hebei Union University, Tangshan (China)

Summary
Purpose: To explore the sensitization of suberoylanilide hydroxamic acid (SAHA) on chemoradiation for cervical cancer cells and its mechanism. Materials and Methods: After human cervical cancer SiHa cells were treated with SAHA and cisplatin (DDP) of different concentrations, inhibition and apoptosis rates, and cell cycle were detected. SiHa cells underwent radiation of various doses after treated with 20% IC50 of SAHA for 24 hours. The survival fraction of SiHa cells was calculated by colony-forming assay, and related parameters were calculated. mRNA and protein expressions of P21, Bax and Ku70 were detected. Results: The inhibition rate was higher in SD (SAHA combined with DDP) group than in D (DDP alone) group ($p < 0.05$). The number of cells in G0/G1 phase was higher, and the number of cells in G2/M+S phase and PI (proliferation index) were lower in S (SAHA), D, and SD groups than in control group, and in SD group than in S and D groups ($p < 0.05$). The apoptosis rate and the expressions of mRNA and protein of Bax and P21 were higher in SD group than in S or D group ($p < 0.05$). The cell survival fraction was lower in SAHA combined with radiotherapy group than in radiotherapy alone group ($p < 0.05$). Do, N, and Dq values were 2.329, 2.761, and 1.721, respectively, in radiotherapy alone group and 1.213, 4.770, and 0.823, respectively, in SAHA combined with radiotherapy group. SER was 1.92. Bax mRNA and protein expressions were higher but Ku70 mRNA and protein expressions were lower in SAHA combined with radiotherapy group than in radiotherapy alone group ($p < 0.05$). Conclusion: SAHA promotes SiHa apoptosis in chemotherapy through up-regulation of mRNA and protein of p21 and Bax which leads to cell cycle arrest in G0/G1 phase. Low dose of SAHA promotes SiHa apoptosis and inhibits cell repair in radiotherapy through Bax up-regulation and Ku70 down-regulation.

Key words: SAHA; DDP; SiHa cell; Apoptosis; Sensitization; Bax; P21; Ku70.

Introduction
The incidence of cervical cancer takes the second place in cancer in women [1, 2]. Its morbidity and mortality are growing in recent years [3]. At present, radiotherapy and surgery are mainly used in the treatment of cervical cancer, supplemented by chemotherapy. Radiotherapy is one of main methods for the treatment of cervical cancer. However, except killing tumor cells, radiotherapy also produces damage on normal tissues such as the bladder and intestinal canal, therefore the radiologic dose is limited. In recent years, more and more attention has been paid to chemotherapy for cervical cancer. Drug resistance is common in the chemotherapy of tumor, and chemotherapeutics not only kill tumor cells, but also produce damage on normal cells [4-6]. The key to improve the therapeutic effects of chemoradiotherapy is to increase the sensitivity of tumor tissue to radiation and chemotherapeutics under the condition that the doses of radiation and chemotherapeutics are not increased.

Histone deacetylase inhibitor (HDACi), a kind of antitumor drug, causes serious concern due to its characteristics of high efficiency and low toxicity [7]. It does not only inhibit tumor cell migration, invasion and metastasis, and the generation of tumor blood and lymph vessels, but also sensitizes chemoradiotherapy [8]. Suberoylanilide hydroxamic acid (SAHA), a kind of HDACi, has proved to be effective for many tumors and has been applied in phase II clinical trial [9, 10]. It has strong specificity and selectivity with fewer side effects. Little research has been done in the application of SAHA combined with radiotherapy or chemotherapy in the treatment of cervical cancer. In this study, the authors explored the inhibitory effects of SAHA combined with cisplatin (DDP) or radiotherapy on human cervical cancer SiHa cells and its potential mechanisms, providing new ideas for the chemoradiotherapy of cervical cancer.

Materials and Methods
Cell culture
Cells were incubated in high glucose-DMEM medium containing 100 U/ml of penicillin, 100 U/ml of streptomycin, and 10% of fetal bovine serum in an atmosphere of 5% CO$_2$ at 37°C. The cells in log phase growth were used for future experiments.
Effects of SAHA, DDP or SAHA combined with DDP on SiHa cell proliferation detected with MTT assay

The cells (5×10⁴/ml, 100 µl per well) were seeded in 96-well plate for incubation in an atmosphere of 5% CO₂ at 37°C. When cells were adherent, different-concentration DDP (1, 2, 4, 6, 8, and 10 µg/ml) and SAHA (0.25, 0.5, 1, 2, 4, and 6 µmol/l) were respectively added for 12, 24, and 36 hours, respectively. In combination group, SAHA (1, 2, and 4 µmol/l) and DDP (1, 2, and 4 µg/ml) were respectively added for 24 hours. There were four wells for each dose. Control group were not administered any drugs. Blank control group had only culture solution. Optical density (OD) value for each well was measured with a microplate reader. The inhibition rate of cell proliferation (%) = (OD value of control group-OD value of experimental group) / OD value of control group×100%. The effects of DDP combined with SAHA were evaluated with the method described by Cao [7].

Cell cycle measured with flow cytometer

There were four groups including control group without any drugs; S groups in which cells were treated with 1, 2 and 4 µmol/l of SAHA, respectively, for 24 hours; D group in which cells were treated with 1, 2, and 4 µg/ml of DDP, respectively, for 24 hours; and SD group in which cells were treated with 1.2, and 4 µmol/l of SAHA for 24 hours, and then 1, 2, and 4 µg/ml of DDP were respectively added. Cells were centrifuged at 800-1,000 r/min for five minutes to get rid of supernatant. After washed three times with PBS, about 1×10⁶ cells were prepared.

Apoptosis rates determined with flow cytometry

There were four groups including group A in which cells were respectively treated with 1, 2, and 4 µmol/l of SAHA for 24 hours; group B in which cells were respectively treated with 1, 2, and 4 µg/ml of DDP for 24 hours; group C in which cells were first treated with 1, 2, and 4 mol/l of SAHA for 24 hours, and then with 2 µg/ml of DDP for 24 hours; group D in which cells were first treated with 2 mol/l of SAHA for 24 hours, and then with 1, 2, and 4 µg/ml of DDP, respectively, for 24 hours. Apoptosis rates were determined with PI/AnnexinV-FITC double labeling method. FITC-/PI- was regarded as living cells, FITC+/PI- as apoptotic cells, FITC+/PI+ as necrotic cells and FITC-+PI+ as mechanical injured cells. Testing was performed in triplicate for each group.

SiHa cells treated with SAHA combined with radiation

There were four groups including control group in which nothing was done, SAHA group in which cells were treated with SAHA of different concentrations (0.5, 1, 2, 4, 6, and 8 umol/l) for 24 hours, irradiation group in which cells underwent irradiation of various doses (2, 4, 6, and 8 Gy), combination group in which cells underwent irradiation of various doses (2, 4, 6, and 8 Gy) after treated with 20% IC50 of SAHA (0.96 µmol/l) for 24 hours. After irradiation, cells were digested with 0.25% of trypsin, and then counted after trypsin blue staining. Cells were seeded in 60-mm culture dish. After culture, cells were washed with PBS twice, fixed with 100% of methanol for 30 minutes, stained with crystal violet for 15 minutes, washed with double distilled water three times, followed by counting the number of colonies containing more than 50 cells. The survival fraction (SF) was calculated based on the following formulas: colony forming efficiency = the number of colony formation / the number of seeded cells ×100%, SF = the number of colonies in each group / the number of seeded cells in this group × colony forming efficiency). Based on the single-hit multi-target (SHMT): S = 1 - (1-eDo/D) n, Dq = Do-logN, the radiological dose-survival curve was drawn. And then the extrapolation number (N), mean lethal dose (Dq), quasi-threshold (Dq) and sensitization enhancement ratio (SER) was calculated (SER = Do value in radiation alone group/Do value in combination group).

mRNA expression of P21, Bax and Ku70 in SiHa cells

Total mRNA was extracted with Trizol from SiHa cells. cDNA was obtained by reverse transcription with 2 µl of total RNA and M-MLv reverse transcriptase. PCR was performed with GAPDH as internal control. PCR conditions were as follows: pre-denaturing at 95°C for two minutes, denaturing at 95°C for 15 seconds, reannealing and elongation at 60-68°C for 20-60 seconds, 15 cycles. Obtained GAPDH fragment was 288 bp, B21 123 bp, Bax 126 bp, and Ku70 487 bp. The primer sequences were as follows: GAPDH: 5'-GGCATTCTGAGGACTCGG-3' 5'-GTGTGAAGGTGACGACG-3' p21: 5'-AAGACCATGTTGACGTCTG-3' 5'-GAGATCGCGCGGCGTGTG-3' Bax: 5'-GCGAGTTGCCTCAAGCCATC-3' 5'-CCATGGTAGCCGCTGCTCAGA-3' Ku70: 5'-CGTGCACACTTCTTTGACGAT-3' 5’-TGGTTCAATTGCTTCCCGATA-3’

Protein expression of P21, Bax and Ku70 in SiHa cells

RIPA (200 u1) was added in Siha cells for 30 minutes followed by centrifugation at 12,000 r/min for 30 minutes at 4°C. The supernatant underwent SDS-PAGE. Membrane blocking was performed, and then antibodies were incubated and stained.

Statistical analysis

Statistical treatment was performed with SPSS13.0 software. Measurement data were expressed as x±s. Normal distribution test was performed in all measurement data. Variance analysis and corresponding t test were used in the measurement data which were in line with normal distribution and Wilcoxon nonparametric test was used in the measurement data which were not consistent with normal distribution. Statistical significance was established at p < 0.05.

Results

Effects of SAHA combined with DDP on SiHa cell proliferation

With the increase in the dose of SAHA, the inhibition rate for SiHa cells was increased. Based on Q = E(AB)/(EA+EB-EA×EB) [7], the Q values of 1 µg/ml DDP combined with 1, 2, and 4 µmol/l SAHA were 1.63, 1.54, and 1.46, respectively; and the Q values of 2 µg/ml DDP combined with 1, 2, and 4 µmol/l SAHA were 1.31, 1.28, and 1.19, respectively. SAHA combined with moderate or low dose of DDP (1 µg/ml and 2 µg/ml) exhibited synergistic effects, but it combined with higher dose (4 µg/ml) of DDP additive effects. The IC50 of SAHA was 4.80 µmol/l. The dose of 20% IC50 of SAHA (0.96 µmol/l) was used in radiosensitization test.
Sensitization of suberoylanilide hydroxamic acid (SAHA) on chemoradiation for human cervical cancer cells and its mechanism

Effects of DDP combined with SAHA on SiHa cell cycle

Flow cytometry indicated that compared with control group, the number of cells in G0/G1 phase was increased, and the number of cells in G2/M+S phase was reduced and PI was decreased in S, D, and SD groups (p < 0.05). Compared with S and D groups, the number of cells in G0/G1 phase was increased, and the number of cells in G2/M+S phase was reduced and PI was decreased in SD group (p < 0.05, Table 1).

Effects of DDP combined with SAHA on SiHa cell apoptosis

Compared with control group, apoptosis was increased in 4 μmol/l SAHA group (p < 0.05). The apoptosis rates were higher in SD groups than in S or D groups (p < 0.05). After SiHa cells were treated with 2 μg/ml of DDP, apoptosis was not marked, but these cells were also treated with SAHA, apoptosis was significantly increased and with the increase in the dose of SAHA, the apoptosis rate was also increased (p < 0.05, Figure 1).

Effects of radiation combined with SAHA on the clone formation of SiHa cells

The number of colonies in radiation groups including 0, 2, 4, 6, and 8 Gy were 120, 225, 576, 480, and 778, and in combination groups were 95, 152, 152, 102, and 163, respectively. SF in each group is shown in Table 2. There were significant differences in SF between different radiological doses (p < 0.05). With the increase in radiological doses, SF was gradually decreased. In the same radiological dose, SF was significantly lower in combination group than in radiation alone group (p < 0.05). SHMT models was drawn using SigmaPlot 2000 Demo software with radiological doses as X axis and with SF as Y axis (Figure 2). Compared with radiation group, the curve of combination group moved left and was relatively flat without marked “shoulder area”. Do, N, and Dq values were 2.329, 2.761, and 1.721 in radiation group and 1.213, 4.770, and 0.823 in combination group. SER was 1.92.

Effects of SAHA combined with DDP or radiation on mRNA expressions of P21, Bax and Ku70 in SiHa cells

Compared with control group, there was no statistical significance in Bax mRNA expression in groups S and D (p > 0.05). However, Bax mRNA expression was higher in SD group than in other groups (p < 0.05). P21 mRNA expression was up-regulated in all groups, and was higher in SD group than in other groups (p < 0.05, Table 3).

Compared with control group, there was no statistical significance in Bax mRNA expression of SAHA group (p >

---

Table 1. — Effects of DDP combined with SAHA on SiHa Cell Cycle (X ± s, n = 4).

<table>
<thead>
<tr>
<th>Group</th>
<th>G0/G1 (%)</th>
<th>G2/M (%)</th>
<th>S (%)</th>
<th>PI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.05±1.44</td>
<td>27.97±3.21</td>
<td>35.98±1.22</td>
<td>63.95±1.72</td>
</tr>
<tr>
<td>S1</td>
<td>47.69±1.14</td>
<td>26.24±2.80</td>
<td>26.07±1.98</td>
<td>52.31±1.38</td>
</tr>
<tr>
<td>S2</td>
<td>50.57±2.53</td>
<td>16.51±0.44</td>
<td>32.92±0.44</td>
<td>49.43±3.57</td>
</tr>
<tr>
<td>S4</td>
<td>54.45±1.20</td>
<td>14.78±3.21</td>
<td>30.77±1.42</td>
<td>45.55±1.28</td>
</tr>
<tr>
<td>D1</td>
<td>55.49±2.87</td>
<td>24.26±2.09</td>
<td>20.25±0.77</td>
<td>44.51±1.54</td>
</tr>
<tr>
<td>D2</td>
<td>58.20±0.77</td>
<td>2.00±1.89</td>
<td>41.80±0.52</td>
<td>42.94±0.87</td>
</tr>
<tr>
<td>D4</td>
<td>58.44±2.43</td>
<td>13.85±1.80</td>
<td>27.72±3.11</td>
<td>41.57±1.39</td>
</tr>
<tr>
<td>S1+D1</td>
<td>57.46±2.00</td>
<td>2.38±0.59</td>
<td>40.16±1.29</td>
<td>42.54±0.69</td>
</tr>
<tr>
<td>S1+D2</td>
<td>61.67±0.97</td>
<td>10.56±3.34</td>
<td>27.76±2.13</td>
<td>38.32±1.22</td>
</tr>
<tr>
<td>S1+D4</td>
<td>63.78±0.41</td>
<td>7.85±1.46</td>
<td>28.37±0.92</td>
<td>36.22±0.99</td>
</tr>
<tr>
<td>S2+D1</td>
<td>61.44±0.16</td>
<td>5.13±2.33</td>
<td>33.42±1.02</td>
<td>38.55±0.75</td>
</tr>
<tr>
<td>S2+D2</td>
<td>63.51±0.25</td>
<td>15.13±1.12</td>
<td>21.36±2.44</td>
<td>36.49±1.23</td>
</tr>
<tr>
<td>S2+D4</td>
<td>64.43±3.22</td>
<td>9.65±3.78</td>
<td>25.92±1.37</td>
<td>35.57±0.98</td>
</tr>
<tr>
<td>S4+D1</td>
<td>62.74±2.66</td>
<td>7.88±2.55</td>
<td>29.38±0.71</td>
<td>37.26±1.22</td>
</tr>
<tr>
<td>S4+D2</td>
<td>63.89±1.63</td>
<td>10.94±1.91</td>
<td>25.17±0.85</td>
<td>36.11±0.98</td>
</tr>
<tr>
<td>S4+D4</td>
<td>65.24±1.17</td>
<td>10.25±2.11</td>
<td>24.50±1.13</td>
<td>34.75±1.77</td>
</tr>
</tbody>
</table>

#: vs control group, p < 0.05; △: vs S group, p < 0.05; ▲: vs D group, p < 0.05;

Table 2. — Cell survival fraction in each group (X ± s).

<table>
<thead>
<tr>
<th>Radiological dose</th>
<th>SF of radiation group</th>
<th>SF of combination group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Gy</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2 Gy</td>
<td>0.750±0.044 ▲</td>
<td>0.645±0.114 ▲</td>
</tr>
<tr>
<td>4 Gy</td>
<td>0.480±0.023 ▲</td>
<td>0.160±0.021 ▲</td>
</tr>
<tr>
<td>6 Gy</td>
<td>0.160±0.011 ▲</td>
<td>0.043±0.008 ▲</td>
</tr>
<tr>
<td>8 Gy</td>
<td>0.065±0.004 ▲</td>
<td>0.009±0.002 ▲</td>
</tr>
<tr>
<td>P</td>
<td>463.942</td>
<td>77.705</td>
</tr>
<tr>
<td>p</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

▲: indicates p<0.05, compared with SF of different doses in the same group.
☆: indicates p<0.05, compared with SF of different groups in the same dose.
SF: survival fraction; Combination group: SAHA combined with radiation

Figure 1. — Effects of DDP, SAHA, and DDP combined with SAHA on apoptosis rates of SiHa cells.
However, Bax mRNA expression was higher in radiation group than in control group and in combination groups than in other groups ($p < 0.05$). Ku70 mRNA expression was lower in SAHA group than in control group, and in combination group than in radiation group; but was higher in radiation group than in control group ($p < 0.05$, Table 4).

### Discussion

The incidence of cervical cancer takes the second place in women cancer [11]. About 78% of patients with cervical cancer are in developing countries where cervical cancer takes the second place in the leading cause of female cancer death. In recent years, its incidence is growing, and the age at onset is younger than ever [7]. Since young patients require high postoperative life quality, treatment methods for cervical cancer remain to be further improved. At present, radiotherapy and surgery are mainly used in treatment of cervical cancer. However, the recurrence rate of cervical cancer is as high as 35%. For the patients with moderate, advanced, recurrent or metastatic cervical cancer, radiotherapy is one of main treatment methods, but chemotherapy is also necessary, so it attracts more and more attention [12].

---

### Table 3 — mRNA and protein expressions of Bax and P21 in S, D, and SD Groups ($\bar{x} \pm s$, $n=3$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bax expressions</th>
<th>P21 expressions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mRNA$_{2^{-\text{Ct}}}$</td>
<td>Protein</td>
</tr>
<tr>
<td>Control</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>S</td>
<td>1.16±0.22</td>
<td>1.37±0.25</td>
</tr>
<tr>
<td>D</td>
<td>1.41±0.54</td>
<td>2.07±0.14#</td>
</tr>
<tr>
<td>SD</td>
<td>4.46±0.48#△</td>
<td>2.63±0.18#△</td>
</tr>
</tbody>
</table>

# indicates $P < 0.05$, compared with control group. 
△ indicates $P < 0.05$, compared with other groups.  
S group: SAHA group; D group: DDP group;  
SD group: SAHA combined with DDP group.

---

### Table 4 — mRNA and protein expressions of Bax and Ku70 in SAHA, radiation, and combination group ($\bar{x} \pm s$, $n=3$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bax expressions</th>
<th>Ku70 expressions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mRNA$_{2^{-\text{Ct}}}$</td>
<td>Protein gray value</td>
</tr>
<tr>
<td>Control</td>
<td>1.14±0.17</td>
<td>7631.19±547.12</td>
</tr>
<tr>
<td>SAHA</td>
<td>1.78±0.74</td>
<td>8122.88±482.40</td>
</tr>
<tr>
<td>Radiation</td>
<td>5.77±1.12#</td>
<td>10541.69±600.08#</td>
</tr>
<tr>
<td>Combination</td>
<td>10.25±1.38△</td>
<td>24345.01±859.138△</td>
</tr>
</tbody>
</table>

#: indicates $P<0.05$, compared with control group.  
△: indicates $P<0.05$, compared with other groups.  
$: indicates P<0.05$, compared with radiation group.  
Combination group: SAHA combined with radiation.
Sodium phenylbutyrate has good effects on cervical cancer cells in vitro [13]. It can inhibit the growth of cervical cancer cells and induce the apoptosis of cervical cancer cells. It is reported that HDACi combined with chemotherapeutics can restore the sensitivity of tumor cells to chemotherapeutics, obtaining synergistic anti-tumor effects [14]. SAHA, a kind of HDACi, has proved to be effective for many tumors and has been applied in phase II clinical trial [15]. SAHA re-activates the expression-blocked genes through histone acetylation, inducing tumor cell apoptosis.

Platinum-based chemotherapeutics play an important role in preoperative adjunctive treatment for bulky cervical cancer, radiosensitization, and improvement of prognosis of the patients with advanced or recurrent cervical cancer. Although the target of DDP is different from that of SAHA, the changes in DNA are closely associated with that in chromatin during tumorigenesis, therefore DDP combined with SAHA play stronger anti-tumor effects probably through DNA and chromatin. Therefore, in this study, SAHA combined with DDP was used in cervical cancer SiHa cells in order to obtain synergetic effects.

In this study, the authors used 20% IC50 of SAHA in radiosensitization experiments. Do value of SAHA-pretreated cells was decreased, suggesting that cellular sublethal damage repair was inhibited.

In this study, SAHA combined with moderate or low dose of DDP exhibited synergistic effects, but it combined with higher dose of DDP additive effects. The number of SiHa cells in G0/G1 phase was higher in SAHA combined with DDP group than in DDP alone ($p < 0.05$), suggesting that SAHA and DDP had synergistic effects.

This study indicated that SAHA combined with DDP could up-regulate the expressions of P21 and Bax genes, and SAHA combined with radiation could up-regulate Bax expression and down-regulate Ku70 expression. P21 is an inhibiting factor of cyclin dependent protein kinase, Bax an apoptotic factor and Ku70 a DNA repair gene. SAHA can inhibit HDAC activity, increasing the expression levels of P21 and Bax genes. P21 and Bax can induce tumor cell differentiation and (or) apoptosis, inhibiting tumor cell proliferation.

DNA is a main target molecular of radiotherapy. DNA damage includes double-strand break, single-strand break,
base damage and protein crosslink. Double-strand break is strongly associated with radiosensitivity. DNA repair system plays a crucial role in radiation resistance. Ku70 is a main repair protein for DNA double-strand break. The radiosensitizing effect of HDACi is closely related to that it can inhibit radiation-sublethal damage repair gene. Munshi et al. [16] have found that NaB can significantly decrease the expressions of repair-related factors such as Ku70, Ku80, and DNA-dependent protein kinase catalytic subunit, exhibiting a radiosensitizing effect. Subramanian et al. [17] have reported that HDAC6-specific inhibitor may induce Ku70 acetylation, Bax release, and neuroblastoma cell death. Cheng et al. [18] described that HDAC inhibitors, HDAC42, MS-275, and TSA can induce Ku70 protein acetylation, inhibiting the repair of DNA double-strand break. In this study, Ku70 expression was higher in radiation alone group than in control group (p < 0.05); was lower in SAHA alone group than in control group (p < 0.05), and in SAHA combined with DDP group than in radiation alone group (p < 0.05). Based on above data, it can be seen that the radiosensitizing effect of SAHA is achieved by down-regulating Ku70 expression, a radiation sub-lethal damage repair gene.

In chemotherapy, all chemotherapeutics have the function to induce tumor cell apoptosis or tumor necrosis. Cell cycle arrest is closely associated with cell apoptosis and differentiation [19]. The present authors found that the cytotoxicity against tumor cells was stronger in SAHA combined with DDP than in either of both alone. The mechanism may be that although the low dose of SAHA fails to markedly kill tumor cells, can quickly increase the level of histone acetylation, which can cause the DNA in chromatin to be fully exposed, promoting the cross-linking of DDP with DNA and enhancing DDP cytotoxicity against tumor cells.

The limitations in this study were that the authors only studied the effects of SAHA combined with chemotherapeutics or radiation on SiHa cells in vitro, hence, in vivo studies on cell line SiHa and other cervical cancer cell lines remain to be further carried out.

References


Address reprint requests to:
J. XING, M.D.
Address: No. 73, Jianshe South Road, Lubei District, Tangshan 063000 (China)
e-mail: gaochunyan1954@163.com
Expression of estrogen receptors in melanoma and sentinel lymph nodes; a “female” clinical entity or a possible treatment modality?

C. Spyropoulos¹, M. Melachrinou², P. Vasilakos³, E. Tzorakoleftherakis¹

¹Department of Surgery, University Hospital of Patras, Rion; ²Department of Pathology, University Hospital of Patras, Rion; ³Department of Nuclear Medicine, University Hospital of Patras, Rion (Greece)

Summary

Purpose: The natural history of human malignant melanoma suggests that steroid hormones may affect the biological behavior of this tumor. The purpose of the current study was to investigate the specific immunostaining patterns of estrogen receptors in malignant melanomas and their sentinel lymph nodes (SLNs), as well as to examine any possible association with patients’ prognosis and overall survival. Materials and Methods: A retrospective analysis of prospectively collected data was conducted during a 12-year period (2001-2012). Sixty patients with mean age of 54.4 ± 14.5 years diagnosed with melanomas of varying depth (Clark) and thickness (Breslow) after excision biopsy of pre-existing melanocytic lesions, were included in the study. All patients underwent wide excision of the primary tumor and SLN identification. Determination of estrogen receptor alpha (ERα) and beta (ERβ) status by immunohistochemistry on tumor and nodal paraffin blocks was performed in all feasible cases. Results: ERβ but not ERα was the predominant estrogen receptor found in all primary tumors and SLNs examined. The most intense ERβ immunostaining was seen in negative SLNs associated with thinner, less invading melanomas. ERβ expression in the primary tumor seems to correlate with the cellular microenvironment, possibly altering the process of SLN invasion. Conclusions: ERβ expression is down-regulated in aggressive melanomas with sentinel nodal metastatic disease, suggesting its possible usefulness as a surrogate marker for metastatic potential and prognosis in malignant melanoma.

Key words: Malignant melanoma; Sentinel lymph node; Estrogen receptors; Immunostaining.

Introduction

The role of estrogens in the cause and progression of many cancers is well documented [1]. The effects of estrogens are mediated by two estrogen receptors, estrogen receptor α (ERα) and estrogen receptor β (ERβ), representing members of the nuclear steroid receptor superfamily. Estrogen receptors classically mediate their action by ligand-dependent binding to the estrogen responsive element, leading to transcriptional regulation of target genes [1]. Both of these proteins have a high degree of homology in the DNA-binding domain but differ considerably in the N-terminal domain (E domain) [2]. These differences suggest that most likely these proteins represent two different subtypes rather than splice variants [3]. Various isoforms of each subtype of the two receptors have been discovered, suggesting that they could have distinct functions in terms of gene regulations and biologic responses or that they could contribute to the selective actions of 17β-estradiol and of other estrogenic molecules on target cells [1, 3, 4].

The skin is an important estrogen-responsive tissue and the fundamental role of estrogen in the regulation of hair follicle cycling and self-renewing is well known [5]. However, the relative contribution of estrogens in skin tumorigenesis remains unclear. This is the reason why there is an extensive debate whether skin cancers, especially their most aggressive form, melanoma, express the two ERs. Faint indications are derived through epidemiological data which clearly state a survival benefit for female patients with metastatic melanoma versus male individuals [4, 6]. Studies based on the immunoreactivity failed to demonstrate a role for ERα in the pathophysiology of either melanoma precursor lesions or melanomas [7-9]. After ERβ was identified in 1995, no controlled, follow-up studies have examined whether ERβ plays a role in the evolution or prognosis of malignant melanomas. Furthermore, no study so far has examined the association between progression of the disease and ER expression in the primary tumor and the sentinel lymph node (SLN) in malignant melanomas; SLN represents the gold-standard procedure in order to estimate the stage of the disease and to determine if further therapeutic actions are required.

For all of these reasons, the present authors embarked on a project of evaluating ER expression in tissues of the primary tumor and the SLN of humans diagnosed with malignant melanoma. The primary target was to identify
whether ERα and mainly ERβ expression has a role in tumor progression and metastatic potential through the lymph route in malignant melanomas.

### Materials and Methods

The protocol of the study was approved by the ethics committee of the present institution. The expression of ERα and ERβ in primary tumors and their representative SLNs was investigated in 60 patients (41 men, 19 women) diagnosed with malignant melanoma on varying locations during a 12-year period (2001-2012). The diagnosis was based on excision biopsy of suspicious pre-existing melanocytic nevi. All patients included in the study had never had any kind of hormonal replacement therapy and none of them was obese.

Patients presenting with clinical or laboratory evidence of lymph node invasion or distal metastatic disease were excluded from the study. Since diagnosis was confirmed, all patients underwent SLN biopsy under general anesthesia and simultaneous lymphatic mapping. An incision was made through the skin and into the subcutaneous tissue overlying the marked radioactive site. If a blue lymphatic vessel was identified in the subcutaneous tissue, this was followed to its respective draining SLN. In each case, any radioactive SLN was identified by the gamma probe alone and removed until less of 10% of the maximum radioactivity level was recorded at the surgical field. Wider excision of the skin and the underlying subcutaneous tissue was then performed in order to achieve 1.5 cm clear margins around the primary lesion and the operation was terminated. The excised lymph nodes were sent for pathological examination. No cases of any type of allergic reactions to the dye product amongst any of the patients in this study were recorded.

### Statistical analysis

Data analysis was performed by means of SPSS software (release 13.0). Clinical and laboratory data were correlated by using the Mann–Whitney test whereas Spearman’s r correlation coefficient was used to detect any potential correlation between ER expressions. Survival curves were estimated using the Kaplan–Meier method and statistical significance was determined by the log rank test. The Cox proportional hazards regression model was used to estimate disease-free survival (DFS) and overall survival (OS) according to ER expression levels. When comparison between groups of lesions was needed, a paired Wilcoxon test was used. Any p-value less than 0.05 was considered statistically significant.
Expression of estrogen receptors in melanoma and sentinel lymph nodes; a “female” clinical entity or a possible treatment modality?

Figure 1. — Immunohistochemical expression of ERβ in three different cases of superficial spreading melanoma (SSM) with radial and vertical growth. A-C: ERβ in a SSM (three mm deep) from the knee of a 49-year-old female. The great majority of melanoma cells show nuclear and/or cytoplasmic immunoreactivity throughout the lesion. Micrographs A and C highlight the stronger immunoreactivity of intraepidermal melanoma cells (KAMs) and of melanoma cells in association with stromal invasion (SAMs), respectively, than the immunoreaction of dermal melanoma cells (MAMs) (B). D-F: ERβ in an 8.5 mm-deep melanoma from the elbow of an 48-year-old male. Micrograph D demonstrates cytoplasmic immunostaining in the majority of keratinocyte-associated melanoma cells (KAMs). Micrographs E and F show nuclear immunostaining in tumor cells. Melanoma cells along tumor perimeter (SAMs) (F) show a higher ERβ immunopositivity compared to melanoma-associated melanoma cells (MAMs) in the centre of the lesion (E). G-J: ERβ in an 1.7 mm-deep melanoma from the trunk of a 62-year-old female and ERβ immunostaining of positive SLN. Micrographs G and I show increased nuclear ERβ immunoreactivity in melanocytic nests located just below the epidermis (G), as well as in melanoma cells that are surrounded by stroma (I). In contrast, MAMs demonstrate low immunopositivity for ERβ (H). Metastatic melanoma in SLN. The minority of tumor cells show a weak nuclear ERβ immunostaining. Note the positive immunostaining of several lymphocytes (J). Original magnification A-G, I, J: x200, H: x400.
Results

Although the original goal of the study was to determine the distribution and levels both of ERα and ERβ receptors in malignant melanomas and their SLNs, immunodetection of ERα was only noted in seven out of 60 patients (11.9%). Therefore, ERβ distribution formed the basic focus of our research.

Sixty cases of primary melanomas were studied. Sentinel node detection was successful in all patients (100%) while the mean number of nodes removed was 1.7 ± 0.88 (range 1-4). Pathological examination revealed nodal metastatic disease in 18 patients (36%) and completion lymph node dissection was performed in these cases with null morbidity and mortality.

The melanomas were divided into three groups, according to the Breslow thickness: group one included thin melanomas (≤ one mm), group two included melanomas of depth one – four mm and group three any deeper lesions (> four mm) (Table 1). A constant observation was that ERβ immunoreactivity was significantly decreasing when Breslow depth was increasing \((p = 0.024\) and \(p = 0.001\) when group one was compared to group two and three, respectively) (Figure 1). Deep melanomas represented the least ERβ immunoreactive lesions in the present series (Figure 2). ERβ expression was significantly more intense in males compared to females, only in deep melanomas \((p = 0.04)\).

When ERβ immunoreactivity was examined in SLN tissues, a constant decrease of ERβ levels was recorded, analogously to increasing Breslow thickness of the primary tumor \((p = 0.017)\). A concomitant decrease in the levels of ERβ expression in the primary tumors and their SLNs was noted when Breslow thickness increased, however a statistically significant difference in ERβ immunoreactivity between primary tumors and SLNs was noted only in Breslow group two \((p = 0.001)\). Nevertheless, a strong association of ERβ immunoreactivity levels and negative SLN status was steadily documented in the present study \((p = 0.006, Figures 1J, 3)\).

During evaluation of the immunostaining patterns of ERβ in the present series, there was a constant finding that the cellular levels of ERβ immunoreactivity depended most upon their microenvironment within the lesion. Based on the methodology by Schmidt et al. [10], the present authors found that the data on ERβ expression varied according to specific cellular spatial associations within the primary tumor; three spatial groups presented reproducible patterns of ER immunostaining: keratinocyte – associated melanoma cells (KAMs), stroma – associated melanoma cells (SAMs) and melanoma – associated melanoma cells (MAMs). KAMs are melanoma cells very close to the epidermal surface and adjacent to keratinocytes. SAMs represent melanoma cells in proximity to the stroma, mostly at the periphery of downwardly progressing nodules. Finally, MAMs are melanoma cells very close to the epidermal surface and adjacent to keratinocytes. SAMs represent melanoma cells in proximity to the stroma, mostly at the periphery of downwardly progressing nodules. Finally, MAMs are melanoma cells which are not in association with keratinocytes or stroma and they are only surrounded by other melanoma cells.

Estrogen receptor β immunoreactivity of KAMs was reversely related to the Breslow thickness of the primary lesion, varying from 60-80% (score 3-4) in thin melanomas (group one) to less than 40% (score 1-2) in thicker melanomas (group three) \((p < 0.004)\) (Figure 4). There was no statistically difference between male and female patients. Interestingly, cytoplasmic ERβ immunoreactivity was more intense than nuclear immunoreactivity in all KAMs studied, however no
Expression of estrogen receptors in melanoma and sentinel lymph nodes; a “female” clinical entity or a possible treatment modality?

127

statistically significant difference was recorded \((p = 0.101)\) (Figures 1A, D, G).

Within the SAMs population there was no statistically significant difference in ERb immunostaining patterns between the three groups of melanomas in both males and females. The SAMs averaged 35-65% immunoreactivity (score 2-3) in both the nuclei and cytoplasm (Figures 1C, F, I).

Melanoma cells located adjacent to one another (MAMs) were also identified in all Breslow groups. Within the MAMs population ERb immunoreactivity levels were low, presenting though a slight gradual increase in melanoma tissues of depth > four mm (Figures 1B, E, H). However, immunoreactivity levels never exceeded 40% (score 2). No difference was recorded in nuclear and cytoplasmic patterns. ERb immunostaining was more intense in male patients with melanomas > four mm deep compared to female patients of this group \((p < 0.001)\).

Among all subpopulations examined, KAMs exhibited the most intense ERb immunopositivity levels in thin melanomas, compared to SAMs and MAMs of all the Breslow groups \((p < 0.001)\). However, in thicker melanomas, immunoreactivity levels presented gradual decrease in all subpopulations examined. Although KAMs and SAMs immunostaining was again more intense compared to MAMs among melanomas of depth > one mm, no statistically significant difference was recorded between these cellular compounds in Breslow groups 2 and 3. Overall immunostaining score among these spatial cellular compounds is summarized in Table 2.

ERb immunoreactivity was mostly identified in thinner melanomas which generally are associated with better prognosis. Although KAMs were the most intense subpopulation documented in this Breslow group, no statistically significant correlation was found between ERb immunostaining of KAMs lesions and SLN infiltration, both in male and female patients \((p = 0.082)\). Interestingly, although MAMs ERb immunostaining was more intense in deeper melanomas (even in lower levels), no significant association between ERb expression in this subpopulation and SLN invasion was also documented \((p = 0.121)\). On the other hand, SAMs ERb immunoreactivity levels in melanomas one to four mm deep was strongly associated with SLN status, indicating a possible crucial step in the metastatic process of the disease \((p = 0.03)\) (Figure 5).

Table 2. — Immunostaining score among cellular compounds studied in primary melanomas (KAMs: keratinocyte-associated melanoma cells, SAMs: stroma-associated melanoma cells, MAMs: melanoma-associated melanoma cells).

<table>
<thead>
<tr>
<th>Immunoreactivity</th>
<th>KAMs</th>
<th>SAMs</th>
<th>MAMs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0 (&lt; 5%)</td>
<td>7</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Score 1 (5 - 25%)</td>
<td>8</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Score 2 (26 - 50%)</td>
<td>12</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>Score 3 (51 - 75%)</td>
<td>25</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Score 4 (≥ 76%)</td>
<td>8</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>
Follow up was feasible for 53/60 patients (88.3%) for a mean period of 8.2 ± 2.8 years [1-11]. As expected, mean survival rate was noticeably decreased in cases of nodal metastatic disease (p = 0.002). On Cox multivariate analysis, a mitotic index > 4/mm² in the primary tumor was a significant, unfavorable prognostic factor for overall survival independently of patients’ age and sex as well as of the location of the tumor, the histological type, and the presence of lymphatic invasion (Table 3). Breslow thickness of the primary tumor was not an independent factor for overall survival, but only for disease-free survival rates (Figure 6).

ERβ immunoreactivity levels in the primary tumors did not affect overall survival. However, when ERβ expression patterns were studied in the specific subpopulations examined, a survival advantage was evident in cases of intense
Expression of estrogen receptors in melanoma and sentinel lymph nodes; a “female” clinical entity or a possible treatment modality?

ERb expression observed in thicker melanomas suggests that it may be relevant to the pathophysiology of malignant melanoma. Noteworthy, similar immunostaining patterns were documented when tissues of SLNs were examined. A constant finding was the decrease of ERb levels in SLNs associated with increasing Breslow thickness of the primary tumor. This was related to the concomitant decrease in the levels of ERb expression in thicker primary tumors as well. The present study demonstrated a strong association of ERb immunoreactivity levels and negative SLNs, as well as improved patients’ survival. This could be a useful tool in the pathological evaluation of SLNs and further clinical assessment of patients with malignant melanomas.

Another captivating finding was that the degree of ERb expression in melanomas depended upon the specific microenvironment of melanoma cells and correlated with its metastatic potential. Melanoma cells in the epidermis (KAMs) were the most ERb immunoreactive compared to the dermal aggregates at the periphery (SAMs) and melanoma cells in large dermal nodules (MAMs). However, ERb immunoreactivity of MAMs only, although in low levels, increased in deeper and more aggressive melanomas. These findings suggest that estrogenic compounds could be influencing the interactions of melanocytes with neighboring cells including keratinocytes, fibroblasts, nerves, capillary and lymphatic endothelial cells, and the extracellular matrix, as indicated by other studies [13-15]. Although KAMs and MAMs represent two cellular compounds related traditionally to favored and poor prognosis, respectively, no correlation was found between their ERb immunostaining levels and SLN infiltration. On the other hand, SAMs were mostly identified in melanomas of one to four mm which represent the most controversial prognostic type of the tumor. Enchantingly, ERb immunoreactivity levels of SAMs were strongly associated with SLN status, indicating a possible crucial step in the metastatic process of the disease at this level. The present findings highlight the importance of carefully examining the microenvironment of melanocytic cells, especially in tumors of one to four mm Breslow thickness [16].

Malignant melanoma is the most aggressive form of skin cancer with a rapidly increasing incidence rate. Although a lot of debate has been conducted in relation to its hormonal behavior compared to other tumors, the role of estrogens in the progression of the disease remains unclear. Some findings that suggest a hormonal role in melanoma include older epidemiologic studies indicating a survival benefit for female patients with metastatic melanoma; the rarity of melanoma prior to puberty; and the peak incidence in women coinciding with the late child bearing years and the beginning of menopause [6]. However, larger, more recent population studies strongly indicate that survival is superior among female patients, independently to Breslow thickness, histological type, and tumor site, suggesting that other tumor-related variables are implicated in the progression of the disease [17, 18].

The data collected by the current study support the hypothesis that melanoma could be classified as an estrogen-
responsive tumor and that ERs expressed in both the primary tumor and the SLN could indicate molecular responses associated with the invasive capacity of melanoma. The effects of estrogens are mediated by different kinds of estrogen receptors (ERs) and probably, as it occurs in breast cancer, not all melanoma cells show the same types of ERs. Therefore, research should focus on melanoma and SLNs themselves, particularly on the subtypes of receptors for estrogens they express. Specific genetic polymorphisms of the ERα and ERβ genes in melanoma patients might correlate with a higher proportion of melanoma [19]. Furthermore, it is nowadays clear that the sole existence of two forms of the ERs is not sufficient to account for the diverse biological roles of estrogens and pharmacological activities of synthetic ER ligands [20]. The discovery of co-regulator complexes in nuclear estrogen receptor action seems to enable the ERs to communicate with the general transcription apparatus, possess the catalytic activities required for chromatin modification, and capacity to integrate extracellular signals and translate them into transcriptional and biological events [21].

Further validation of these results may lead to altered targeted therapies as well as more radical surgical therapy (therapeutic lymph node dissection) even in primary stages of melanomas with disease-negative SLNs which exhibit altered expression of ERβs.

**Conclusion**

The current study indicates a role for the estrogen receptors expressed in the sentinel node of malignant melanomas during the metastatic process. These results pinpoint at the possibility of using ERβ expression as a prognostic indicator of melanoma spreading through the lymph route. The possibility of distinguishing proliferative melanomas, which are associated with dismal prognosis, from the so-called dormant melanomas opens up novel avenues in tailoring individual treatments, as already occurs for other tumors.

**References**


Address reprint requests to: C. SYPROPULOS, M.D.
4, Foskolou Street
15232, Chalandri, Athens (Greece)

e-mail: xspiropupatras@gmail.com
Isolated axillary nodal swelling and cancer of unknown primary

S. Bertozzi1, A.P. Londero2, R. Petri1, S. Bernardi1

1 Department of Surgery, Azienda Ospedaliero-Universitaria "Santa Maria della Misericordia", Udine
2 Clinic of Obstetrics and Gynecology, Azienda Ospedaliero-Universitaria "Santa Maria della Misericordia", Udine (Italy)

Summary

Introduction: The literature reports rare cases of isolated axillary lymph node metastasis from cancer of unknown primary (CUP). The authors reviewed the prevalence and outcome of patients with isolated axillary nodal swelling suspicious for malignancy affected or not by isolated axillary node metastasis from CUP. Materials and Methods: The authors collected data about 65 patients presented with isolated axillary lymph node swelling who underwent axillary lymph node excisional biopsy for malignancy suspicion, between January 2005 and December 2011, in the absence of any specific diagnosis. Results: Histological examination revealed a metastatic infiltration by an occult solid cancer in 16 cases (24%), ten of which were occult breast cancers. Histological patterns and molecular markers allowed in all cases of occult cancer a probable identification of the primary tumor site, while a definitive diagnosis was possible only in the 56.25% of cases (9/16). The prognosis of these patients was very poor with a five-year overall survival of 28%, and thus very similar to patients affected by Stage IV overt breast cancer. Conclusions: Among occult malignancies presenting with sole axillary lymph node metastasis, breast cancer remains the more probable primary cancer, but many other sites should be taken into consideration by negative breast imaging. Positron-emission tomography computed tomography (PET-CT) resulted helpful in the primary site detection, but has nonetheless a margin of failure. Occult breast cancers behave very similar to Stage IV overt breast cancers, and should be treated accordingly.

Key words: CUP syndrome; Cancer of unknown primary; Axillary lymph node metastasis; Occult tumor; Breast cancer.

Introduction

Cancer of unknown primary (CUP) is defined when cancer is found in one or more metastatic sites but the primary site is unknown. These cancers are characterized by early dissemination and unpredictable metastatic pattern coupled to dormancy or regression of the primary tumor and aggressive biologic behavior [1]. For its rarity, axillary node metastasis from CUP represents a diagnostic and therapeutic challenge being mostly, but not exclusively, the sole clinical symptom of non-palpable breast cancers.

Occult breast cancer accounts for less than 0.5% of all breast cancers, is located in the upper-outter quadrant in the 50% of cases and in the lower outer one in the 20% [2, 3]. Also lymphoma, melanoma, adenocarcinomas of the lung or gastrointestinal tract are known to metastasize to the axilla [1].

Determination of the occult primary tumor site is essential to lead the treatment and usually requires a multidisciplinary approach. Many molecular markers can be useful to investigate tumor tissue properties in order to state its probable origin. In particular, estrogen receptor (ER) expression results a helpful marker in case of occult breast cancer [4, 5], but also gross cystic disease fluid protein 15 (GCDFP-15) and mammaglobin have been tested with the same target [6, 7].

The radiodiagnostic approach in metastasis from CUP with the target of finding the primary site and staging the patient consists of bilateral mammography, computed tomography (CT) of chest/abdomen/pelvis supplemented by additional imaging or endoscopic studies [1]. Although mammography is still considered the gold standard among breast diagnostic tools, its ability to detect occult breast tumors is disappointing [8], and despite the progresses of modern imaging techniques that enable to find and biopsy many early non-palpable breast lesions [9], there are many limiting factors such as radiologist’s inexperience, small lesion size, and lesion localization [9–11]. Most of the imaging techniques, such as CT, magnetic resonance imaging (MRI), and positron-emission tomography (PET), had their sensitivity and specificity strongly limited by the lesion size [12].

Finally, it is important to remember that only the histopathological examination of the surgical specimen allows a definitive diagnosis. Unfortunately, if the tumor size is smaller than the pathological section interval the primary tumor escapes the histological detection and this may justify the fact that in approximately the 30% of cases undergoing mastectomy the tumor is not found [13–16].

The authors reviewed their cases with isolated axillary nodal swelling suspicious for malignancy and affected or not by isolated axillary node metastasis from CUP, focusing on their diagnostic and therapeutic management.

Materials and Methods

The authors collected retrospective data about all patients who underwent an axillary lymph node excisional biopsy for isolated axillary nodal swelling suspicious for malignancy, in their De-
Table 1. — *Population characteristics.*

<table>
<thead>
<tr>
<th>Site</th>
<th>Gender</th>
<th>Age at op. (years)</th>
<th>Side</th>
<th>Mx</th>
<th>Us</th>
<th>MRI</th>
<th>CT</th>
<th>PET-CT</th>
<th>Adjuvant therapies</th>
<th>Follow up</th>
<th>ER</th>
<th>PR</th>
<th>Mib1</th>
<th>Her-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>F</td>
<td>78</td>
<td>left</td>
<td>pos</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
<td>Died within 1 month</td>
<td>pos</td>
<td>pos</td>
<td>30</td>
<td>neg</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>F</td>
<td>77</td>
<td>bilat</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>RT, CHT</td>
<td>Alive</td>
<td>pos</td>
<td>pos</td>
<td>10</td>
<td>neg</td>
</tr>
<tr>
<td>Breast</td>
<td>F</td>
<td>84</td>
<td>left</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>RT, CHT</td>
<td>Died after 4 years</td>
<td>pos</td>
<td>neg</td>
<td>5</td>
<td>neg</td>
</tr>
<tr>
<td>Breast</td>
<td>F</td>
<td>61</td>
<td>right</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>RT, CHT</td>
<td>Alive</td>
<td>pos</td>
<td>70</td>
<td>pos</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>F</td>
<td>46</td>
<td>left</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>RT, CHT</td>
<td>Alive</td>
<td>pos</td>
<td>pos</td>
<td>80</td>
<td>neg</td>
</tr>
<tr>
<td>Breast</td>
<td>F</td>
<td>53</td>
<td>left</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>RT, CHT</td>
<td>Alive</td>
<td>pos</td>
<td>pos</td>
<td>70</td>
<td>neg</td>
</tr>
<tr>
<td>Breast</td>
<td>F</td>
<td>48</td>
<td>right</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>RT, CHT</td>
<td>Alive</td>
<td>pos</td>
<td>pos</td>
<td>25</td>
<td>neg</td>
</tr>
<tr>
<td>Breast</td>
<td>F</td>
<td>87</td>
<td>left</td>
<td>pos</td>
<td>pos</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Alive</td>
<td>pos</td>
<td>pos</td>
<td>pos</td>
<td>neg</td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>F</td>
<td>56</td>
<td>right</td>
<td>neg</td>
<td>neg</td>
<td>–</td>
<td>pos</td>
<td>pos</td>
<td>CHT</td>
<td>Died after 2 years</td>
<td>pos</td>
<td>neg</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ovary</td>
<td>F</td>
<td>50</td>
<td>right</td>
<td>neg</td>
<td>neg</td>
<td>–</td>
<td>pos</td>
<td>CHT</td>
<td>Alive</td>
<td>pos</td>
<td>neg</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>M</td>
<td>71</td>
<td>right</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>–</td>
<td>Alive</td>
<td>pos</td>
<td>neg</td>
<td>5</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>M</td>
<td>59</td>
<td>left</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>–</td>
<td>Died after 2 years</td>
<td>neg</td>
<td>neg</td>
<td>50</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Bladder</td>
<td>F</td>
<td>71</td>
<td>left</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
<td>pos</td>
<td>CHT</td>
<td>Died after 2 years</td>
<td>neg</td>
<td>neg</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Bronchus</td>
<td>F</td>
<td>59</td>
<td>right</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
<td>CHT</td>
<td>Alive</td>
<td>neg</td>
<td>70</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Results

During the considered period, 65 patients underwent axillary node excisional biopsy for malignancy suspicion. Mean patients age was 53.75 years (±19.33), and 63% of them were females. The right axilla was seized with illness in more than half cases (51%, 33/65), while 23 patients had a left axillary lymphadenomegaly (35%), and in the remaining nine (14% of cases) there was a bilateral involvement of axillary lymph nodes (Table 1).

Histological examination was negative for neoplastic infiltration in 11 cases (17%, including reactive and granulomatous adenosis and a case angioleiomyoma), whereas it revealed a hematologic malignancy in 38 cases (59%) and a metastatic infiltration by an occult solid cancer in 16 cases (24%). In particular, there were ten cases of occult breast carcinoma, two occult malignant melanomas, two ovarian carcinomas, one bladder urothelial carcinoma, and one bronchus neuroendocrine cancer. The diagnostic and therapeutic management of these 11 patients is summarized in Table 2.
isolated axillary nodal swelling and cancer of unknown primary

133

CUP syndrome represented the 3% of all patients receiving an axillary lymphadenectomy in the study period, and breast cancer axillary metastasis from an occult primary site were 0.48% of all breast cancers operated in the same period. Histological patterns and molecular markers allowed in all cases of occult cancer a probable identification of the primary tumor site, while a certain diagnosis on the primary cancer specimen was possible only in the 56% of cases (9/16). The authors found ER positivity to have, respectively, a sensitivity and a specificity of 80% (CI.95 49-94%) and 67% (CI.95 30-90%) for identification of a CUP as originating from a breast cancer primary. Furthermore, they found progesterone receptor (PR) positivity to have a sensitivity and a specificity of 60% (CI.95 31-83%) and 100% (CI.95 61-100%).

Taking into consideration only occult breast cancers, mammography and breast ultrasound examination showed always according findings, but succeeded in detecting the occult primary site only in three women with breast cancer (30%, 3/10) (Table 3). Moreover, 80% (8/10) of women affected by breast cancer had a previous regular screening and the last screening examination was performed at a median of 12 months (9-16) before finding CUP. A fourth case of occult breast cancer was detected only by PET-CT scan, while additional breast MRI was not routinely performed, and in any case never detected the occult breast primary site. Finally, a fifth case was found out only by the histological examination of the breast surgical specimen, and in only one of 103 histological sections.

The 80% of women with occult breast cancer underwent quadrantectomy for a suspected mass, but in only three cases it resulted to be a primary tumor. Two patients underwent mastectomy and in both cases the primary tumor was successfully found. Five patients underwent further ipsilateral breast irradiation and chemotherapy (50%), three underwent only adjuvant radiation therapy (30%), and the two remaining cases did not receive any adjuvant therapy (20%). In addition, five patients also received lymph node radiation therapy.

Considering together patients with occult solid breast and non-breast cancers, chest-abdominal CT scan and total-body PET-CT scan found the occult primary site in respectively, the 25% (3/12) and 64% (7/11) of cases.

Comparing patients with an hematologic malignancy and those with an occult solid cancer, mean age of these last results significantly higher (63.75 vs 55.50) (Table 4). Moreover, considering only patients who died for cancer during the follow up, they are significantly older in the group of patients with an occult solid tumor ($p = 0.239$). No significant difference was observed according to gender and the axilla side.

The OS at two years for patients with an axillary metastasis from an occult solid primary tumor was 70% (CI.95 50-100%), and thus lower ($p = 0.192$) if compared to that of patients affected by hematologic malignancies who developed an axillary isolated lymphadenopathy (79%, CI.95 67-94%). Furthermore, the 70% (CI.95 55%-90%) of patients affected by hematologic malignancies resulted alive at five years, whereas the five-year OS in patients with an occult solid primary tumor diagnosis resulted in only 28% (CI.95 9-87%) (Figure 1A).

Table 3. — Diagnostic tools used in the group of patients with occult solid malignancies.

<table>
<thead>
<tr>
<th></th>
<th>Breast</th>
<th>Non breast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammography</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not performed</td>
<td>0% (0/10)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>Negative</td>
<td>70% (7/10)</td>
<td>100% (6/6)</td>
</tr>
<tr>
<td>Positive</td>
<td>30% (3/10)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>Breast ultrasound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not performed</td>
<td>0% (0/10)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>Negative</td>
<td>70% (7/10)</td>
<td>100% (6/6)</td>
</tr>
<tr>
<td>Positive</td>
<td>30% (3/10)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>Breast magnetic resonance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not performed</td>
<td>40% (4/10)</td>
<td>100% (6/6)</td>
</tr>
<tr>
<td>Negative</td>
<td>60% (6/10)</td>
<td>0% (0/10)</td>
</tr>
<tr>
<td>Positive</td>
<td>0% (0/10)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>Chest-abdominal CT scan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not performed</td>
<td>40% (4/10)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>Negative</td>
<td>60% (6/10)</td>
<td>50% (3/6)</td>
</tr>
<tr>
<td>Positive</td>
<td>0% (0/10)</td>
<td>50% (3/6)</td>
</tr>
<tr>
<td>Total-body PET-CT scan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not performed</td>
<td>50% (5/10)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>Negative</td>
<td>20% (2/10)</td>
<td>33% (2/6)</td>
</tr>
<tr>
<td>Positive</td>
<td>30% (3/10)</td>
<td>67% (4/6)</td>
</tr>
</tbody>
</table>

Table 4. — Comparison among patients with negative axillary findings, axillary manifestation of a hematologic malignancy, and axillary metastasis from an occult solid primary malignancy.

<table>
<thead>
<tr>
<th></th>
<th>Hematologic malignancy</th>
<th>Occult solid cancer</th>
<th>Benign/Reactive</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at surgery (ys)</td>
<td>55.5 (±18.33)</td>
<td>63.75 (±12.77)</td>
<td>33.18 (±16.35)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Median follow up (months)</td>
<td>28 (15-48)</td>
<td>30 (14-44)</td>
<td>40 (24-62)</td>
<td>0.310</td>
</tr>
<tr>
<td>Mean age at surgery (pts dead for cancer)</td>
<td>59 (±22.43)</td>
<td>69.14 (±11.13)</td>
<td>0.239</td>
<td></td>
</tr>
<tr>
<td>Median follow up (pts dead for cancer)</td>
<td>16 (7-26)</td>
<td>21 (17-45)</td>
<td>0.282</td>
<td></td>
</tr>
<tr>
<td>Female gender</td>
<td>55% (21/38)</td>
<td>88% (14/16)</td>
<td>55% (6/11)</td>
<td>0.066</td>
</tr>
<tr>
<td>Axilla side</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td>16% (6/38)</td>
<td>12% (2/16)</td>
<td>9% (1/11)</td>
<td>0.838</td>
</tr>
<tr>
<td>Right</td>
<td>50% (19/38)</td>
<td>44% (7/16)</td>
<td>64% (7/11)</td>
<td>0.591</td>
</tr>
<tr>
<td>Left</td>
<td>34% (13/38)</td>
<td>44% (7/16)</td>
<td>27% (3/11)</td>
<td>0.661</td>
</tr>
</tbody>
</table>
Comparing patients with breast CUP and those with overt breast cancers at the same stage, the majority of histological types were ductal invasive carcinoma in both cases (Table 5). Furthermore, the authors found hormonal status to be similar to TNM II and III, while Her-2 positivity was significantly higher in CUP compared to TNM II, breast cancer (p < 0.05), and Mib-1 was not significantly higher in CUP compared to TNM II. TNM stage at diagnosis of breast CUP was in 50% of cases TNM II and in 50% TNM III. In Figure 1B the authors found CUP to have a significantly lower OS than TNM II and III of other breast cancers. Moreover, the survival pattern of CUP was similar to TNM Stage IV of other breast cancers (Figure 1B).

Table 5. — Characteristics of breast cancer and breast cancer found by axillary isolated lymph node metastasis.

<table>
<thead>
<tr>
<th></th>
<th>TNM II</th>
<th>TNM III</th>
<th>TNM IV</th>
<th>Axillary isolated lymph node metastasis as first presentation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woman age (years)</td>
<td>61.53 (±12.99)</td>
<td>59.8 (±14.56)</td>
<td>64 (±12.12)</td>
<td>65.4 (±14.98)</td>
<td>0.610</td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal invasive carcinoma</td>
<td>82% (193/235)</td>
<td>85% (47/55)</td>
<td>100% (3/3)</td>
<td>90% (9/10)</td>
<td>0.723</td>
</tr>
<tr>
<td>Lobular invasive carcinoma</td>
<td>13% (30/235)</td>
<td>13% (7/55)</td>
<td>0% (0/3)</td>
<td>0% (0/10)</td>
<td>0.596</td>
</tr>
<tr>
<td>Ductal and lobular invasive carcinoma</td>
<td>3% (6/235)</td>
<td>2% (1/55)</td>
<td>0% (0/3)</td>
<td>0% (0/10)</td>
<td>0.934</td>
</tr>
<tr>
<td>Other invasive carcinoma</td>
<td>3% (6/235)</td>
<td>0% (0/55)</td>
<td>0% (0/3)</td>
<td>0% (0/10)</td>
<td>0.621</td>
</tr>
<tr>
<td>Ductal in situ carcinoma</td>
<td>0% (0/235)</td>
<td>0% (0/55)</td>
<td>0% (0/3)</td>
<td>10% (1/10)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ER positivity</td>
<td>89% (196/221)</td>
<td>79% (41/52)</td>
<td>67% (2/3)</td>
<td>80% (8/10)</td>
<td>0.183</td>
</tr>
<tr>
<td>PgR positivity</td>
<td>77% (171/222)</td>
<td>71% (37/52)</td>
<td>33% (1/3)</td>
<td>60% (6/10)</td>
<td>0.167</td>
</tr>
<tr>
<td>Ki-67/Mib-1 &gt;30</td>
<td>21% (43/207)</td>
<td>39% (20/51)</td>
<td>33% (1/3)</td>
<td>43% (3/7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Her-2 positivity</td>
<td>7% (16/217)</td>
<td>18% (9/51)</td>
<td>67% (2/3)</td>
<td>30% (3/10)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Discussion

Among 65 patients operated for a suspicious axillary metastasis from an occult primary cancer, 16 (24%) presented a metastatic infiltration by an occult solid cancer: ten breast cancers, two malignant melanomas, two ovarian cancers, one bladder urothelial cancer, and one bronchus neuroendocrinous cancer. Histological patterns and molecular markers allowed in all cases of occult cancer a probable identification of the primary tumor site, while certain diagnosis was possible only in the 56% of cases (9/16). The prognosis of these patients was very poor with an OS at five years of 28%.

According to the literature, isolated axillary nodal mass represented the sole presentation of metastatic cancer in about 0.3-1% of cases [2, 7, 18]. If the authors consider, for example, the 2,097 women operated for breast cancer in their Department of General Surgery in the study period, occult breast carcinoma presenting with sole axillary node metastasis represented the 0.48% of cases (about one case every 200).

Even by the presence of an axillary specimen strongly suggestive for a breast origin, and independently from imaging findings, the histological examination succeeded in finding the primary localization of the occult breast carcinoma in only five cases (50%), and in one of them only in one section to 103. Actually, many studies demonstrate that if the tumor size is smaller than the pathological sections interval, primary tumor usually escapes detection by histology [2, 6, 7, 13–16]. Hence this is regarding small tumors that have already metastasized axilla and axillary nodal status in breast cancer is one of the most important prognostic factors for patient survival [19].

Although the breast cancer is the most common cancer among women in Western countries [20] and represents the most frequent site of occult primary tumor in case of isolated axillary node metastasis, many cancers have the potential to spread to the axilla, such as lung, thyroid, gastrointestinal, and gynecological carcinomas [1]. Therefore, despite the rarity of this condition, it is important to exclude a primary tumor other than breast.

The literature reports some cases of ovarian and peritoneal serous carcinoma metastatic to the breast and/or to the axillary lymph nodes [21–27], and sometimes very difficult to diagnose while mimicking inflammatory breast cancer [28–30]. The authors also reported one case of occult ovarian cancer presenting with an axillary mass, accompanied by a suspicious lymphangitis carcinomatosa of the breast.

Also malignant melanoma has a recognized tendency to early lymph-mediated distant dissemination [31], which seems to be more aggressive from the axilla than from the groin [32, 33]. In some cases, the absence of a visible primary site may be explained by a progressive physiological depigmentation during the natural history of melanoma [34], as well as by its mis-recognition and involuntary previous excision.

The most likely mechanism for axillary nodes involvement by lung cancer is intercostal lymphatics pathway spread from mediastinal lymph node metastasis [35]. Instead, it results more difficult to explain the lymphatic dissemination to the axilla in case of solid intra-abdominal malignancies.

Among women with occult breast cancer, mammography and breast ultrasound examination resulted false negative in the 70% of cases [7/10]. Actually, although mammography represents the current gold standard of breast imaging, and together with breast ultrasound examination has a very high accuracy for lesions of at least five mm, in the present group of patients, its ability in detecting breast occult tumor remains disappointing, mainly depending on tumor size [8], and in some cases requires many years of regular follow up in order to identify the primary tumor location [36].

Although some experiences in the literature demonstrate that MRI successfully detected occult breast cancers in about 70-83% of cases [37, 38], in other studies breast MRI resulted very weak in the detection of an occult breast primary tumor site [39], and in the present population it could not find any primary tumor site in all the cases where it was performed.

According to other recent studies, PET-CT results to be the more helpful imaging technique in identifying the primary tumor [40–43], but its diagnostic performance should be maximized by its appropriate use and interpretation [44]. Moreover, a recent meta-analysis assessed FDG-PET/CT sensitivity and specificity to be respectively, 84% (CI.95 77-88%) and 84% (CI.95 78-89%) [45]. In the present population, it succeeded in finding the primary tumor in the 64% of cases, with a diagnostic gain of the 12% (2/16). In fact, it detected one more case among occult breast cancers and one more case among non-breast occult cancers (the primary lung cancer site). Moreover, it confirmed CT finding in the patient affected by ovarian cancer, but was useless in both cases of malignant melanoma, the primary localization of which remained unknown.

In accordance with the literature, the OS of patients affected by hematologic malignancies at two and five years was respectively, 79% and 70% [46], showing a stabilization of the curve after the third follow up year. On the other hand, the prognosis of patients with an occult solid cancer was very poor, with an OS of 70% at two years and 28% at five years from the diagnosis, and a median survival of 20 months (14-44). In the current literature, CUP represents the seventh to eighth most frequent type of cancer, but the fourth most common cause of cancer-related death; it is characterized by a very aggressive natural history, early dissemination, and metastatic pattern unpredictability [47–50], with a median survival of 16 months with 10% five-year survival [49, 50].

Treatment and prognosis of occult cancer mainly depends on its histological type and clinical pathological stage [18, 51], remaining particularly controversial in case of occult breast location, because outcome are not significantly different between radical mastectomy and conservative
treatments such as quadrantectomy and radiation [18, 27, 52–58]. In the present population, all women with a suspicion of occult breast cancer were operated in their breasts: eight received a breast-conserving intervention, while two had a mastectomy performed. Then, five patients underwent further ipsilateral breast irradiation and chemotherapy, three underwent only adjuvant breast radiation therapy, five received also additional lymph node radiation therapy as appropriate, and two underwent a watchful policy (one for personal choice and the second because of her advanced age and bad general conditions).

Also in these cases of breast cancer presenting as isolated axillary swelling, biological characteristics represented an important predictive factor for axillary metastasis [59]. In fact, cancers that presented as isolated axillary swelling had high proliferation index and high prevalence of Her2 positivity. Generally the more axillary lymph node involvement is associated with high T [60] in these cases small non-palpable breast cancers or unknown primary breast cancers were so aggressive to give overt axillary metastasis as first sign.

Finally, breast cancer axillary metastasis from unknown primary site resulted associated in the literature with similar presentation, biology, and outcome to node-positive overt breast cancer, and should be treated accordingly [1]. Nonetheless in the authors’ opinion, although a recent review compares breast CUP with Stage II overt breast cancer [61], occult breast cancers with axillary metastasis behave more similarly to stage IV overt breast cancers than to stage II or III.

Conclusions

In case of an occult primary tumor presenting with a sole axillary node metastasis, breast cancer should always be excluded, before taking into consideration other possible primary malignancies. In this perspective, immunohistochemistry could be helpful with a detailed portrait of the cancer molecular expression, in order to detect the occult primary site and identify some favorable therapeutic targets. In addition, the modern imaging diagnostic tools may support primary site suspicion, although with an insufficient accuracy. PET-CT seems to be the more helpful imaging technique in this particular group of patients, but has however a margin of failure.

In the authors’ opinion, focusing on occult primary breast cancers, there may be a particular type of cancer with an important lymphatic tropism, and a very poor prognosis, which do not depend on the primary tumor growth. Moreover, along with the very low imaging detection rate of the primary breast cancer, and its even lower detection rate with histological examination of the specimen, even by radical breast surgery, it is very discouraging that many of the women included in this report were regularly undergoing the suggested screening for breast cancer and in this group of patients was completely helpless.

References

Isolated axillary nodal swelling and cancer of unknown primary


S100P is a useful marker for differentiation of ovarian mucinous tumors

Y. Umezaki¹, M. Ito¹, M. Nakashima², Y. Mihara¹, Y. Naruke¹, H. Kurohama¹, N. Yatsunami³, I. Yasuhi³

¹Department of Pathology, National Hospital Organization Nagasaki Medical Center, Nagasaki
²Department of Tumor and Diagnostic Pathology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki
³Department of Obstetrics and Gynaecology, National Hospital Organization Nagasaki Medical Center, Nagasaki (Japan)

Summary

The S100P protein stimulates cell proliferation and survival, thereby contributing to tumor progression. The purpose of this study was to evaluate S100P expression in the three subtypes of mucinous cystic tumors, cystadenomas, borderline tumors, and adenocarcinomas. The authors examined nuclear S100P expression in 60 mucinous ovarian tumor specimens, including 24 specimens of mucinous cystadenoma, 15 of borderline tumors, and 21 of adenocarcinomas. Immunohistochemistry revealed S100P expression followed one of three patterns: (1) Expressed in most nuclei of mucinous epithelial cells, (2) sporadic (spotted or patchy) expression, or (3) absent or rarely expressed in the nuclei of mucinous epithelial cells. Most adenomas showed the first expression pattern, and borderline tumors often showed a patchy expression pattern. Adenocarcinomas generally demonstrated absence of S100P expression. These data suggest that S100P is a useful histological marker to differentiate between benign, borderline, and malignant mucinous tumors of the ovary.

Key words: Mucinous tumor; Ovary; S100P; Immunohistochemistry.

Introduction

Morphological differentiation of ovarian mucinous cystic tumors is sometimes difficult, and distinction between benign, borderline, and malignant lesions is important for determining prognosis and treatment. For instance, pathologists waver in diagnosis between mucinous benign tumors and slightly atypical borderline tumors or between highly atypical borderline tumors and adenocarcinomas. The present authors examined the usefulness of S100P immunohistochemistry in improving the diagnostic accuracy of ovarian cancer.

S100P protein is a member of the S100 subfamily of calcium-bound proteins; its expression is predominantly observed in the placenta, as well as various human tissues and tumors. S100P promotes cancer progression via its specific roles in cell proliferation, survival, angiogenesis, and metastasis. Signal transduction pathways and the regulatory molecules that mediate these effects include Ca²⁺ ions [1], the receptor for advanced glycation end products (RAGE)-dependent pathway proteins [2], ezrin [3], calcyclin-binding protein/Siah-1-interacting protein (CacyBP/SIP) [4], and cathepsin D [5].

Although the molecular function of S100P is hitherto unclear, an association between S100P overexpression and poor prognosis of pancreatic [6], colorectal [7], lung [8], breast [9], and prostate [10] cancers has been reported. However, only one report has demonstrated S100P expression in the ovaries; Surowiak et al. briefly described expression of this protein in malignant ovarian tumors [11].

The present authors investigated S100P expression in the three subgroups of ovarian mucinous cystic tumors, namely, cystadenoma, borderline tumors, and adenocarcinomas, in order to characterize S100P expression during tumor progression toward malignancy and to assess whether this protein can serve as a clinical biomarker for differentiation of benign, borderline, and malignant tumors.

Materials and Methods

Tissue samples

Tumor tissue specimens were collected from patients who underwent standard surgical treatment and histopathological examination at the National Hospital Organization Nagasaki Medical Center between 2001 and 2010. The present study protocol conformed to the ethics guidelines of the 1975 Declaration of Helsinki, and informed consent was obtained from all patients before these specimens were used for the study.

The examined specimens of ovarian mucinous tumors included 24 cystadenomas, 15 borderline tumors, and 21 adenocarcinomas. Tumor definition was based on the World Health Organization classification and the specimens were evaluated by two pathologists (Y.U. and M.I.).

When a given specimen contained a mixture of two or more tumor subtypes, the histological diagnosis with the higher or highest malignancy level was adopted, respectively. Patients with ovarian and multiple other coexistent cancers and those who had undergone preoperative chemotherapy were excluded.

Revised manuscript accepted for publication December 30, 2013
Immunohistochemical staining and evaluation

Each tumor tissue was fixed with 10% formalin, embedded in paraffin, and cut into three-μm slices. Immunohistochemistry was performed with a specific system and an autostainer after quenching of endogenous peroxidase with 0.3% aqueous hydrogen peroxide solution. Antigen retrieval was performed by heating samples in citrate buffer (pH 6.0) using a pressurized heating chamber. The rabbit polyclonal anti-S100P antibody (1:100 dilution) was used as the primary antibody. Immunostaining images were analyzed to determine the distribution and percentage of positively stained cells, and the intensity of chromatic responses. The results were classified into three staining patterns, as shown in Figure 1. S100P was either expressed in most nuclei of mucinous epithelial cells (Figure 1a), sporadically expressed (spotted or patchy) (Figure 1b), or absent or rarely expressed in the nuclei of mucinous epithelial cells (Figure 1c).

The stains were evaluated by two pathologists (Y.U. and M.I.) who were not informed about the clinical course (including treatment) of the concerned patients. The chromatic responses were also classified into the three aforementioned patterns.

Table 1. — Immunohistochemical staining patterns of S100P in ovarian mucinous tumors.

<table>
<thead>
<tr>
<th>S100P expression</th>
<th>Pattern 1</th>
<th>Pattern 2</th>
<th>Pattern 3</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystadenoma (n = 24)</td>
<td>19</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Borderline Tumor (n = 15)</td>
<td>0</td>
<td>13</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adenocarcinoma (n = 21)</td>
<td>0</td>
<td>8</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

Pattern 1: Positive staining in almost all neoplastic cells.
Pattern 2: Staining present in some or even most but not in all neoplastic cells.
Pattern 3: Isolated positive cells or absent in most neoplastic cells.

Statistical Analysis

Intergroup differences were analyzed by Chi square analysis. A $p < 0.05$ was regarded as statistically significant. Statistical analysis was performed using the computer program Prism 5.0d.
Results

Table 1 shows the chromatic responses, which were divided into three patterns, in cystadenomas, borderline malignant tumors, and adenocarcinomas of ovarian mucinous cystic tumors. Most mucinous cystadenomas showed pattern 1 (79%, 19/24), borderline tumors predominantly demonstrated pattern 2 (86.7%, 13/15), and adenocarcinomas showed either pattern 2 (38%, 8/21) or pattern 3 (61.9%, 13/21). S100P expression patterns significantly differed between the 3 cancer subtypes (p < 0.01).

Figure 2 shows a case of mucinous borderline tumor associated with carcinoma in situ. S100P was absent in the carcinoma in situ region (pattern 3), and patchy expressed was observed in the borderline malignant tumor (pattern 2), clearly highlighting the immunohistochemical difference between borderline malignant tumors and adenocarcinomas.

Discussion

Morphological differentiation between ovarian mucinous cystic tumors is sometimes difficult, especially when differentiating between benign and slightly atypical borderline malignant tumors or between highly atypical borderline malignant tumors and adenocarcinomas. To achieve accurate diagnosis for mucinous cystic tumors, auxiliary diagnostic techniques, such as immunohistochemistry, if available, are extremely helpful.

This study revealed two new findings regarding S100P expression in ovarian mucinous tumors. First, S100P expression decreased with increasing degrees of atypia and polarity turbulence (benign < borderline < malignant). Second, morphological distinction between benign, borderline malignant, and malignant tumors was possible on the basis of S100P expression profile. Differences in S100P expression between the three different mucinous tumors were statistically significant (Table 1). These findings suggest that the pattern of S100P expression is useful as an auxiliary means of diagnosis for ovarian mucinous cystic tumors.

Although the exact molecular function of the S100P protein is unknown, the present results suggest a possible involvement of S100P in the differentiation and polarity of ovarian mucinous tumors.

To date, associations between increased S100P expression in tumor tissues and low survival rates have been reported for a variety of malignancies, such as pancreatic, breast, and colorectal cancers. In bile duct lesions, S100P expression increases with the degree of atypia and is reduced in cholangiocarcinomas [12]. These findings suggest that the role of S100P varies in different tumor histology and organs.

The significance of S100P expression in ovarian cancer has not been clarified. Surowiak et al. studied 73 cases of ovarian cancer; they reported that excessive S100P expression was associated with tumor recurrence and shortened progression-free survival, although their analysis was not based on the histological types of ovarian cancer [11]. In vitro analyses conducted by Gao et al. [13] and Wang et al. [14] indicated that cultured ovarian cancer cells with low S100P expression were resistant to paclitaxel, whereas those with high S100P expression rendered the cells more susceptible to paclitaxel and carboplatin. These reports suggest that S100P may play a role in tumor susceptibility to chemotherapy. However, the molecular mechanisms of S100P and tumor susceptibility have not yet been further elucidated.

In conclusion, the present authors showed that S100P expression in ovarian mucinous tumors decreases as tumor atypia increases and that its differential expression profiles allow distinction between benign, borderline, and malignant tumors. These findings suggest that S100P is associated with tumor differentiation, and S100P immunohistochemistry is a useful marker for differentiation between the different subtypes of ovarian mucinous cystic tumors.

References


Address reprint requests to:
Y. UMEZAKI, M.D.
National Hospital Organization
Nagasaki Medical Center
2-1001-1 Kubara, Omura 856-0835
Nagasaki (Japan)
e-mail: umezaki@nagasaki-mc.com
Pelvic exenteration – our initial experience in 15 cases

M.E. Căpîlna1, B. Moldovan2, B. Szabo1
1First Clinic of Obstetrics and Gynecology, University of Medicine and Pharmacy, Târgu-Mureş; 2“Sf. Constantin” Hospital, Braşov (Romania)

Summary

Objective: To analyse the initial experience of pelvic exenteration for gynaecological malignancies in a tertiary referral center. Materials and Methods: Between 2011 and 2013, 15 patients underwent a pelvic exenteration for gynaecological malignancies. Results: Out of the 15 exenterations, six were total, four anterior, and five posterior. The indication was cervical (nine patients), advanced vaginal (one patient), and ovarian cancer (in five patients). A Bricker non-continent ileal urinary conduit was performed in all ten anterior and total exenterations. In-hospital complications occurred in six patients (40%) of whom two perioperative deaths (13%). Among the 15 patients, at this moment, eight are alive and six died because of the disease, and one was lost to follow-up. Conclusion: Pelvic exenteration for recurrent or advanced pelvic malignancies can be associated with long-term survival and even cure without high perioperative mortality in properly selected patients. However, postoperative complications are common and can be lethal.

Key words: Pelvic exenteration; Gynecologic malignancies; Perioperative mortality.

Introduction

After initially published by Alexander Brunschwig in 1948 [1] with a palliative intent and described as “the most radical surgical attack against the pelvic cancer”, pelvic exenteration became an ultimate, salvage therapy for patients with advanced or recurrent pelvic cancers. It is considered an extremely difficult and demanding procedure for both surgeon and anaesthesiologist, with an intra- and perioperative mortality between 0 and 9% [2-12], but, if succeeded, for those patients without other alternative curative option, the five-years survival rate ranges between 20% and 60% [2-13].

The main indications are the central pelvic recurrences after gynaecologic, urologic or rectal cancers. In later years, the indications have expanded to include also lateral recurrences involving the pelvic side wall when resection with clear margins is achievable, making it possible to offer salvage therapy to selected patients previously regarded to be incurable [14]. Occasionally, pelvic exenteration is performed as primary treatment for advanced pelvic malignancies with the intent of excising the malignancy en bloc [15], as well as for palliation in patients with severe symptoms, like intense pelvic pain, bleeding difficult to control, fistulas or grossly changes of local anatomy, where no other treatment options exist.

Pelvic exenterations may be total (removal of urinary bladder, rectum, vagina, tumour), anterior (urinary bladder, vagina, tumour) or posterior (rectum, vagina, tumour). In all three situations, it is mandatory to remove the uterus and the adnexae, if not previously removed. An anterior exenteration generates the need for a urinary diversion, which can be incontinent or continent. Also, the continent urinary diversion may be heterotopic, when the reservoir is placed under the abdominal wall and the patient has to catheterize herself, or orthotopic, when the new reservoir is placed in the pelvis and the patient voids through her preserved urethra [13, 16, 17]. The procedure can be classified also as suprarectal, infrarectal or infrarectal with vulvectomy [3] depending on the resection lines in relation to the levator ani muscles.

An infrarectal excision including the removal of the anal canal requires a permanent colostomy, and a total colpectomy requires the creation of a neo-vagina for the patients who desire to maintain their sexual function [13].

The objective of this study was to review the authors’ pelvic exenteration initial experience for patients with gynaecologic cancers, in terms of patient selection, indications, surgical technique, and complications.

Materials and Methods

Between August 2011 and September 2013, 15 patients were submitted for a pelvic exenteration in the First Clinic of Obstetrics and Gynaecology, University of Medicine and Pharmacy Târgu-Mureş, Romania. This procedure was initially considered feasible in 18 patients, but it succeeded only in 15. Even when complete tumour resection was assessed as possible after preoperative staging, the surgical procedure was abandoned in three patients. In two patients the tumour was found impossible to be removed because of sidewall involvement with extension to the bony structure or tumour involving the neurovascular structures of the sciatic foramen (especially the first sacral plexus root), and in one patient, multiple metastases have been discovered in the omentum and peritoneum. Patients’ age ranged between 36 and 73 years. All the procedures were considered with a curative intent. The preoperative assessment included mandatory a computed tomography (CT) or magnetic resonance imaging (MRI), for exclusion of extrapelvic disease and evaluation of operability. All
M.E. Căpîlna, B. Moldovan, B. Szabo

143

patients proposed for a total or anterior exenteration underwent cystoscopy, and for a total or posterior exenteration a colonoscopy. Two patients with cervical cancer Stage IVa (bladder mucosa involvement and unilateral hydronephrosis) decided for primary anterior exenteration as treatment and refused radiochemotherapy, when they asked for the treatment options. One patient with a Stage IVa vaginal cancer was treated 19 years before with surgery and radiotherapy for a cervical cancer. Also, in two patients with pelvic advanced ovarian cancer, the authors considered as posterior exenteration en-bloc removal of uterus, adnexae, recto-sigmoid junction together with the tumours of the pouch of Douglas; the procedure necessitating a retroperitoneal and pelvic side-wall dissection. All the other cases were exenterations performed for recurrent or persistent cervical cancer after radiochemotherapy or for central pelvic recurrent ovarian cancer. The authors did not consider mandatory to obtain a histopathologic confirmation of all recurrences or persistent cervical cancers when the clinical or imaging were doubtful. In all cases when, during the procedure, a complete resection was considered impossible with macroscopically no residual tumour (R0), the surgery was abandoned. A detailed informed consent was obtained for each patient before surgery. Complications were divided as early (< 30 days) or late (> 30 days). For each patient, only the highest complication was recorded when a complication clearly occurred as a consequence of a prior complication of a lower grade.

Results

Out of the 15 exenterations, six were total, four anterior, and five posterior. The indication was recurrent (for seven patients) or advanced (for two) cervical cancer, vaginal (for one) and ovarian cancer (in five patients). All ten total or anterior exenteration underwent a urinary diversion by Bricker
Table 1. — Oncologic indications, type of exenteration, and early and late complications for the 15 patients who suffered a pelvic exenteration.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>36-73 (median 54.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gynecological cancer</td>
<td></td>
</tr>
<tr>
<td>Cervix - recurrent or persistent</td>
<td>7 (46.6%)</td>
</tr>
<tr>
<td>- Stage IVA</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>Vagina</td>
<td>1 (6.6%)</td>
</tr>
<tr>
<td>Ovary - recurrent</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>- Stage IV</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>Type of exenteration</td>
<td></td>
</tr>
<tr>
<td>Supralevatorian</td>
<td>11 (73.3%)</td>
</tr>
<tr>
<td>Infraclevatorian</td>
<td>4 (26.6%)</td>
</tr>
<tr>
<td>Complications</td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Late</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>Patients’ status</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>8 (53.3%)</td>
</tr>
<tr>
<td>Dead of disease</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>1 (6.6%)</td>
</tr>
</tbody>
</table>

Pelvic exenteration – our initial experience in 15 cases

Discussion

Analyzing the results of pelvic exenteration series, it must be keep in mind that this procedure remains the only option and the only potentially curative treatment for these patients with recurrent or advanced pelvic malignancy. Even when a tendency to push forward the indications occurs, the medical (poor general conditions or all other illness causing problems for a long and difficult surgical procedure and recovery) and surgical-oncologic contraindications, like extrapelvic metastases (exception – isolated hepatic or pulmonary one), distance lymph nodes metastases (inclusive para-aortal), sidewall involvement with extension to the bony structures of the pelvis or tumour involving the neurovascular structures of the sciatic foramen, must be respected. Nonetheless, considerable differences exist between indications and contraindications for exenteration within and between countries.

The mainstay for treatment success in terms of locoregional control and long-term survival is resection of the pelvic tumour with clear margins [14, 16, 19]. In this series, the procedure was abandoned in three patients when complete tumour removal was considered impossible. Margin status appears to be the factor most consistently associated with prognosis [14, 19]. Pelvic sidewall involvement was previously considered a contraindication for exenterations with curative intent [14, 20], but since then, studies have shown equal results as for central recurrences when a complete resection can be performed [14, 19, 21-23]. However, resections including pelvic side wall are technically demanding and may be associated with increased risks. Patients considered for exenterations with curative intent should be properly selected based on thorough clinical and imaging assessment to minimize the risk of performing resections with involved margins or to abandon the procedure based on intraoperative findings.

Perioperative mortality in more recent studies ranges between 0% and 9% [5-9, 14]. In the present authors’ initial series, they had two deaths in the first 30 days after the surgery in 15 patients - a higher perioperative mortality of 13%. The present department is the first gynaecological one in Romania, a country with an extremely high incidence of cervical cancer, to perform such hyper-radical procedures. These are the authors’ initial results; probably, by achieving more experience in all the steps already mentioned, the morbidity...
and mortality related to pelvic exenteration will decrease and the survival will be better.

Introducing exenteration is paramount for a group of cases. It is a complicated procedure, needs special training, surgical devices (as staplers, vessel sealing devices, etc), and special postoperative care. Introducing this procedure has a learning curve, and thus an initial relative risk. It seems that this experience (with acceptable morbidity and mortality rate) might encourage other services to begin using exenterative procedures. For certain, an international experience is needed in teaching and learning complicated and infrequent surgical procedures. Aiming to obtain maximum results in terms of patients cure and survival, clear protocols must be established for all the steps to be followed in the management of such a case: patient selection, preoperative assessment, surgical procedure, intensive care support, and recovery period.

The major limitations of this report are the retrospective nature of the study, the small number of patients included, the limited follow-up period, and the heterogeneity of diagnoses for which the exenterations were performed. These drawbacks restricted a statistical analysis and major conclusions should be drawn with cause.

Overall, pelvic exenteration for recurrent or advanced pelvic malignancies can be associated with long-term survival and even cure without high perioperative mortality in properly selected patients [24]. New devices, such as the harmonic scalpel, new vessel sealants, and mechanical staplers have diminished the operative time dramatically, increasing the safety of the vascular ligatures at the same time. However, postoperative complications are common and can be lethal. Complete surgical resection with negative margins is associated with sustained survival and should be the goal of surgery. An international experience is needed in teaching and learning complicated and infrequent surgical procedures.

Conclusions

Pelvic exenteration for recurrent or advanced pelvic malignancies can be associated with long-term survival and even cure without high perioperative mortality in properly selected patients. However, postoperative complications are common and can be lethal.

References


Address reprint requests to: M.E. CĂPÎLNA, M.D.
First Clinic of Obstetrics and Gynecology, University of Medicine and Pharmacy, str. Gheorghe Marinescu no. 50, 540136, Târgu-Mureș (Romania)
e-mail: mcapilna@gmail.com
Introduction

Endometrial carcinoma is one of the common malignant tumors in female genital tract, with increased morbidity and mortality in recent years. According to dependency on sex hormone, endometrial carcinoma is divided into type I and type II [1], and the former is estrogen-dependent tumor, accounting for 80%-85% of endometrial carcinoma. The carcinogenesis mechanism of endometrial carcinoma on molecular level is not clear. At present, it was considered to be associated with the abnormal expression of oncogene, tumor suppressor gene and DNA repair gene. Detection of tumor suppressor gene, oncogene or cytokine helps in analyzing the properties of endometrial carcinoma and understanding the relationship between tumor and tissue differentiation. This has great significance and value for further revealing the molecular biological mechanism of endometrial carcinoma and exploring new treatment strategies including combined gene therapy [2].

Oncogene C-erbB-2, also known as neu or HER-2, was discovered in 1981. It plays an important role in cell signal transduction, and is an important regulator in cell growth, differentiation, and survival. The overexpression of C-erbB-2 has important regulatory effect on tumor formation and growth process, and has a certain relationship with some biological behaviors of tumor. It exists in breast cancer, ovarian cancer, and renal cell carcinoma [3]. Macrophage migration inhibitory factor (MIF) is a type of multifunctional cytokine, and is an important regulatory factor in inflammatory and immune responses. MIF is related with growth factor-dependent cell proliferation, cell cycle change, angiogenesis, and tumor formation. At the same time, it directly influences the division of normal cells and oncogene-induced malignant transformation. MIF can also regulate immune response, inhibit function of tumor suppressor gene, and promote tumor angiogenesis, thus promoting the occurrence and development of tumor from multiple levels. In recent years, MIF has been a hotspot in tumor research [4]. At present, the correlations of C-erbB-2 and MIF with endometrial carcinoma have not been reported. Elucidation of these problems has important significance for early detection and treatment of endometrial carcinoma.

Endometrial hyperplasia is the most common cause of dysfunctional uterine bleeding in women of childbearing age. Part of endometrial hyperplasia lesions are precancerous lesions of endometrial carcinoma; thus a precise diagnosis and prompt treatment will be of great significance in preventing the progression of the disease [5]. Growth factors are a class of polypeptide substances, which widely exist in various tissues of the body. Endometrial hyperplasia is closely associated with the dysregulation of growth factors. Various kinds of growth factors and their related peptides, combining with various components of endometrial and the hormones secreted by...
ovarian cells and body, constitute a complicated network to regulate the orderly cyclical changes of endometrium and maintain the stability of the endometrium [6]. Recently, the relationship between the growth factors and endometrium has drawn wide attentions. Thus, it is very important to carry out in-depth studies of the effects of growth factors on endometrial hyperplasia disorders and diseases. Leukemia inhibitory factor (LIF) and MIF have extensive biological effects including promoting cell proliferation and differentiation [7]. Recently, the relationship between growth factors and endometrial carcinoma has attracted more and more attentions. In this study, the correlations of LIF and MIF with endometrial carcinoma were investigated, in order to obtain new progress in studying the mechanism of endometrial hyperplasia.

Materials and Methods

Specimens

The study included 113 paraffin-embedded specimens from the department of Pathology, submitted from May 2006 to October 2008, collected for detection of LIF and MIF. All the 113 specimens were classified pathologically into normal endometrium (n=13), hyperplastic endometrium [(n=55, including simple hyperplasia (n=17), complex hyperplasia (n=18), atypical hyperplasia (n=20)] and endometrial carcinoma (n=45). All the subjects had not been treated with any hormone replacement therapy, chemotherapy or radiotherapy before they underwent curettage or surgery. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Harbin Medical University. Written informed consent was obtained from all participants. Specimens of endometrial carcinoma were further staged according to the FIGO staging criteria into Stage I (n=14), Stage II (n=1), and Stage III (n=13). They could also be further staged according to the FIGO staging criteria into Stage I (n=14), Stage II (n=1), and Stage III (n=13). They could also be further graded into G1 (n=12), G2 (n=14), and G3 (n=19).

Immunohistochemistry

Immunohistochemistry analysis of 113 endometrium specimens was carried out with SP kits. Five μm-thick slices of paraffin-embedded specimens were dewaxed to hydrophile according to the routine procedure. Then the slides were incubated with 3% H2O2 in dark at room temperature for 15 minutes, followed by microwave heating antigen retrieval in 0.01 M citrate buffer (pH 6.0) for 20 minutes. After cooling at room temperature, non-specific binding was blocked with normal goat serum for 30 minutes. Incubation with rabbit anti-human MIF or LIF was carried out at 4°C overnight before the slides were treated with SP kit according to the manufacturer’s instruction, followed by sequential incubation with horseradish peroxidase-streptavidin and the peroxidase substrate 3’-diaminobenzidine. Nucleus was counterstained with hematoxylin.

Assessment of the results

Results were analyzed semi-quantitatively. The degree of the positive expression of LIF and MIF was the sum of the number and the intensity of the positive cells. No brown granules appearing in the endometrial tissue were regarded as negative (-); 1% to 5% of the endometrial cells appearing brown were regarded as weakly positive (+/-); 6% to 15% of the positive endometrial cells was regarded as moderate positive (+), and more than 16% of the positive endometrial cells were regarded as strong positive (++).

Statistical analysis

Results were analyzed using Chi-square test and p < 0.05 was considered as significant.

Results

Expression of MIF

MIF primarily expresses in the cytoplasm of glandular epithelial cells and some mesenchymal cells. Sometimes the nucleus of glandular epithelial cells may be stained. Thus, the cytoplasm of glandular epithelial cells or mesenchymal cells appearing brown were considered to be positive (Figure 1A, B). According to the pathological classification, 113 specimens were classified into normal endometrium, simple hyperplasia, complex hyperplasia, atypical hyperplasia, and endometrial carcinoma. As can be seen in Table 1, among the 45 cases of endometrial carcinoma, there were 42 MIF-positive cases. Its positive rate (91.11%) was obviously higher than that in the normal endometrium group and hyperplastic endometrium group (23.07% and 65.45%, p < 0.001 and p < 0.05, respectively). As compared with the positive rate in the normal endometrium group (23.07%), the MIF-positive rates in both the complex hyperplasia group (66.67%) and the atypical hyperplasia group (75.00%) increased remarkably (p < 0.05), but there was no statistical difference in the simple hyperplasia group (p > 0.05). As far as the hyperplastic endometrium was concerned, no significant difference existed in the MIF-positive rates of the three groups (p > 0.05) (Table 1).

Figure 1. — Expressions of MIF and LIF in normal endometrium and endometrial carcinoma. A: Expression of MIF in normal endometrium; B: Expression of MIF in endometrial carcinoma; C: Expression of LIF in normal endometrium; D: Expression of LIF in endometrial carcinoma.

If grading endometrial carcinoma into G1, G2, and G3, the MIF-positive rate was highest in the G3, but there was no statistical difference between G1, G2, and G3 (Table 2).
Table 1. — Expression of MIF in the endometrium of the different groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cases</th>
<th>Intensity of the positive expression</th>
<th>Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal endometrium</td>
<td>13</td>
<td>10 2 1 0</td>
<td>23.07</td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>17</td>
<td>8 6 3 0</td>
<td>59.24</td>
</tr>
<tr>
<td>Complex hyperplasia</td>
<td>18</td>
<td>6 5 5 2</td>
<td>66.67</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>20</td>
<td>5 4 5 6</td>
<td>75.00</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>45</td>
<td>3 9 15 18</td>
<td>91.11</td>
</tr>
</tbody>
</table>

Table 2. — Expression of MIF in endometrial carcinoma.

<table>
<thead>
<tr>
<th>Grades</th>
<th>Total cases</th>
<th>Intensity of the positive expression</th>
<th>Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>12</td>
<td>1 3 4 4</td>
<td>91.66</td>
</tr>
<tr>
<td>G2</td>
<td>14</td>
<td>1 3 6 4</td>
<td>92.86</td>
</tr>
<tr>
<td>G3</td>
<td>19</td>
<td>1 2 8 8</td>
<td>94.74</td>
</tr>
</tbody>
</table>

Table 3. — Expression of LIF in the endometrium of the different groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cases</th>
<th>Intensity of the positive expression</th>
<th>Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal endometrium</td>
<td>13</td>
<td>9 2 2 0</td>
<td>30.76</td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>17</td>
<td>8 7 2 0</td>
<td>52.94</td>
</tr>
<tr>
<td>Complex hyperplasia</td>
<td>18</td>
<td>7 5 4 2</td>
<td>61.10</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>20</td>
<td>5 6 6 3</td>
<td>70.00</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>45</td>
<td>14 15 13 3</td>
<td>68.88</td>
</tr>
</tbody>
</table>

Table 4. — Expression of LIF in endometrial carcinoma.

<table>
<thead>
<tr>
<th>Grades</th>
<th>Total cases</th>
<th>Intensity of the positive expression</th>
<th>Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>12</td>
<td>5 2 2 3</td>
<td>58.33</td>
</tr>
<tr>
<td>G2</td>
<td>14</td>
<td>4 3 4 6</td>
<td>71.43</td>
</tr>
<tr>
<td>G3</td>
<td>19</td>
<td>5 3 7 8</td>
<td>73.68</td>
</tr>
</tbody>
</table>

Expression of LIF

LIF also expresses mainly in the cytoplasm of glandular epithelial cells and some mesenchymal cells. Thus, the cytoplasm of glandular epithelial cells or mesenchymal cells appearing brown was considered to be positive (Figure 1C, D). There were 31/45 cases of endometrial carcinoma, 30/55 cases of hyperplastic endometrium and 4/13 cases of normal endometrium expressing LIF (Table 3). The positive rate of LIF in the normal endometrium group (30.76%) was remarkably lower than that in the atypical hyperplasia group (70.00%) and the endometrial carcinoma group (68.88%) \( p < 0.05 \), but it showed no statistical difference from that in the simple hyperplasia group (52.94%) or the complex hyperplasia group (61.10%). The positive rates of LIF in the three hyperplasia groups showed no significant difference between each other and were not different significantly from that in the endometrial carcinoma group \( p > 0.05 \) (Table 3). In the different grades of endometrial carcinoma, the LIF positive rate increased with the grade, but showed no statistical significance between the three grades (Table 4).

Discussion

The main purpose of this study was to investigate the correlations of LIF and MIF with endometrial carcinoma. Results of immunohistochemical experiments show that the positive rate and intensity of MIF and LIF expression increase with the elevation of histological grade of endometrial carcinoma, but the differences are not statistically significant \( p > 0.05 \). This conforms to the authors’ original experimental expectation and assumption, and is consistent with existing viewpoints and results of other researchers. LIF is a cytokine with multiple biological functions. Currently, its biological effects in reproductive medicine have been a hot point in China [6-8]. LIF expresses in the endometrial glandular epithelial cells and increases in the secretory phase. In the reproductive cycle, LIF is associated with the uterine function and the regulation of endometrial growth. Endometrial glandular epithelial cells are the main production of LIF, which changes cyclically with the menstrual cycle and reaches a peak in the secretory phase, especially in the mid-secretory phase, suggesting that the expression of LIF may be directly or indirectly controlled by ovarian hormones [9, 10]. Some studies showed that adding exogenous LIF to the cultured sheep endometrial cells can stimulate cell proliferation in a dose-dependent manner, indicating that LIF may relate to the regulation of endometrial growth, self-renewal, and uterine functions [11]. In this study, the positive rate of LIF in endometrial cancer was only 70%, which may be explained by several reasons. Firstly, in the past, despite that blood LIF changes cyclically with the menstrual cycle has usually been described, no detection of LIF in tissues has virtually been reported. LIF may express weakly in normal tissue and thereby the positive rate of LIF in endometrial cancer cannot be higher than that in the normal endometrium [12]. Secondly, in the hyperplastic conditions, LIF is regulated abnormally by hormones, and thus it may be reduced in the endometrial tissues. It was reported that the expression of LIF shows no significant difference in various types of endometrial carcinoma [13]. Thus, the roles of LIF in the endometrial carcinoma cannot be verified by the results obtained in this study.

MIF is a protein comprising 115 amino acids. It is a multifunctional cytokine controlled by the hypothalamic-pituitary. Besides the activated T lymphocytes, LIF also can be produced by numerous tissue cells. It can promote the occurrence of various inflammatory diseases. Some scholars believe that MIF inhibits the cytotoxicity of NK cell against tumor cells via the inactivation of p53 and induces neovascularization for tumor formation, and thus plays an important role in the occurrence and development of tumor. With the effects of MIF, tumor cells and macrophages are involved in tumor angiogenesis, and thereby promoting tumor growth and metastasis [14]. It was also reported that MIF can stimulate macrophages to produce...
several kinds of inflammatory mediators, which could kill tumor cells [15]. Thus, MIF may play a dual role in the occurrence and development of tumor. In the researches about the relationship between MIF and endometriosis, it has been suggested that MIF limits macrophages in the ectopic foci and enhances the secretory function of macrophages to produce the growth factor and angiogenesis factor. Furthermore, MIF can directly stimulate the proliferation of vascular endothelial cell, promoting the formation of blood vessel, and thus being conducive to the maintenance and growth of ectopic endometrium [16]. Apoptosis plays an important role in maintaining the normal cyclical change of endometrial cells and the apoptosis of endometrium is regulated by apoptosis-related genes and associated with the function of sex hormone and its receptor, cytokines, and enzymes. Apoptosis disorders may be one of the causes of endometrial carcinoma [17]. MIF can inhibit apoptosis through some ways, such as inhibiting the p53 gene [18]. In this study, the positive rate of MIF reached more than 90%, indicating that there is a close relationship between MIF and endometrial cancer. However, it acts by which means still requires further study. The positive rates of MIF were high in atypical hyperplasia and endometrial carcinoma, indicating that MIF not only is related to the abnormal endometrial hyperplasia, but also plays an important role in the process of cellular malignant change [19]. A study by Levy et al. showed that MIF also expresses highly in ovarian cancer cells [20]. In recent years, with the development of genomics and molecular biological technology, it has been realized that the formation and development of malignant tumors is a process with multi-step and multiple genes involved. More and more tumor-associated factors have been discovered, and conducted to many in-depth researches. Therefore, to identify a key factor in all aspects of tumor progression has a very important practical significance. It can facilitate the detection of a certain type of tumor and quantify the indicators, and thus provides a meaningful reference to the diagnosis of cancer, the differential diagnosis, prognostic assessment, and the selection of an individualized treatment. In-depth study of LIF and MIF is expected to get some new developments in the research field of the mechanism of endometrial carcinoma.

Acknowledgements

This work was supported by the Specialized Research Fund for the Natural Science Foundation of Heilongjiang Province, People’s Republic of China (nr. H201429).

References


Address reprint requests to: W. XIAO, M.D., Department of Obstetrics and Gynecology, Forth Affiliated Hospital of Harbin Medical University, 31 Yinhang Street Harbin 150001 (China) e-mail: weixiaovcn@yeah.net
Clinical significance of ASC-US and ASC-H cytological abnormalities: a six-year experience at a single center

G.S. Demirtaş1, L. Akman2, O. Demirtaş1, B.S. Hursitoglu2, M.C. Terek2, O. Zekioglu3, H. Yılmaz2, A.A. Ozsaran2

1 Igdir State Hospital, Department Of Obstetrics and Gynecology, Igdir
2 Ege University Medical School, Department Of Obstetrics and Gynecology, Izmir
3 Ege University Medical School, Department Of Pathology, Izmir (Turkey)

Summary

Background: To evaluate colposcopic biopsy results of patients with cervical cytological findings of atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells with high-grade lesions that cannot be excluded (ASC-H). Materials and Methods: A retrospective evaluation of data from 358 patients, who had cervical cytological findings of ASC-US (n=335) and ASC-H (n=23), and had colposcopic assessments between 2005 and 2011. Results: Cervical biopsy results of patients diagnosed with ASC-US cytology (n=335) revealed cervical squamous cell carcinoma 0.9 % (n=3) at biopsy, cervical intraepithelial neoplasia 3 (CIN 3) in 3.8 % (n=13), cervical intraepithelial neoplasia 2 (CIN 2) in 1.1 % (n=4), cervical intraepithelial neoplasia 1 (CIN 1) in 35.2 % (n=118), and benign lesions in 59 % (n=197). Cervical biopsy results of patients diagnosed with ASC-H cytology (n=23) revealed CIN 3 at biopsy in 39.3% (n=9), CIN 2 in 21.7% (n=5), CIN 1 in 26% (n=6), carcinoma in situ in 8.7% (n=2), and squamous cell cancer in one patient (4.3%). Conclusion: The cytological diagnosis of ASC-US may lead to the diagnosis of cervical intraepithelial lesion of higher grades as well as cervical cancer and should be evaluated by colposcopic cervical biopsy.

Key words: ASCUS; ASC-H; Cervical cytology; Colposcopy.

Introduction

Cervical cancer is the third most commonly diagnosed cancer in women [1]. An important part of the cases of death related to cervical cancer can be prevented by the identification, using specific diagnostic method, of pre-invasive cervical lesions and their adequate treatment. Cytologic examination plays the principal role in screening for cervical pathologic conditions. The Bethesda System is used for classifying abnormalities of cervical cytology [2].

The Bethesda System published in 2001 contains major changes concerning atypical squamous cells of undetermined significance (ASC-US). Two classes are distinguished: ASC-US, which addresses the difficulty in distinguishing between reactive changes and “low grade squamous intraepithelial lesions” (LSIL) and on the other hand, the difficult distinction between reactive metaplasia and high grade squamous intraepithelial lesions (HSIL), underlined by “atypical squamous cells – cannot exclude HGSIL” (ASC-H). Patients with ASC-H have a higher proportion of moderate or severe dysplasia and a higher probability of more severe lesions compared to ASC-US.

Cytologic examination, colposcopy, and cervical biopsy are methods complementing each other. Colposcopy is useful in evaluating positive cytological results. It is a direct examination performed under bright light, with the help of a stereoscopic microscope which provides magnification by six to 40 times. The objective of cervical colposcopic assessment is the identification of lesions in the transformation zone to guide the cervical biopsy. The correct approach to the patients with abnormal cytological results consists of a combination of the cytologic, colposcopic, and histologic data.

The authors’ objective was to evaluate the colposcopic biopsy results in patients whose cervical cytologic examination showed ASC-US or ASC-H.

Materials and Methods

A total of 60,450 results of cytologic examination of the cervix, performed during the years 2005-2011 at the Ege University Gynecology and Obstetrics Department, either for various complaints including vaginal discharge, bleeding during intercourse, and menometrorrhagia or for a check-up. The authors retrospectively evaluated data from 358 patients, who had cervical cytological findings of ASC-US (n=335) and ASC-H (n=23), and had colposcopic assessments between 2005 and 2011. Cervical biopsy and endocervical curettage results were obtained from the pathology records. Colposcopic findings were obtained from the patients’ files.

Results

The mean age of the patients with ASCUS cytology result was 45.2 years and with ASC-H result, it was 39.6 years.
Cervical biopsy results of patients diagnosed with ASC-US cytology (n=335) revealed cervical squamous cell carcinoma 0.9 % (n=3) at biopsy, cervical intraepithelial neoplasia 3 (CIN 3) in 3.8% (n=13), cervical intraepithelial neoplasia 2 (CIN 2) in 1.1% (n=4), cervical intraepithelial neoplasia 1 (CIN 1) in 35.2 % (n=118), and benign lesions in 59 % (n=197). Table 1 summarizes the clinical characteristics of patients with CIN 2 and CIN 3 histopathology results after ASCUS cytology. The histopathological sections of cervical smear, cervical biopsy, conization, and hysterectomy specimen of the case 3 in Table 1 are shown in Figure 1. Cervical biopsy results of patients diagnosed with

<table>
<thead>
<tr>
<th>Cases No</th>
<th>Age</th>
<th>Cervical biopsy</th>
<th>Endocervical canal curettage</th>
<th>First procedure/ Histopathology</th>
<th>HPV status</th>
<th>Colposcopic findings</th>
<th>Second procedure/ histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>CIN 3</td>
<td>CIN 3</td>
<td>Conization/ CIN 3</td>
<td>6 (+), 16 (-)</td>
<td>Acetowhite area</td>
<td>Hysterectomy/ CIN3</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>CIN 3</td>
<td>CIN 3</td>
<td>Conization/ CIN 3</td>
<td>6 (+), 16 (+)</td>
<td>Normal</td>
<td>Hysterectomy/ CIN2</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>CIN 3</td>
<td>CIN 3</td>
<td>Conization/ CIN 3</td>
<td>-</td>
<td>Abnormal vascularity</td>
<td>Hysterectomy/ CIN3</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
<td>CIN 3</td>
<td>CIN 3</td>
<td>Hysterectomy/ CIN 3</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>CIN 3</td>
<td>Normal</td>
<td>Conization/ CIN 3</td>
<td>6 (+), 16 (+)</td>
<td>Abnormal vascularity</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>43</td>
<td>CIN 3</td>
<td>CIN 2</td>
<td>Conization/ CIN 3</td>
<td>6 (+), 16 (-)</td>
<td>Mosaic pattern</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>36</td>
<td>CIN 3</td>
<td>CIN 3</td>
<td>Hysterectomy/ CIN 3</td>
<td>6 (+), 16 (-)</td>
<td>HGL</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>64</td>
<td>CIN 3</td>
<td>CIN 3</td>
<td>Hysterectomy/ CIN 3</td>
<td>-</td>
<td>TZ not completely seen, nodular appearance of cervix</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>51</td>
<td>CIN 3</td>
<td>Normal</td>
<td>Hysterectomy/ CIN 3</td>
<td>-</td>
<td>HGL</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>38</td>
<td>CIN 3</td>
<td>CIN 3</td>
<td>LEEP/ CIN 3</td>
<td>-</td>
<td>HGL</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>44</td>
<td>CIN 3</td>
<td>CIN 3</td>
<td>Hysterectomy/ Ca in situ</td>
<td>-</td>
<td>HGL</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td>CIN 3</td>
<td>CIN 3</td>
<td>LEEP/ CIN 2</td>
<td>-</td>
<td>Dense acetowhite lesion</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>41</td>
<td>CIN 3</td>
<td>CIN 3</td>
<td>Hysterectomy/ CIN 1</td>
<td>-</td>
<td>Mosaic pattern</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>36</td>
<td>CIN 2</td>
<td>Normal</td>
<td>LEEP/ CIN 2</td>
<td>-</td>
<td>Mosaic pattern</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>CIN 2</td>
<td>Normal</td>
<td>LEEP/ CIN 2</td>
<td>-</td>
<td>Aceto-white area and punctuation</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>35</td>
<td>CIN 2</td>
<td>Normal</td>
<td>LEEP/ CIN 2</td>
<td>-</td>
<td>Aceto-white area</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>26</td>
<td>CIN 2</td>
<td>CIN 1</td>
<td>LEEP/ CIN 1</td>
<td>6 (+), 16 (+)</td>
<td>Normal</td>
<td>-</td>
</tr>
</tbody>
</table>

CIN: cervical intraepithelial neoplasia; LEEP: loop electrosurgical excision procedure; ECC: endocervical curettage; HGL: high grade lesion.

**Table 1.** The clinical characteristics of patients with CIN 2 and CIN 3 histopathology results after ASCUS cytology.

**Cervical biopsy results of patients diagnosed with ASC-US cytology**

**Figure 1.** In a 36-year-old patient (a) cervical cytology shows atypical squamous cells demonstrating minimally increased nucleus/cytoplasm ratio, minimal nuclear hyperchromasia, and nuclear shape irregularity (ASC-US cytology) (x40). (b) Cervical biopsy specimen shows cervical intraepithelial neoplasia 3 in surface epithelium (x20). (c) Conization specimen shows diffuse CIN 3 in surface epithelium (x20). (d) Hysterectomy specimen shows carcinoma in situ with dyskeratotic cells.
ASC-H cytology (n=23) revealed CIN 3 at biopsy in 39.3% (n=9), CIN 2 in 21.7% (n=5), CIN 1 in 26% (n=6), carcinoma in situ in 8.7% (n=2) and squamous cell cancer in one patient (4.3%). Table 2 summarizes the clinical characteristics of patients with CIN 2 and CIN 3 histopathology results after ASC-H cytology. The histopathological sections of cervical smear, cervical biopsy, and hysterectomy specimen of the case 3 in Table 2 are shown in Figure 2.

Discussion

The diagnosis of ASC-US is confusing because of the circumstances of its use and the reality it is supposed to represent [3]. The use of this term allows a solution for classifying cases which cannot in reality be allocated to either the low or the high risk groups. Over two million women are diagnosed with ASC-US in the USA as a result of cervical cytology screening [4]. A survey of different lab-
oratories shows a frequency of ASC-US diagnosis varying from 1.07 to 9% [5-9]. This frequency was determined as being 5% in a study by Gerber et al. [6] in 29,829 patients. According to Turkish Cervical Cancer and Cervical Cytology Research Group, the prevalence in Turkey is 1.07%. Lee et al. [9] reported a diagnosis of ASC-US in 1,035 (2%) of 49,882 cytology results. According to certain authors, the frequency of diagnoses of ASC-US should not exceed two to three times that of LGSIL [10, 11]. While the number of ASC-US was 335 among a total of 60,450 cases (0.55%) between 2005 and 2011, there were 118 (0.19%) patients with LGSIL in the same period. These findings do not seem to correspond to those in other published reports.

A major cause of discrepancies in the abnormal cervical cytology frequencies is the absence of agreement among pathologists. The present authors correlate the low rate of ASC-US diagnosis in this study to a correct transmission of patient data between clinician and pathologist and also to the evaluation of the preparations by experienced pathologists. Another possible reason for the lower ASC-US rate in the present study as compared to the levels generally found in Europe or America might have been the fact that this population is in a relatively low risk group for cervical cancer. In Turkish literature, the range is also lower compared to other countries [8]. The present data, however, are similar to the target frequencies published by the National Cancer Institute (NCI) in 1992. According to the latter, the rate of ASC-US in low risk populations should be < 5% [12,13].

The Bethesda System published in 2001 contains major changes with regards to (ASC-US). Approximately 5-10% of all cervical cytologic examinations should be diagnosed as ASC-US when evaluated by the Bethesda System. A higher frequency of ASC-US diagnosis would require a check of the particular laboratory’s quality control. According to this classification, atypical squamous cells are divided into two classes: ASC-US (the group at the origin of the difficulty in distinguishing between reactive changes and LGSIL), and ASC-H (the group creating difficulty in distinguishing between reactive metaplasia and high grade squamous intraepithelial lesions) [9,14]. Morin et al. [15] reported 22.2% CIN in 360 patients with an ASC-US diagnosis with CIN 1 in 16% of these and CIN 2-3 in 5.3%.

In the present study, the authors determined the presence of 118 (35.2%) CIN 1, 17 (5%) CIN 2, and CIN 3 among the present 335 patients with ASC-US. The authors observed that this CIN 1 proportion is higher if compared to published data, while the high grade lesion (CIN 2 and CIN 3) frequency approximates that available rates in the literature.

Three choices are available for follow up of ASC-US diagnosis. The first of these is to perform monthly follow-up cervical cytologic examinations, the second a human papillomavirus (HPV) typing test, and the third is that of a colposcopy to define the areas at risk with cervical biopsies as needed. The authors of the ASCUS-LSIL Triage Study (ALTS) have indicated that HPV DNA typing followed by colposcopy for patients who are positive for HPV DNA is as effective as immediate colposcopy for purposes of identifying patients with CIN I or cancer [16].

Shalini et al. [16] studied the cost-effectiveness of HPV DNAtyping of conservative treatment and immediate colposcopy based on the ALTS. They concluded that testing for HPV DNA may be an economically preferable solution for the triage of ASC-US. In the present authors’ opinion, colposcopy has a great and certain place for the triage of cervical pathologies in developing countries. Colposcopic biopsy is used in the present clinic for patients with ASC-US diagnosis, because of the presence of physicians experienced in colposcopic diagnosis of preinvasive cervical lesions and cervical cancer. Based on ALTS, more recent studies have recommended using HPV DNA positivity more in patients with an ASC-H diagnosis, those with ASC-US, and, due to the higher probability, in the former of an underlying HGSIL, to perform direct colposcopic biopsy instead of HPV DNA testing [17].

In the present series, one case of squamous cell cervical cancer was encountered in the pathology results of the 23 patients with a diagnosis of ASC-H in the years 2005-2011. In the authors’ opinion, this is due to the low number of patients. As for the 335 patients diagnosed with ASC-US, three patients had cervical carcinoma. The authors therefore think that patients in whom a diagnosis of ASC-US is made, should be carefully evaluated. In conventional cervical cytology, the physician may not obtain the correct specimen or the cells may be lost during processing. These may be the results of cervical cancer causes diagnosed by ASCUS cytology.

In cytologic diagnosis, ASC-US may be confused with reactive changes. Given the knowledge of the predominantly LGSIL type of underlying disease, the approximately 50% probability of spontaneous regression of the lesions, the slow progression, the very low concomitance of carcinoma in situ, and the low negative cervical cytology rate on repetition, a histologic evaluation is not recommended for patients with a diagnosis of ASC-US [18]. The difference, however, is in the 9.2% frequency of CIN when considering all cases of ASC-US. Several reports indicate a CIN frequency of 10-45% in the follow-up of cases with a diagnosis of ASC-US [19, 20]. Barreth et al. reported 2.9% cervical cancer, 1.7% adenocarcinoma in situ, and 65.6% HSIL in 517 patients histologically after ASC-H cytology result. Patients with an ASC-H result should undergo timely colposcopic and histologic assessment to rule out invasive cervical disease [21].

In conclusion, ASC-US is a relatively frequent finding especially in gynecology clinics; the testing sensitivity can be influenced by the cytologic sampling method and the pathologist’s experience in evaluating the result and high grade cervical lesions may be identified after ASC-US cytology result. Even though the morphologic criteria have been
painstakingly defined, the possibility of diagnosing ASC-US in cases in which HGSIL or other cervical epithelial lesions are present and the absence of certainty in this regard leads to wariness with regards to a diagnosis of ASC-US. It is therefore recommended to follow up cases with a diagnosis of ASC-US with repeat cytology six months later, and colposcopy with colposcopic biopsy if the suspicious finding is repeated. Colposcopic examination of cervix in cases of atypical cervical cytology is cost-effective and should be performed liberally especially in developing countries. Colposcopic evaluation is important and patients should be evaluated whenever possible by colposcopy-directed biopsy or biopsy should be repeated following treatment of infection and reactive changes and should the abnormal cytologic picture persist, the patient is referred in any case to a center equipped for colposcopic examination.

References


Address reprint requests to: L. AKMAN, M.D.
Ege University Medical School
Department Of Obstetrics and Gynecology
Bornova, 35100 - Izmir (Turkey)
e-mail: leventakman@gmail.com
Disease-free ovarian cancer patients report severe pain and fatigue over time: prospective quality of life assessment in a consecutive series

S. Shinde1, T. Wanger2, P. Novotny3, M. Grudem1, A. Jatoi1,2
1 Department of Oncology, Mayo Clinic, Rochester, Minnesota; 2 Cancer Education Center, Mayo Clinic Cancer Center, Rochester, Minnesota; 3 Department of Biostatistics, Mayo Clinic, Rochester, Minnesota (USA)

Summary
Objective: Among ovarian cancer patients, cancer treatment is aggressive and yet survival is often so limited; hence, this study sought to measure quality of life with the ultimate goal of identifying ways of improving it over the duration of these patients’ lives. Materials and Methods: The medical records of all ovarian cancer patients who received some/all of their initial chemotherapy at the Mayo Clinic in Rochester, Minnesota from late 2010 through 2012 were reviewed. Patient-reported quality of life was derived from the following ten-point linear analogue scale questions which had been administered to all patients: 1) How would you describe your degree of pain, on average? 2) How would you describe your level of fatigue, on average? 3) How would you describe your overall quality of life? Quality of life data were censored upon cancer recurrence. Results: Among 59 eligible patients, the median cumulative interval during which quality of life was serially assessed was 1.15 years (range: three months, 3.2 years). Area under the curve for pain, fatigue, and global quality of life showed no statistically significant differences between patients treated with dose-dense chemotherapy with carboplatin/paclitaxel (n=10) versus three-week chemotherapy with carboplatin/paclitaxel (n=36) versus other (n=13). Although pain, fatigue, and global quality of life improved over time, 35 of 59 (59%) patients reported grade 4 or worse pain during follow up, and 47 of 59 (80%) reported grade 4 or worse fatigue (higher scores denote worse pain or fatigue). After completion of cancer treatment, 30 (51%) described grade 4 or worse pain or fatigue. The most common pain site was the abdomen/pelvis, followed by the back, followed by the hands, feet, fingers, and toes. Conclusion: In ovarian cancer patients who remain cancer-free, severe pain and fatigue occur years after cancer treatment. Further research should focus on how best to address these symptoms.

Key words: Pain; Fatigue; Quality of life; Ovarian cancer.

Introduction
Over 70% of ovarian cancer patients are diagnosed with late-stage disease, undergo an extensive lymphadenectomy and an omentectomy in conjunction with extirpation of multiple pelvic organs, and then go on to receive further cancer treatment in the form of several cycles of chemotherapy — only to die, oftentimes within five years, of recurrent cancer [1]. This sobering pattern of events has prompted contemporary, large-scale therapeutic trials in ovarian cancer patients to integrate quality of life measurements into their study design: if cancer treatment is so aggressive and survival often so limited, it seems appropriate to measure quality of life with the ultimate goal of improving it over the duration of these patients’ short lives. These large-scale clinical trials have provided salient observations on quality of life. First, in ovarian cancer patients receiving potentially curative therapy, symptoms appear to diminish as cancer treatment continues. For example, in a phase III trial that assessed the role of neoadjuvant chemotherapy for the treatment of ovarian cancer, Greimel et al. observed that, among 404 patients, cancer symptoms such as pain and fatigue decreased over time [2]. Second, maintenance therapy confers a negative impact on quality of life. Monk et al. examined quality of life among 1,693 ovarian cancer patients who participated in Gynecological Oncology Group Study 0218 and found that patients who received maintenance bevacizumab manifested an approximately 10% decline in global quality of life over time [3]. Similar results were reported in the quality of life assessment from the ICON7 trial, which also tested maintenance bevacizumab [4]. Finally, quality of life in the Gynecological Oncology Group Study 0172 showed that intraperitoneal chemotherapy leads to “more health-related quality-of-life disruption,” specifically more abdominal pain and peripheral neuropathy, compared to a more typically-administered intravenous chemotherapy regimen [5]. Thus, quality of life assessment has become an important part of prospectively-conducted clinical trials and serves an important role in the assessment of new cancer treatments.

Nonetheless, gaps exist. First, weekly, dose-dense chemotherapy with carboplatin and paclitaxel has gained notable recognition based on its conferred survival advan.
tage over three-week chemotherapy, as reported in the Japanese Gynecological Oncology Group 3016 study [6, 7]. To the present authors’ knowledge, however, few previous studies have examined whether dose-dense chemotherapy is associated with a comparative change in global quality of life. Second, although quality of life assessment is increasingly integrated into prospectively-conducted clinical trials, only a relatively small number have focused on long-term quality of life in patients who are not enrolled in a clinical trial [8-20]. This distinction between whether or not patients had enrolled in a trial is not trivial, as the latter group often has a more advanced age, an inferior performance score, a greater number of co-morbid conditions and, hence, not surprisingly, a greater number of severe treatment-related adverse events [21, 22].

In view of the foregoing, the purpose of the current study was twofold. First, the authors sought to explore quality of life differences between patients who received dose-dense chemotherapy as part of their initial cancer treatment versus other patient groups. To their knowledge, these data may be among the first to examine comparative quality of life with this regimen. Second, the authors sought to describe prospectively derived quality of life data from a more typical group of ovarian cancer patients, the majority of whom had not been enrolled in a clinical trial. Because over 90% of ovarian cancer patients are not enrolled in clinical trials, such descriptive data would be invaluable to understand what most ovarian cancer patients are experiencing [22].

Materials and Methods

Overview

The Mayo Clinic Institutional Review Board approved this study. A study nurse, who was affiliated with the Mayo Clinic Ovarian Specialized Program of Research Excellence (SPORE) grant and routinely recorded the names of all patients treated for ovarian cancer at the Mayo Clinic in Rochester, Minnesota, provided a list of patients from late 2010 through 2012. This starting date was chosen because the Japanese Gynecological Oncology Group Study 3016 with dose dense chemotherapy was published shortly prior and because the Mayo Clinic Medical Oncology Clinic began prospectively to capture and record quality of life data in late 2010 [7]. Patients were deemed eligible for inclusion in the current study if they had a diagnosis of ovarian cancer and had received their initial peri-operative chemotherapy at the Mayo Clinic in Rochester, Minnesota.

Data Acquisition

All records were reviewed in depth by two investigators (SS and TW) with spot checks for accuracy by another (AJ). Extracted data included patients’ date of birth, vital status at time of medical record review, date of death or last follow up, cancer stage and histology, date of surgery, dates of chemotherapy, type of chemotherapy initially administered (weekly, dose dense carboplatin/paclitaxel versus three-week carboplatin/paclitaxel versus other), number of completed cycles, and whether recurrent cancer had been diagnosed, and, if so, when. If a patient needed to switch to a different regimen, such information was also recorded.

<table>
<thead>
<tr>
<th>Table 1. — Baseline and treatment demographics.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Age,* median (range)</td>
</tr>
<tr>
<td>Cancer Stage**</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Tumor histology</td>
</tr>
<tr>
<td>Serous</td>
</tr>
<tr>
<td>Endometrioid</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Neoadjuvant chemotherapy? Yes</td>
</tr>
<tr>
<td>Median number of chemotherapy cycles (range)****</td>
</tr>
<tr>
<td>Switched chemotherapy? Yes</td>
</tr>
<tr>
<td>Recurrent cancer? Yes</td>
</tr>
</tbody>
</table>

* denotes age at start of chemotherapy; ** numbers may not sum to 100% because of rounding and numbers in parentheses denote percentages unless otherwise specified; *** one patient who received 3-week chemotherapy did not undergo surgery; **** if maintenance non-cytotoxic agents were used per a clinical trial, these agents were not counted in the cycle.

Quality of life information

Patient-reported quality of life was extracted from each medical record along with the date the patient completed the previous validated questionnaire items [23]. This patient-reported information was derived from the following three questions: 1) How would you describe your degree of pain, on average? 2) How would you describe your level of fatigue, on average? 3) How would you describe your overall quality of life? Patients were asked to “check only one” option on a scale from 0-10 that used verbal descriptions on the ends of the scale to denote the severity of each specific symptom or condition. These quality of life questions and responses were administered with paper and were provided to each patient at each visit if it occurred within two weeks or longer of the previous visit; clinic staff recorded patients’ questionnaire responses in the medical record.

If a patient had graded her pain as a 4 or worse, as per the patient-reported pain question described above, that patient’s medical record from the date of the severe pain was re-reviewed to learn the source of pain. This pain score threshold was chosen because it has precedent for denoting clinically significant, severe pain [24]. Starting at the time of recurrent disease, quality of life data were censored to avoid the confounding negative effect of recurrent cancer.

Data analyses

Data are presented descriptively with means, medians, standard deviations, ranges, percentages, and graphics, as appropriate. For the primary analysis of quality of life based on type of peri-operative chemotherapy, area under the curve (AUC) was calculated for each of the three patient-reported quality of life questions over time [25]. These AUC values were based on the time from first chemotherapy until last-reported quality of life score. To adjust for differences in patient follow-up, the AUC was then divided by the time from chemotherapy to last reported value. Analysis of vari-
Disease-free ovarian cancer patients report severe pain and fatigue over time: prospective quality of life assessment in a consecutive series

Results

Demographics and treatment summary
Fifty-nine ovarian cancer patients met the study eligibility criteria. Baseline and treatment demographics appear in Table 1.

Comparisons of AUC for pain, fatigue, and global quality of life scores showed no statistically significant differences between patients treated with dose-dense chemotherapy (1) versus three-week chemotherapy (2) versus other (3). Boxplots show 95% confidence intervals.

Figure 1. — Comparisons of area under the curve for pain, fatigue, and global quality of life scores showed no statistically significant differences between patients treated with dose-dense chemotherapy (1) versus three-week chemotherapy (2) versus other (3). Boxplots show 95% confidence intervals.

Figure 2. — Thirty-five of 59 patients reported grade 4 or worse pain (higher scores denote worse pain) at some point during follow-up. Each dot represents a pain score and the inserted line shows the trend.

Quality of life assessment
Patients completed an assessment of pain, fatigue, and global quality of life at one time point or more. The number of quality of life assessments per patient ranged from 1 to 20 over time. Although the frequency of assessment varied widely even intra-patiently, the median cumulative interval during which quality of life was serially assessed was 1.15 years (range: three months, 3.2 years).

Comparisons of AUC for pain, fatigue, and global quality of life scores showed no statistically significant differences between patients treated with dose-dense chemotherapy versus three-week chemotherapy versus other (Figure 1). For pain, AUC (standard deviation) was 2.6 units/year (2.1) versus 3.4 units/year (3.9) versus 4.6 units/year (4.4), for patients who had received dose-dense, three-week, and other chemotherapy, respectively ($p = 0.51$). For fatigue, 2.5 units/year (2.0) versus 2.6 units/year (1.8) versus 2.7
units/year (1.8) was observed for patients who received dose-dense, three-week, and other chemotherapy, respectively; \( p = 0.95 \). Finally, for global quality of life, 7.3 units/year (2.5) versus 7.2 units/year (2.4) versus 6.7 units/year (2.0) was observed for patients who received dose-dense, three-week, and other chemotherapy, respectively (\( p = 0.78 \)).

**Descriptive quality of life data**

Trends suggest that pain, fatigue, and global quality of life improved over time (Figures 2-4). However, 35 of 59 (59%) patients reported grade 4 or worse pain at some point during follow-up, and 47 of 59 (80%) reported grade 4 or worse fatigue (higher scores denote worse pain or fatigue). Of note, after completion of cancer treatment, 30 patients (51%) described grade 4 or worse pain or fatigue. Seventeen (29%) described grade 4 or worse general quality of life at some time point (lower scores denote worse quality of life) (Figures 2-4).

The most common site of pain was in the abdomen/pelvis and was cited 37 times in the medical records within the cohort. This site was followed by back pain, which was cited 20 times. Pain in the hands, feet, fingers, and toes was cited 20 times.

**Discussion**

This study first sought to explore whether quality of life over time was markedly different in patients treated with dose-dense chemotherapy with paclitaxel and carboplatin, as per the Japanese Gynecological Oncology Group 3016, versus three-week chemotherapy versus some “other” regimen. Secondly, it sought to provide serial, prospective, descriptive data on patient-reported quality of life in a group of patients, the majority of whom had received care outside a cancer therapeutics trial. With regards to this first goal, this study found no glaring differences in quality of life between treatment groups. These findings suggest that current practice when prescribing dose-dense chemotherapy should not be modified but that further study of quality of life with dose-dense chemotherapy in ovarian cancer patients is warranted.

Importantly, this study’s secondary goal uncovered the most noteworthy observations. Similar to other studies, this study observed trends of symptom improvement over time [2]. However, trends do not always tell the whole story. For some patients within this cohort, severe pain and fatigue persisted for years after completion of initial cancer therapy and occurred in the absence of recurrent cancer. Indeed, the observation that 30 of 59 patients suffered grade 4 or worse pain and/or fatigue after completion of cancer treatment underscores the fact that, as a cohort, ovarian cancer patients have major unmet needs that persist over time. Furthermore, the fact that most of these patients will likely die from recurrent cancer in the near future only further points to the urgency of working to address these needs.

How does this study differ from other quality of life studies in ovarian cancer patients? First, in contrast to
Disease-free ovarian cancer patients report severe pain and fatigue over time: prospective quality of life assessment in a consecutive series

many previous studies, the authors of this report provided more than averages and trends when we reported on quality of life. Indeed, a recent review from Lorusso et al. advocates for moving beyond reporting trends in global quality of life assessment [26]. By identifying large subgroups of patients who had severe pain and fatigue, the current study is in keeping with this recommended approach. Second, this study’s retrospective design, which included a prospective evaluation of quality of life in all patients as part of routine clinical care, is another major strength. As a result of this study design, the present results are not biased from patient selection. Third, in the current study, the authors distinguished between patients who were cancer-free and those who had developed recurrent cancer, censoring the latter. To their knowledge, relatively few quality of life studies have scrutinized patients’ health status to the point of being able to make this distinction. This distinction is important because it shifts the emphasis away from cancer therapy towards treating the patient for the residual effects of surgery and chemotherapy, a focus that has perhaps received less attention in the past. Finally, the current study focused on pain in contrast to some earlier studies that focused only on global quality of life. Acknowledged as the fifth vital sign, pain is often highly treatable yet often ignored [27]. The present observation that this highly treatable symptom is severe and prevalent in long-term ovarian cancer survivors can potentially foster changes in clinical practice and hence relieve suffering for patients with ovarian cancer.

This study has both limitations and strengths that revolve primarily around its limited sample size. Because only a small number of patients had received dose-dense chemotherapy, some of our preliminary conclusions on comparative quality of life with dose-dense chemotherapy must be viewed with caution. However, this relatively smaller sample size is also a strength. It allowed the authors to examine long-term quality of life data in much greater detail, to identify subgroups of patients who suffer from severe pain and fatigue long-term, and to even report on the physical location of that pain. As a result, we are able to clearly articulate this study’s most salient finding that severe pain in contrast to some earlier studies that focused only on disease-free ovarian cancer patients report severe pain and fatigue over time: prospective quality of life assessment in a consecutive series.

References


Address reprint requests to:
A. JATOI, M.D.
200 First Street SW
Rochester, Minnesota 55905 (USA)
e-mail: jatoi.aminah@mayo.edu
Primary fallopian tube carcinoma - a retrospective analysis of 66 cases

L. Liu¹, X. Xu²*, L. Jia³*, M. Wei⁴, B. Qian⁴, Y. Wu⁴, Y. Shen⁴, X. Wang⁴, H. Pei⁴, X. Chen⁴,⁵,⁶

¹ Department of Obstetrics and Gynecology, Sihong People’s Hospital, Sihong
² Department of Chemotherapy, Jiangsu Institute of Cancer Research, Nanjing, Jiangsu
³ Department of Obstetrics and Gynecology, The Affiliated People’s Hospital of Inner Mongolia Medical College, Inner Mongolia Autonomous Region
⁴ Department of Gynecologic Oncology, Jiangsu Institute of Cancer Research, Nanjing, Jiangsu
⁵ State Key Laboratory of Bioelectronics, Southeast University, Nanjing, China
⁶ Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX (USA)

Summary

Background: Primary fallopian tube carcinoma (PFTC) is a rare malignant gynecologic oncology. There was no consensus on the outcome related clinicopathological characteristics. Present study aims to determine the prognosis associate factors in PFTC. Materials and Methods: In this retrospective study, the authors identified 50 PFTC patients in Jiangsu Institute of Cancer Research and 16 cases in the Affiliated People’s Hospital of Inner Mongolia Medical College between 1988 and 2013. Disease surveillance was conducted based on the follow-up protocol of MD Anderson Cancer Center. Cox proportional hazards model and log-rank test were used to assess the associations between potential clinicopathologic characteristics and the survival durations. Results: The median progression free survival (PFS) and overall survival (OS) of PFTC were 36.9 and 62.7 months, respectively. FIGO Stage (p < 0.01, 0.01), grade (p = 0.02, 0.03), tumor residual after initial debulking surgery (p = 0.05, 0.01), nadir CA-125 (p = 0.01, 0.01) were independently related with PFS and OS. The PFS and OS of patients with Stage II PFTC were similar as those with Stage III-IV (30.7 vs 28.3 and 61.9 vs 49.2 months, respectively) but poorer than those of Stage I cases (N/A). The PFS of patients with paclitaxel-based chemotherapy was longer than those with other regime (51.3 vs 33.1 months), but not OS (62.7 vs 42.6 months). The outcome of patients underwent optimal initial cytoreduction surgery was better than those of suboptimal ones (PFS 56.4 vs 21.2 months and OS 65.3 vs 47.9 months, respectively). Conclusion: PFTC patients with FIGO Stage II disease should be regarded as advanced disease. Paclitaxel based chemotherapy was associated with longer PFS but not OS in PFTC.

Key words: Fallopian tube carcinoma; Prognosis; Nadir CA-125.
was followed the International Federation for Obstetrics and Gynecology (FIGO) in 1991. Avidin-biotin peroxidase system was routinely performed for immunohistochemical staining. The primary antibodies were: p53 (1:100, DO-7), Ki67 (1:100, clone MIB-1), epidermal growth factor receptor (EGFR clone 3C6, 3mg/ml) and CA-125 (1:100, EPR1020(2)).

The primary therapy mostly included cytoreductive surgery (CRS) and adjuvant chemotherapy. The predominant adjuvant chemotherapy protocols included: CAP (cyclophosphamide-500 mg/m², doxorubicin-30 mg/m², and cisplatin-75 mg/m²), CP (carboplatin AUC-6 plus cyclophosphamide-500 mg/m²) and TP (carboplatin AUC-6, paclitaxel 135-175 mg/m²). The authors used the Response Evaluation Criteria in Solid Tumors (RECIST) criterion and adapted WHO standard to assess objective response and tumor progression which took into account the measurement of the longest diameter for all target lesions [15-18]. Complete response (CR) was thought to be the disappearance of all target lesions; partial response (PR) was at least a 30% decrease in the sum of the longest diameter of target lesions, the baseline sum longest diameter as reference; progressive disease (PD) was at least a 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded, since the treatment started or the appearance of one or more new lesions; stable disease (SD) was defined to be neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, the smallest sum longest diameter since the treatment started as reference. The criteria of complete clinical remission included: (1) no residual tumor on physical examination and imaging studies; (2) absence of tumor-associated clinical symptoms; and (3) serum CA-125 concentration of less than or equal to 35 U/ml. Pathological complete remission was judged by laparoscopy and/or laparotomy. Optimal CRS was defined as the threshold of ≤ 1 cm of the residual tumor, and suboptimal debulking was determined as having more than one cm of nodules left. Overall survival (OS) was defined as the time interval from diagnosis until death, or until last follow-up examination of patients who are still alive. Progression-free survival (PFS) was the length of time during and after primary treatment wherein the patient’s condition did not worsen.

Statistical analysis
Cox proportional hazards model was used to assess the relationship between the clinical characteristics and the survival. Stepwise regression was conducted to build the univariate and multivariate analysis models. The log-rank test and Kaplan-Meier curve were used to assess this relationship. Logistic regression analysis was used to explore outcome related factors. The p values < 0.05 was considered statistically significant. All analyses were conducted using the SPSS statistical software program (version 18.0).

Results
Patient characteristics
There were 46 (69.7%) serous subtype of PFTC in present study. Median follow-up time was 65.0 months (interquartile range, 46.9 months to 88.4 months). Thirty patients (45.5%) reported experiencing vaginal bleeding and discharge, 25 (37.8%) abdominal colicky pain, 16 (24.2 %) abdominal mass, and four (6.1 %) gastrointestinal dysfunction. There were nine (13.6 %) cases experiencing typical Latzko’s triad of symptoms which consisted of intermittent profuse serosanguineous vaginal discharge,

Materials and Methods
Study population
This study was approved by the institutional review boards of Jiangsu Institute of Cancer Research (JICR) and Affiliated People’s Hospital of Inner Mongolia Medical College (APHIMMC). The authors identified 50 PFTC patients in JICR and 16 cases in APHIMMC from clinical stations between January 1, 1988 and September 1, 2013. Those who did not undergo the standard first line treatment in the present centers were excluded. After primary therapy, the routine follow-up protocol was conducted according to the surveillance protocol of MDACC. The relevant clinic pathological data included: age, presenting symptoms, past medical history, family history, preoperative investigations (including tumor markers), the histological type and FIGO Stage and grade of the tumor, volume of ascites, details of the primary surgical procedure, management protocols of primary and recurrent disease, and follow-up information as shown in Table 1. All of the cases of PFTC were independently reviewed by L. Hou from JICR, who is a lead gynecological pathologist.

Diagnosis criteria and therapy principals
The pathological criteria of PFTC for differentiating it from ovarian and other gynecological malignancies were raised by Hu et al. (1950) and modified by Sedlis et al. (1978) [14]. This widely accepted proposal for diagnosis of PFTC includes these four essentials: the main tumor arises from the endosalpinx; the histological pattern reproduces the epithelium of tubal mucosa; transition from benign to malignant tubal epithelium is demonstrable; the ovaries or endometrium are either normal or contain tumor smaller than the tumor in the tube. System staging of PFTC was followed the International Federation for Obstetrics and Gynecology (FIGO) in 1991. Avidin-biotin peroxidase system was routinely performed for immunohistochemical staining. The primary antibodies were: p53 (1:100, DO-7), Ki67 (1:100, clone MIB-1), epidermal growth factor receptor (EGFR clone 3C6, 3mg/ml) and CA-125 (1:100, EPR1020(2)).

The primary therapy mostly included cytoreductive surgery (CRS) and adjuvant chemotherapy. The predominant adjuvant chemotherapy protocols included: CAP (cyclophosphamide-500 mg/m², doxorubicin-30 mg/m², and cisplatin-75 mg/m²), CP (carboplatin AUC-6 plus cyclophosphamide-500 mg/m²) and TP (carboplatin AUC-6, paclitaxel 135-175 mg/m²). The authors used the Response Evaluation Criteria in Solid Tumors (RECIST) criterion and adapted WHO standard to assess objective response and tumor progression which took into account the measurement of the longest diameter for all target lesions [15-18]. Complete response (CR) was thought to be the disappearance of all target lesions; partial response (PR) was at least a 30% decrease in the sum of the longest diameter of target lesions, the baseline sum longest diameter as reference; progressive disease (PD) was at least a 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded, since the treatment started or the appearance of one or more new lesions; stable disease (SD) was defined to be neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, the smallest sum longest diameter since the treatment started as reference. The criteria of complete clinical remission included: (1) no residual tumor on physical examination and imaging studies; (2) absence of tumor-associated clinical symptoms; and (3) serum CA-125 concentration of less than or equal to 35 U/ml. Pathological complete remission was judged by laparoscopy and/or laparotomy. Optimal CRS was defined as the threshold of ≤ 1 cm of the residual tumor, and suboptimal debulking was determined as having more than one cm of nodules left. Overall survival (OS) was defined as the time interval from diagnosis until death, or until last follow-up examination of patients who are still alive. Progression-free survival (PFS) was the length of time during and after primary treatment wherein the patient’s condition did not worsen.

Statistical analysis
Cox proportional hazards model was used to assess the relationship between the clinical characteristics and the survival. Stepwise regression was conducted to build the univariate and multivariate analysis models. The log-rank test and Kaplan-Meier curve were used to assess this relationship. Logistic regression analysis was used to explore outcome related factors. The p values < 0.05 was considered statistically significant. All analyses were conducted using the SPSS statistical software program (version 18.0).

Results
Patient characteristics
There were 46 (69.7%) serous subtype of PFTC in present study. Median follow-up time was 65.0 months (interquartile range, 46.9 months to 88.4 months). Thirty patients (45.5%) reported experiencing vaginal bleeding and discharge, 25 (37.8%) abdominal colicky pain, 16 (24.2 %) abdominal mass, and four (6.1 %) gastrointestinal dysfunction. There were nine (13.6 %) cases experiencing typical Latzko’s triad of symptoms which consisted of intermittent profuse serosanguineous vaginal discharge,
Primary fallopian tube carcinoma - a retrospective analysis of 66 cases

colicky pain relieved by discharge, and abdominal or pelvic mass. There were 35 (53.0%) patients with pelvic or abdominal mass and only six (9.1%) cases with ascites in physical examination. A definite or suspected preoperative diagnosis of PFTC was made only in eight (12.1%) of all patients.

Objective tumor response of primary treatment

CRS as the definite treatment which involves total abdominal hysterectomy with bilateral salpingo-oophorectomy and omentectomy in 48 (72.7%) PFTCs. Lymphadenectomy was performed in 34 (51.5%) selection patients as shown in Table 2. There were 41 (62.1%) patients that met optimal outcome by initial CRS and 40 (60.6%) cases that underwent chemotherapy including paclitaxel. At the end of initial treatment, there were 45 (68.2%) PFTCs meet the criteria of CR, nine (13.6%) cases that met PR, five (7.6%) cases that met SD, and seven (10.6%) cases that were PD.

Survival related factors

The median PFS and OS of PFTC were 36.9 months (18.8 - 55.0) and 62.7 months (48.1 - 77.3), respectively. Univariate Cox proportional hazards model revealed that FIGO Stage, pathological grade, outcome of CRS, nadir CA-125 level, ascites, and chemotherapy protocol were associated with OS and PFS while lymph node metastatic was associated with PFS but not OS (Table 3). Multivariate analysis revealed that FIGO Stage, nadir CA-125 level, outcome of CRS, and chemotherapy protocol were independent OS and PFS predictors in PFTC (Table 4).

The OS and PFS of FIGO Stage II patients with were poorer than those who were Stage I, but not Stage III-IV in PFTC (30.7 vs 28.3 and 61.9 vs 49.2 months, respectively; Figures 1A and 1B). Unlike in EOC, Stage II cases indicated poorer outcome than those of Stage I. In patients underwent paclitaxel based chemotherapy, the PFS but not OS durations were longer than those of non-paclitaxel ones (75.2 vs 48.4 months and 91.8 vs 62.3 months, respectively; Figures 2A and 2B).

Validation sets

To validate the present results from JICR in China, the authors analyzed another set of data from MDACC. Thirty-four PFTC patients at MDACC were identified between January 1, 1990 and February 1, 2011 as shown

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percentage (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neochemotherapy</td>
<td>Yes 6 (9.1%), No 60 (90.9%)</td>
</tr>
<tr>
<td>Surgery</td>
<td>Biopsy or BSO 6 (9.1%), TAH &amp; BSO 9 (18.2%), TAH &amp; BSO &amp; omentectomy 14 (21.2%), TAH &amp; BSO &amp; omentectomy &amp; lymphadenectomy 34 (51.5%)</td>
</tr>
<tr>
<td>Tumor residual</td>
<td>Optimal (≤ 1 cm) 41 (62.1%), Suboptimal (&gt; 1 cm) 18 (27.2%), Unknown 7 (10.6%)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>Positive 6 (17.6%), Negative 26 (76.5%), Unknown 2 (5.9%)</td>
</tr>
<tr>
<td>Front line chemotherapy</td>
<td>Non-paclitaxel 24 (36.4%), Including paclitaxel 40 (60.1%), Unknown 2 (3.0%)</td>
</tr>
<tr>
<td>No. of front line chemotherapy cycles (course)</td>
<td>&lt; 6 5 (7.6%), ≥ 6 61 (92.4%)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>2 (3.0%)</td>
</tr>
</tbody>
</table>

TAH & BSO: total abdominal hysterectomy with bilateral salphingo-oophorectomy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OS OR 95% CI</th>
<th>PFS OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO Stage</td>
<td>I 1.000 Reference 1.00 Reference</td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td>I 1.000 Reference 1.00 Reference</td>
<td></td>
</tr>
<tr>
<td>Tumor residual</td>
<td>2 1.5–14.1 2.2 0.9–21.3</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>2.1 0.9–6.9 1.7 0.8–5.3</td>
<td></td>
</tr>
<tr>
<td>Tumor residual</td>
<td>6.2 3.2–12.8 5.3 2.7–11.3</td>
<td></td>
</tr>
<tr>
<td>Non-paclitaxel</td>
<td>2.3 1.5–5.2 1.5 1.3–3.9</td>
<td></td>
</tr>
<tr>
<td>Nadir CA-125</td>
<td>1.03 1.0–1.06 1.04 1.01–1.08</td>
<td></td>
</tr>
</tbody>
</table>

OS: overall survival; PFS: progression-free survival; OR: odds ratio; CI: confidential interval

<table>
<thead>
<tr>
<th>Variable</th>
<th>OS OR 95% CI</th>
<th>PFS OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO Stage</td>
<td>I 1.000 Reference 1.00 Reference</td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td>I 1.000 Reference 1.00 Reference</td>
<td></td>
</tr>
<tr>
<td>Tumor residual</td>
<td>2 1.5–14.1 2.2 0.9–21.3</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>2.1 0.9–6.9 1.7 0.8–5.3</td>
<td></td>
</tr>
<tr>
<td>Tumor residual</td>
<td>6.2 3.2–12.8 5.3 2.7–11.3</td>
<td></td>
</tr>
<tr>
<td>Non-paclitaxel</td>
<td>2.3 1.5–5.2 1.5 1.3–3.9</td>
<td></td>
</tr>
<tr>
<td>Nadir CA-125</td>
<td>1.03 1.0–1.06 1.04 1.01–1.08</td>
<td></td>
</tr>
</tbody>
</table>

OS: overall survival; PFS: progression-free survival; OR: odds ratio; CI: confidential interval

Table 2. — Primary therapy of PFTC.

Table 3. — Univariate analysis of survival-related characteristics in PFTC.

Table 4. — Multivariate analysis of survival-related characteristics in PFTC.
Figure 1. — PFTC patients with Stage II disease had poorer OS and PFS than those of Stage I but not Stage III-IV (1A, 1B).

Figure 2. — PFTC patients who underwent paclitaxel based chemotherapy had longer PFS and but not OS than counterparts (2A, 2B).

Figure 3. — PFTC patients from MDACC with Stage II disease had poorer OS and PFS than those of Stage I but not Stage III-IV (S1A, S1B).

Figure 4. — PFTC patients who underwent optimal CRS had longer PFS and OS than counterparts (S2A, S2B).
in Table 5. Twenty-four patients (70.6%) were high-grade. Most of them (73.7%) were serous cancer. There were four (11.8%) patients who were reported to experience Latzko’s triad of symptoms. Lymphadenectomy was performed in 20 (58.8%) selection patients. Cox proportional hazards model revealed that outcome of CRS and FIGO Stage were also associated to OS and PFS (Tables 6, 7). The PFS and OS durations of PFTC patients with Stage II disease were poorer than those of Stage I (p = 0.01 and p = 0.04, respectively; Figures 3A and 3B). The PFS and OS durations of PFTC patients who underwent optimal CRS were longer than those who did not undergo it (p = 0.01 and p = 0.05, respectively; Figures 4A and 4B).

Discussion

Primary fallopian tube carcinoma is infrequent and little information can be derived from a single institution. To the authors’ knowledge, this is the first study from three institutions of PRC and USA to evaluate the survival associated clinical-pathological factors for this disease. The clinicopathologic features and biological behavior of PFTC is similar to EOC. Both tumors also show an increase among nulliparous women, are frequent of serous papillary histology, have advanced stage with a poor outcome, and mostly well respond to initially platinum-based chemotherapy [11, 19, 20]. Nevertheless, some differences appear between the two diseases: the median age of PFTC is younger than those of ovarian cancer in the present centers. PFTC is more often diagnosed in an earlier stage. There is also different regarding the need for routine lymphadenectomy and postoperative therapy of early stage disease.

The preoperative diagnosis ratio of PFTC is low and it was 12.1% in the present report [21]. The main clinical symptoms were abdominal pain (30-50%), vaginal bleeding and drainage (50-60%), and abdominal mass (12-61%) [22-23]). In this study, the patients that underwent typical Latzko’s triad was 18.0%. Huang et al. argued that the so-called “triad” was inflammatory fallopian tube changes, but not specific symptoms of PFTC, and may cause unnecessary check [24]. Radiological study is helpful to find early stage PFTC. Neovascularization in fallopian tube cavity could be revealed by transvaginal Doppler ultrasound. MRI was more sensitive than CT and ultrasound for local invasion of this disease [25]. The positive rate of cervical/vaginal smears was only 0-23% and should not regarded as conventional measures [26]. The misdiagnosis of PFTC may reduce the actual prevalence rate of this disease. It is reported that serial sections of fallopian tube in EOC or primary peritoneal carcinoma will improve the detection rate for PFTC [27].

Table 5. — The characteristic of PFTC.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Percentage (%) / Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53 (38-77)</td>
</tr>
<tr>
<td>Baseline CA-125 level (U/ml)</td>
<td>685 (7-5880)</td>
</tr>
<tr>
<td>Nadir CA-125 level (U/ml)</td>
<td>10 (4-35)</td>
</tr>
<tr>
<td>Ethnic group</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>22 (64.7%)</td>
</tr>
<tr>
<td>Black</td>
<td>6 (17.6%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3 (8.8%)</td>
</tr>
<tr>
<td>Eastern Asian</td>
<td>2 (5.9%)</td>
</tr>
<tr>
<td>Middle east</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>23 (67.6%)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>9 (26.5%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>2 (5.9%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>4 (11.8%)</td>
</tr>
<tr>
<td>High</td>
<td>30 (88.2%)</td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7 (20.6%)</td>
</tr>
<tr>
<td>II</td>
<td>6 (17.6%)</td>
</tr>
<tr>
<td>III</td>
<td>17 (50.0%)</td>
</tr>
<tr>
<td>IV</td>
<td>4 (11.8%)</td>
</tr>
</tbody>
</table>

FIGO: International Federation of Gynecology and Obstetrics.

Table 6. — Univariate analysis of survival-related characteristics in PFTC.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OS OR 95% CI</th>
<th>PFS OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1.000 Reference</td>
<td>1.00 Reference</td>
</tr>
<tr>
<td>II</td>
<td>4.1 0.9–38.9</td>
<td>6.5 0.9–37.9</td>
</tr>
<tr>
<td>III</td>
<td>6.8 1.1–47.5</td>
<td>7.7 1.0–54.7</td>
</tr>
<tr>
<td>IV</td>
<td>9.7 1.3–57.1</td>
<td>11.5 1.2–97.2</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.000 Reference</td>
<td>1.00 Reference</td>
</tr>
<tr>
<td>High</td>
<td>2.3 0.9–25.1</td>
<td>3.2 0.9–35.7</td>
</tr>
<tr>
<td>Tumor residual</td>
<td>7.4 1.3–38.3</td>
<td>9.5 1.5–46.0</td>
</tr>
<tr>
<td>Ascites</td>
<td>1.4 1.1–28.1</td>
<td>1.5 1.2–38.5</td>
</tr>
<tr>
<td>Nadir CA-125</td>
<td>1.02 1.0–1.05</td>
<td>1.03 1.0–1.06</td>
</tr>
</tbody>
</table>

OS: overall survival; PFS: progression-free survival; OR: odds ratio; CI: confidential interval.

Table 7. — Multivariate analysis of survival-related characteristics in PFTC.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OS OR 95% CI</th>
<th>PFS OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1.00 reference</td>
<td>1.00 reference</td>
</tr>
<tr>
<td>II</td>
<td>3.4 0.9–26.7</td>
<td>4.8 0.9–36.9</td>
</tr>
<tr>
<td>III</td>
<td>6.1 0.9–46.1</td>
<td>5.2 1.0–43.0</td>
</tr>
<tr>
<td>IV</td>
<td>9.7 1.3–70.3</td>
<td>8.8 1.1–63.9</td>
</tr>
<tr>
<td>Tumor residual</td>
<td>4.7 1.3–24.2</td>
<td>6.2 1.4–32.1</td>
</tr>
<tr>
<td>Nadir CA-125</td>
<td>1.01 1.0–1.03</td>
<td>1.02 1.0–1.03</td>
</tr>
</tbody>
</table>

Primary fallopian tube carcinoma - a retrospective analysis of 66 cases 165
Due to the limited quantities, long time span and without unified treatment plan in most retrospective studies of PFTC, there was no consensus on outcome related factors. Presently, extent of disease is the only well-established prognosis indicator. FIGO Stage, pathological subtype, and grade were reported to be the main prognostic factors of PFTC from some single center studies [28, 29]. The present authors found that the survival duration of Stage II PFTC was poorer than that of Stage I, but similar to that of Stage III and IV cases. The present authors consider that PFTC confines to the fallopian tube for a comparatively long period before the breakthrough tubal and then relatively quickly spread to the ovarian, abdominal cavity or the distance. Considering that fallopian tube origin model of ovarian cancer, it may imply that part of the serous tubal intraepithelial carcinoma or locally invasive carcinoma spread to ovarian or pelvic was not thought to be Stage II PFTC, but ovarian or peritoneal carcinoma. Previous studies revealed that the prognosis of patients with Stage I and II PFTC was comparatively good, and should be regarded as the “early stage” [30], however there was controversy; the other study reported that the prognosis of Stage II PFTC was similar to those of advanced stage, like the present study [31]. The present authors further found that the nadir CA-125 level after primary therapy was independent prognostic indicator of this disease like that of EOC [32]. They did not find the relationship between immunosaying results of p53, CA-125, EGFR, and Ki67 and the prognosis of PFTC [33].

There was no prospective trial on the most preferred surgical procedure and adjuvant chemotherapy. The management principal of this disease followed those of EOC. Today, initial CRS and adjuvant chemotherapy including carboplatin/paclitaxel were regarded as standard primary management of PFTC [34-35]. The objective response rate of carboplatin/paclitaxel as initial adjuvant chemotherapy in present research was similar to those of other studies (53-92%) [36-37]. There was no consensus on abdominal and pelvic lymph node dissection and the proportion of this procedure was 51.5%, higher than that of other reports [38]. The present authors found that tumor residual after initial CRS was independent prognosis factor.

There are limitations to the present study. Firstly, unavoidable selection biases inherent to its retrospective design. Age, initial CRS, chemotherapy protocols, and some additional salvage therapy may have reflected certain selected factors that may influence prognosis. Secondly, given the long term follow up and the heterogeneity of therapy strategies used throughout the 25 years study period, including the emergence of new protocols such as paclitaxel based chemotherapy and molecular targeted therapy and so on, it was impossible to unify the therapy strategy. Thirdly, the limited sample size may have also caused selection bias. Evaluating patients from China with validation set from America may have assisted in lessening this unfavorable effect.

In summary, in this study including patients from two centers with same recruited standard, the authors found that the prognosis of PFTC was associated with FIGO Stage, pathological grade, and surgical outcome. They also found that the prognosis of patients with Stage II disease was similar to those of Stage III and IV but not Stage I. Stage II of PFTC cannot be regarded as early stage.

Acknowledgements

This work was supported by the Natural Science foundation of Jiangsu (grant number: BK20131439) and the Jiangsu Province Institute of Cancer Research Foundation (grant number: ZK201203) and the 2012 International Exchange Support Program of Jiangsu Health.

References

Primary fallopian tube carcinoma - a retrospective analysis of 66 cases


Chemotherapy-induced thrombocytopenia and clinical bleeding in patients with gynecologic malignancy

Department of Obstetrics and Gynecology, Osaka City University, Graduate School of Medicine, Osaka (Japan)

Summary
Objectives: Chemotherapy-induced thrombocytopenia seems to be a relevant problem and the risk of clinical bleeding in patients with gynecologic malignancy is reported to be higher than other malignancy. In this study, the authors investigated chemotherapy-induced thrombocytopenia recently performed in all patients with gynecologic malignancy. Materials and Methods: Between January 2009 and December 2011, the authors examined chemotherapy-induced thrombocytopenia using the Common Terminology Criteria for Adverse Events (CTCAE) v.4.0. They analyzed the incidence and clinical features of chemotherapy-induced thrombocytopenia in patients with gynecologic malignancy. Results: During this period they administered over 1,614 infusions (29 regimens) to 291 patients. Chemotherapy-induced thrombocytopenia occurred in 43 (14.8%) patients over 56 (3.5%) chemotherapy cycles. Bleeding occurred in 13 (4.5%) patients over 14 (0.9%) cycles. Platelet transfusions were administered for eight (2.7%) patients over eight (0.5%) cycles. Median platelet count at platelet transfusions was 17,000 /µl. Chemotherapy-induced thrombocytopenia was associated with more than five previous chemotherapy cycles, previous radiotherapy, disseminated disease, distant metastatic disease, poor performance status, and taxane-including regimens. Clinical bleeding was associated with previous radiotherapy, distant metastatic disease, poor performance status, and taxane-including regimens. Conclusions: Estimating bleeding risk factor such as previous radiotherapy, distant metastatic disease, poor performance status, and taxane-including regimens seem to be important for safe management of chemotherapy-induced thrombocytopenia.

Key words: Chemotherapy-induced thrombocytopenia; Clinical bleeding; Gynecologic malignancy.

Introduction
Patients with gynecologic malignancy often receive several kinds of systemic chemotherapy throughout primary therapy and recurrent therapy. Moreover, new drugs (pegylated liposomal doxorubicin, gemcitabine, etc) were to be used recently in gynecologic malignancy [1, 2].

American Society of Clinical Oncology (ASCO) guideline concluded that the clinical benefit of prophylactic transfusion was at a threshold of 10,000 /µl platelets or less [3]. Gary et al. concluded that routine prophylactic platelet transfusion was unnecessary in patients with counts ˃10,000 /µl [4]. There are many reports investigating chemotherapy-induced thrombocytopenia since first report in 1962, but most reports were studied in patients with leukemia or aplastic anemia [5-7]. The risk of clinical bleeding in patients with gynecologic malignancy reported to be higher than other malignancy [6]. Elting et al. concluded that genitourinary and gynecologic neoplasm was one of bleeding risk index [7]. To the present authors’ knowledge, the last comprehensive study of thrombocytopenia in patients with gynecologic malignancy was published in 1994 [4]. There are insufficient data from patients who have received modern chemotherapy regimens to address these issues.

In this study, the authors investigated chemotherapy-induced thrombocytopenia recently performed in all patients with gynecologic malignancy with no exception.

Materials and Methods
This retrospective study was approved by Osaka City University, Graduate school of Medicine Institutional Review Board. Using the available electronic medical record data between January 2009 and December 2011, the authors examined their reported chemotherapy-induced thrombocytopenia using the Common Terminology Criteria for Adverse Events (CTCAE) v.4.0. Complete blood cell counts, including platelet counts were performed on all patients at least once a week. They analyzed the incidence and clinical features of chemotherapy-induced thrombocytopenia in patients with gynecologic malignancy.

Episodes of bleeding were categorized as either no bleeding, minor bleeding or major bleeding. Major bleeding was defined as gastrointestinal bleeding, gross hematuria, genital bleeding or intracranial bleeding. Minor bleeding was defined as petechiae, epistaxis, gingival bleeding or blood-tinged sputum. Performance status was measured on day 1 of each cycle using the Eastern Cooperative Oncology Group (ECOG) score. Performance status 3-4 was considered as a poor performance status. Disease sites were categorized as no evidence of disease, local disease, distant metastatic disease, disseminated disease, and both distant metastatic and disseminated disease. Computed tomography (CT) examination
of the abdomen and chest or magnetic resonance imaging (MRI) examination of pelvis was performed on all patients at least once per chemotherapy cycles. Disease sites and bone marrow metastasis were evaluated such a variety of imaging examination including positron emission tomography (PET). Febrile neutropenia was defined as an oral temperature >38.3°C or two consecutive readings of >38.0°C for two hours and an absolute neutrophil count < 0.5 × 10⁹/l, or expected to fall below 0.5 × 10⁹/l.

Statistical analysis

The relationship between each clinical group was analyzed using the Fisher’s exact probability test. A p value of less than 0.05 was considered significant.

Table 1. — Number of patients with gynecologic malignancy.

<table>
<thead>
<tr>
<th>Gynecologic malignancy</th>
<th>No. of patients</th>
<th>No. of thrombocytopenia</th>
<th>% of thrombocytopenia</th>
<th>No. of bleeding</th>
<th>% of bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian cancer</td>
<td>111</td>
<td>17</td>
<td>15</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>75</td>
<td>9</td>
<td>12</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>73</td>
<td>13</td>
<td>18</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Peritoneal cancer</td>
<td>14</td>
<td>2</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uterine carcinosarcoma</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vaginal cancer</td>
<td>4</td>
<td>1</td>
<td>25</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>2</td>
<td>1</td>
<td>50</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Uterine sarcoma</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clinical invasive mole</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Endometrial stromal sarcoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vaginal melanoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bartholin gland carcinoma</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ovarian &amp; endometrial cancer</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Thrombocytopenia: chemotherapy-induced thrombocytopenia (CTCAE v.4.0: grade 3-4).

Table 2. — Regimen of chemotherapy performed between January 2009 and December 2011.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. of courses</th>
<th>No. of patients</th>
<th>No. of thrombocytopenia</th>
<th>% of thrombocytopenia</th>
<th>No. of bleeding</th>
<th>% of bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (PTX+CBDCA)</td>
<td>677</td>
<td>152</td>
<td>21</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>DC (DTX+CBDCA)</td>
<td>176</td>
<td>40</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>weekly CDDP</td>
<td>124</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CPT-11+CDDP</td>
<td>119</td>
<td>31</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CDGP</td>
<td>91</td>
<td>26</td>
<td>6</td>
<td>7</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>PLD</td>
<td>86</td>
<td>22</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CDDP</td>
<td>50</td>
<td>14</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CPT-11+CDGP</td>
<td>43</td>
<td>16</td>
<td>6</td>
<td>14</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>CPT-11</td>
<td>42</td>
<td>18</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DTX+CDGP</td>
<td>31</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PTX</td>
<td>31</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AP (ADR+CDDP)</td>
<td>27</td>
<td>8</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>THP-ADR+CDDP</td>
<td>22</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EMA/CO (VP-16+MTX+ACD+CPA+VCR)</td>
<td>16</td>
<td>2</td>
<td>5</td>
<td>31</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>CDDP+S-1</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DTX</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GEM</td>
<td>9</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CPT-11+PTX</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EP/MEA (VP-16+CDDP+MTX+ACD)</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ADR</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CPT-11+MMC</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DAVFeron (DTIC+ACNU+VCR+IFN-beta)</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MTX</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BEP (BLM+VP-16+CDDP)</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>weekly CDGP</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DTX+GEM</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CAP (CPA+ADR+CDDP)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>50</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>CPT-11+VP-16</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TP (PTX+CDDP)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>1</td>
<td>100</td>
</tr>
</tbody>
</table>

Chemotherapy-induced thrombocytopenia and clinical bleeding in patients with gynecologic malignancy

Results

Incidence of chemotherapy-induced thrombocytopenia and clinical bleeding

The patients with gynecologic malignancy and chemotherapy regimens are shown in Tables 1 and 2. During this period the authors administered over 1,614 infusions (29 regimens) to 291 patients with gynecologic malignancy. Median age was 60 years (24-84). The most common gynecologic malignancies were ovarian cancer (111 patients: 38%), endometrial cancer (75 patients: 26%), and cervical cancer (73 patients: 25%). All patients had received conventional cytotoxic chemotherapy. There was no use of targeted treatment, such as monoclonal antibodies or tyrosine kinase inhibitors. The most common chemotherapy regimen was TC (paclitaxel and carboplatin) therapy; 152 patients (52%) received 677 courses (42%) of TC therapy in total. Chemistry-induced thrombocytopenia occurred in 43 (14.8%) patients over 56 (3.5%) chemotherapy cycles. Clinical bleeding occurred in 13 (4.5%) patients over 14 (0.9%) cycles. Major bleeding occurred in seven (2.4%) over seven (0.4%) cycles (gastrointestinal bleeding: four, genital bleeding: two, gross hematuria: one). In other seven cycles, clinical bleeding were minor bleeding (petechiae: four, epistaxis: one, gingival bleeding: one, blood-tinged sputum: one). No life-threatening bleeding occurred in any patient.

Clinical features of chemotherapy-induced thrombocytopenia and clinical bleeding

Risk of chemotherapy-induced thrombocytopenia and clinical bleeding related clinical characteristics are shown in Table 3. Chemotherapy-induced thrombocytopenia was associated with more than five previous chemotherapy cycles.
(p = 0.03), previous radiotherapy (p = 0.0001), disseminated disease (p = 0.006), distant metastatic disease (p = 0.02), and poor performance status (p = 0.0001). Chemotherapy-induced thrombocytopenia was not related with age or bone marrow metastases. Clinical bleeding was associated with previous radiotherapy (p = 0.003), distant metastatic disease (p = 0.03), and poor performance status (p = 0.02). Clinical bleeding was not related with more than five previous chemotherapy cycles, disseminated disease, age or bone marrow metastases.

Both chemotherapy-induced thrombocytopenia and clinical bleeding were not related with platinum-based regimens or number of anti-cancer drug of regimens. Taxane-including regimens were associated with lower rate of thrombocytopenia (p = 0.01) and clinical bleeding (p = 0.002).

Febrile neutropenia was complicated with 19 (35%) of thrombocytopenia cycles and six (43%) of clinical bleeding cycles. Infection was complicated with 29 (52%) of thrombocytopenia cycles and nine (64%) of clinical bleeding cycles.

Platelet transfusion

The patients with platelet transfusion are shown in Table 4. Platelet transfusions were administered for eight (2.7%) patients over eight (0.5%) cycles. Clinical bleeding was observed in four cycles and febrile neutropenia was observed in six cycles. In four cycles with clinical bleeding, platelet transfusions were administered after their bleeding episode and clinical bleeding stopped in a few days. In four cycles without clinical bleeding, platelet transfusions were administered before their bleeding episode began. Median platelet count at platelet transfusions was 17,000 /µl (6,000 - 29,000). Median number of platelet units for each cycle was 25 units (10-40). Platelet transfusion reactions were not observed in any patient.

Discussion

The patients with gynecologic malignancy often received systemic chemotherapy as one of primary therapy. Moreover, most patients with recurrent disease received chemotherapy. As a result, patients with gynecologic malignancy received several kinds of chemotherapy and received frequent chemotherapy per patient in clinical practice.

On the other hand, it is to be assumed that chemotherapy-induced thrombocytopenia seemed to be more problematic in the safe management of chemotherapy as the outpatient chemotherapy is performed more frequently. Chemotherapy-induced thrombocytopenia is a known source of great stress to physicians and patients. Major bleeding during chemotherapy-induced thrombocytopenia is a serious clinical problem. In some cases, platelets are administered to patients with malignancy for preventing such events. The risk of clinical bleeding in patients with gynecologic malignancy reported to be higher than other malignancies [6, 7].

There were many reports investigating chemotherapy-induced thrombocytopenia. Several clinical trials have demonstrated the potential for a 10,000 platelet /µl threshold in patients with acute leukemia [8-10]. Although there were many reports investigating chemotherapy-induced thrombocytopenia, most reports were studied in patients with leukemia or aplastic anemia [5-7]. Although there were some reports investigating chemotherapy-induced thrombocytopenia in patients with solid tumors, only a small number of patients with gynecologic malignancy were included [6, 7]. To the present authors’ knowledge, the last comprehensive study of thrombocytopenia in patients with gynecologic malignancy was published by Gary et al. in 1994 [4]. They concluded that routine prophylactic platelet transfusion was unnecessary in patients with counts >10,000 /µl [4]. In their study, taxane was not yet used and chemoradiotherapy was not performed frequently in patients with cervical cancer. There are insufficient data from patients who have received modern chemotherapy regimens to address these issues. Primary purpose of this study was to clarify risk factor of clinical bleeding during chemotherapy-induced thrombocytopenia.

In the present study, chemotherapy-induced thrombocytopenia was defined as platelet count <50,000 /µl (grade 3-4) using the CTCAE v.4.0. In previous reports, chemotherapy-induced thrombocytopenia was defined as platelet count <50,000 /µl, <75,000 /µl or <100,000 /µl [4, 6, 7, 11]. In clinical practice, there are only a few cases of severe bleeding and platelet transfusions in patients with platelet count >50,000 /µl. For this reason, the present authors’ definition of chemotherapy-induced thrombocytopenia was reasonable.

In this study, chemotherapy-induced thrombocytopenia occurred in 14.8% of patients over 3.5% of chemotherapy cycles. Gary et al. reported that chemotherapy-induced thrombocytopenia (platelet count <100,000 /µl) occurred in 36.3% of patients with gynecologic malignancy and over 52% of cycles with these patients resulted in thrombocytopenia [4]. Hitron et al. reported that chemotherapy-induced thrombocytopenia (platelet count <75,000 /µl) occurred in 10.1% of patients with solid tumors [11]. The present findings were similar to previous reports.

On the other hand, clinical bleeding occurred in 4.5% of patients over 0.9% of cycles in the present study. Major bleeding occurred in 2.4% patients over 0.4% of cycles (gastrointestinal bleeding, genital bleeding, and gross hematuria). Gary et al. reported that clinical bleeding was in 23.6% of patients and over 6.7% of cycles in patients with chemotherapy-induced thrombocytopenia (platelet count <100,000 /µl) with gynecologic malignancy. In their report, major bleeding was in 4.9% of patients and over
1.3% of cycles in patients with thrombocytopenia [4]. Elting et al. reported that clinical bleeding was in 9% of cycles and major bleeding was in 3% of cycles in patients with chemotherapy-induced thrombocytopenia (platelet count <50,000/µl) with solid tumors [6]. The present data showed lower rate of clinical bleeding than these reports. These reports were investigated before 1995 and taxanes were not used. Moreover, new drugs (pegylated liposomal doxorubicin, gemcitabine, etc.) were used recently in gynecologic malignancy [1, 2]. Major change in chemotherapy regimens seemed to affect lower rate of clinical bleeding with patients in gynecologic malignancy. In this study, no life-threatening bleeding occurred in any patient. Gary et al. reported that no intracranial bleeding or other serous clinical effects from hemorrhage were observed in their study [4]. The present data was similar to their report. Furthermore, the present authors investigated risk of thrombocytopenia and clinical bleeding related clinical characteristics. Especially, disease sites were categorized as no evidence of disease, local disease, distant metastatic disease, disseminated disease, and both distant metastatic and disseminated disease in this study. In Elting’s reports, disease sites were categorized as no evidence of disease, local disease, one metastatic disease, disseminated disease [6]. Chemotherapy-induced thrombocytopenia was associated with more than five previous chemotherapy cycles, previous radiotherapy, disseminated disease, distant metastatic disease, and poor performance status. Clinical bleeding was associated with previous radiotherapy, distant metastatic disease, and poor performance status. Both chemotherapy-induced thrombocytopenia and clinical bleeding were not related with age, bone marrow metastases or platinum-based regimens. Elting et al. reported that bleeding was associated with previous bleeding episodes, baseline platelet count less than 75,000/µl, disseminated disease, poor performance status, bone marrow metastases, and cisplatin, carboplatin, carmustine or lomustine administration [6]. Most clinical bleeding before primary therapy was from uterus in patients with gynecologic malignancy and hysterectomy was performed in most cases. Therefore, the present authors did not consider previous bleeding episodes as a risk factor related with chemotherapy-induced thrombocytopenia and clinical bleeding. This study included no cycles with baseline platelet count less than 75,000/µl because the authors performed chemotherapy on patients with baseline platelet count more than 75,000/µl. In their report, disseminated disease was defined as one or more site of metastasis. The present results combined with their report confirm that bleeding is associated with metastatic disease and poor performance status. Although clinical bleeding was not related with bone marrow metastases, clinical bleeding was associated with previous radiotherapy. The present results combined with their report confirm that bleeding is associated with poor bone marrow reserve. This data also suggested that chemotherapy-induced thrombocytopenia was associated with poor bone marrow reserve (five previous chemotherapy cycles and previous radiotherapy) and poor general condition (disseminated disease, distant metastatic disease, and poor performance status). In this study, both chemotherapy-induced thrombocytopenia and clinical bleeding were not related with platinum-based regimens. Taxane-including regimens were associated with lower rate of thrombocytopenia (p = 0.01) and clinical bleeding (p = 0.002). Elting et al. reported that bleeding was associated with cisplatin, carboplatin, carmustine or lomustine administration [6]. Although most of platinum-based regimens did not include taxane in their report, more than 60% of platinum-based regimens included taxane in the present study. This seemed to be a reason for no relationship between thrombocytopenia and platinum-based regimens. To the present authors’ knowledge, there was no comprehensive study investigating a relationship between taxane-including regimens and chemotherapy-induced thrombocytopenia in patients with gynecologic malignancy. Despite more than 90% of taxane-including regimens included platinum drug in the present study, taxane-including regimens were associated with lower rate of chemotherapy-induced thrombocytopenia and clinical bleeding. This seemed to be necessary for taking into consideration of using taxane-including regimens in high risk cases of bleeding. Platelet transfusions were administered in eight (2.7%) patients over eight (0.5%) cycles in this study. Median platelet count at platelet transfusions was 17,000/µl (6,000-29,000). ASCO guidelines [3] concluded the risk of bleeding in patients with solid tumors during chemotherapy-induced thrombocytopenia is related to the depth of the platelet nadir, although other factors contribute as well. Evidence obtained from observational studies supports the clinical benefit of prophylactic transfusion at a threshold of 10,000/µl platelets or less. The Panel suggests, however, on the basis of expert clinical opinion, that prophylactic transfusion at a threshold of 20,000/µl be considered for patients receiving aggressive therapy for bladder tumors, as well as those with demonstrated necrotic tumors, owing to their presumed increased risk of bleeding at these sites. Platelet transfusions were administered in the present hospital according to ASCO guideline. In this study, clinical bleeding was observed in four cycles and febrile neutropenia was observed in six cycles. In any cases with platelet transfusion, at least one of these states was filled as followed; platelet count <10,000/µl, bleeding episode or febrile neutropenia. In four cycles with clinical bleeding, platelet transfusions were administered after their bleeding episode and clinical bleeding stopped in a few days. In four cycles without clinical bleeding, platelet transfu-
sions were administered before their bleeding episode began. Platelet transfusions may appear to be any benefit in the prevention of the subsequent bleeding. In conclusion, chemotherapy-induced thrombocytopenia and clinical bleeding are not so frequent in patients with gynecologic malignancy. Estimating risk factor of clinical bleeding such as previous radiotherapy, distant metastatic disease, and poor performance status seemed to be important for safe management of chemotherapy-induced thrombocytopenia without unnecessary platelet transfusion.

References


Address reprint requests to:
Y. HASHIGUCHI, M.D.
Department of Obstetrics and Gynecology,
Osaka City University, Graduate School of Medicine,
1-4-3 Asahimachi, Abeno-ku,
Osaka 545-8585 (Japan)
e-mail: cbl37090yh@nifty.com
Metabolomics analysis of cervical cancer, cervical intraepithelial neoplasia and chronic cervicitis by $^1$H NMR spectroscopy

N. Ye, C. Liu, P. Shi
Department of Chemistry, Capital Normal University, Beijing (China)

Summary
Metabolomics profiles of serum samples from women with chronic cervicitis, cervical intraepithelial neoplasia (CIN), and cervical cancer were characterized by proton nuclear magnetic resonance ($^1$H NMR). These spectral profiles were subjected to partial least-squares discriminant analysis (PLS-DA), and good discriminations between cancer and non-cancer groups (chronic cervicitis and CIN) were achieved by multivariate modeling of serum profiles. The main metabolites contributing to these discriminations, as highlighted by multivariate analysis and confirmed by spectral integration, were formate, tyrosine, $\beta$-glucose, inositol, glycine, carnitine, glutamine, acetate, alanine, valine, isoleucine, and very-low-density lipoprotein (VLDL). Metabolomics analysis for chronic cervicitis, CIN, and cervical cancer is significant, which give a systemic metabolic response of these female diseases. The systemic metabolic response may be used to identify the potential biomarkers for the diseases.

Key words: Metabolomics; Cervical cancer; Cervical intraepithelial neoplasia; Chronic Cervicitis; $^1$H NMR spectroscopy; PLS-DA.

Introduction
Cervical cancer is a kind of malignant tumor and represents a significant disease burden. This cancer is serious harm for the majority of women’s health, and its incidence rate is second only to breast cancer for women [1-5]. With the work of mass screening and treatment of cervical cancer, the incidence rate of cervical cancer in the world has declined in near 40 years. However, due to the worse environmental pollution and personal hygiene practices, the incidence trend of the patients with cervical cancer was younger, and the incidence of precancerous was increased [6]. Cervical intraepithelial neoplasia (CIN), a common type of precancerous disease of cervical cancer, has significantly increased the risk of cervical cancer, and the same is true of chronic cervicitis for CIN. Therefore, early diagnosis and detection of cervical cancer is more critical for much higher survival rate. Currently, cervical cancer can be characterized by the Papanicolaou (Pap) smear test [7, 8], thin layer of liquid crystal cytology [9-11], and colposcopy [12]. However, these cytological screenings have inherent defects that produce false-negative/positive results and subjective judgment [13, 14], which tend to result in insufficient diagnostic sensitivity and specificity. To improve survival rate, more sensitivity and specificity method should be developed for early detection and treatment of cancer successfully, which may identify novel biomarkers and molecular targets. Proteomics and metabolomics are powerful analytical tools that can provide worthy information on complex biological samples. Proteomics has more advantages of analyzing various biological samples, and detecting some differently expressed proteins. Serum protein profiling of patients with cervical cancer has been screened by using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry [15], and the differentially expressed proteins between cervical cancer and healthy samples have been discovered.

Metabolomics is the study of metabolic processes in biological systems, which goals are to identify metabolic biomarkers or predictors associated with a specific biochemical event and its effect. Some work has been reported regarding such applications in this field [16, 17]. Ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC/QTOF/MS) [18] has been used for identifying metabolic biomarkers to diagnose epithelial ovarian cancer, and the metabolism of diabetic urine samples [19] has been studied by liquid chromatography–mass spectrometry (LC/MS) and $^1$H Nuclear magnetic resonance (NMR) spectroscopy method.

NMR spectroscopy is one of the main research methods of metabolomics [20-22]. Recent literature also used $^1$H NMR-based metabolomics to detect epithelial ovarian cancer [23]. The results of previous study by using $^1$H NMR revealed the relationships of choline:creatinine ratio, and lactate levels with cervical cancer [24].
Recent researches have mainly focused on cytology of cervical cancer in order to understand the metabolic processes and mechanisms during the development of cancer [25, 26], and during the radiotherapy of cervical cancer, the changes had been measured polarographically [27, 28]. Magic angle spinning magnetic resonance spectroscopy was applied to characterize cervical cancer tissue [29, 30]. There are also some literatures that researched the phospholipid metabolism by $^{31}$P NMR spectroscopy [31]. However, these papers of tissue studies may not be non-destructive for patients, analyzing samples of serum or urine could be given more attention [32-34]. Furthermore, the metabolomic analysis of the serum of patients with cervical cancer and its precancerous diseases is not enough by far.

In this study, serum samples from patients with chronic cervicitis, CIN, and cervical cancer were subjected to metabolomic analyses by $^1$H NMR spectroscopy, followed by partial least-squares discriminant analysis (PLS-DA) to analysis the serum metabolites in the three groups and identify the potential biomarkers.

**Materials and Methods**

**Sample collection**

All the diagnoses of chronic cervicitis, CIN, and cervical cancer were confirmed by histopathology. A written informed consent was obtained from all patients, voluntarily. Number of patients with chronic cervicitis was 22, and the average age was 31 years (22-43 years). Number of patients with CIN was nine, and the average age was 33 years (24-43 years). Number of patients with cervical cancer was 18, and the average age was 40 years, (35-46 years), and the total number of patients was 49. All samples were collected from 2010 to 2012. The blood sample was taken at around the same time prior to breakfast for each patient. The serum was obtained by centrifugation of the blood sample in tube at 1,300 g for 20 minutes at 4°C. The treated serum samples were stored at -60°C until NMR analysis.
Preparation of serum samples for $^1$H NMR spectroscopic analysis

The serum samples were prepared according to literature [21]. In brief, the frozen serum samples were thawed prior to use. Then the serum samples were prepared for NMR analysis by mixing 200 μL of serum with 400 μL D$_2$O. The serum-D$_2$O mixture was then centrifuged at 12,000 g for ten minutes. The clear supernatant (550 μL) was then transferred to a five-mm NMR tube, which was used for NMR analysis later.

Acquisition $^1$H NMR spectroscopy of serum

NMR data were acquired on a Varian Unity Inova 600 spectrometer operating at 600.00 MHz $^1$H observation frequency. Water signals and broad protein resonances were suppressed by a combination of presaturation and the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. Typically, 64 free induction decays (FIDs) were collected into 32 k data points using a spectral width of 8,000 Hz and acquisition time of two seconds and a relaxation delay of four seconds. Spectroscopy were acquired at 298 K. The data were zero filled, and the FIDs were multiplied by an exponential weighting function equivalent to a line broadening of 0.3 Hz prior to Fourier transformation (FT). The assignments of $^1$H NMR spectroscopy were made, which referenced to published literatures [16, 34, 35].

$^1$H NMR spectroscopy were processed and corrected for phase and baseline with MestReNova 6.1.0.6224 software. Chemical shifts were referenced to the anomeric proton of lactate at δ1.336. The region 5.20-4.60 ppm was removed in order to avoid the effects of water suppression. The spectra of δH=9.0 to 0.02 ppm were put into 1,675 integrated regions of 0.005 ppm, and the data needed to be normalized by peak area. The data were then imported into Microsoft Excel.

Statistical analysis of metabolites

The normalized integral values were imported into the SIMCA-P+11.5 software as variables for the multivariate pattern recognition analysis. Partial least-squares discriminant analysis (PLS-DA) method was used for the class discrimination and the identification of metabolites. The normalized NMR data were then subjected to classical statistical analysis using SPSS 19.0 software. The statistically significant result was indicated by $p < 0.05$. Student’s $t$-test also gave a significant difference of the metabolite content for the different stage of these diseases.

Results

$^1$H NMR spectroscopic analysis of serum

The $^1$H NMR spectra of chronic cervicitis, CIN, and cervical cancer are shown in Figure 1, respectively. According to the published literatures, some metabolites in serum of patients with cancer or inflammation may be metabolic abnormalities, such as lactate, serine, alanine, glycine, phenylalanine, and glucose. By analyzing the different spectra of serum, it would be found that there were some changes of metabolites existed between cervical cancer and chronic cervicitis or CIN.

Difference between cervical cancer and its precancerous diseases using pattern recognition analysis

For obtaining an objective statistical estimation, PLS-DA for a model discriminating was used between the samples from patients with chronic cervicitis, CIN, and cervical cancer (shown in Figure 2). Figure 2a shows that the PLS-DA
scatter plot of the cervical cancer and chronic cervicitis patients ($R^2_X=0.225$, $R^2_Y=0.992$, $Q^2=0.527$). Obviously, Figure 2b shows the scatter plot of cervical cancer and CIN patients ($R^2_X=0.147$, $R^2_Y=0.932$, $Q^2=0.168$) which were located in different clusters. The PLS-DA scatter plot for chronic cervicitis and CIN patients (Figure 2c, $R^2_X=0.142$, $R^2_Y=0.911$, $Q^2=0.260$) also shows clear separation and discrimination. These results demonstrated a different metabolic profile in patients with cervical cancer and non-cancer groups, which indicated that there were some serum metabolites contributing to these discriminations. Furthermore, there were several points far away from the center for the serum samples; it may be attributed to a slightly longer period of sample storage [36, 37].

According to the statistical analysis using PLS-DA as unsupervised and supervised methods, respectively, the samples of patients with cervical cancer, CIN, and chronic cervicitis were all scattered into their regions. Figure 2 represented good discrimination of the cancer from chronic cervicitis and CIN with the pattern of metabolites and these results suggested that patients with cervical cancer have a specific profile which was different from patients with chronic cervicitis and the CIN.

Loading plots calculated from the PLS-DA models were to identify metabolites for different models. There were 20 metabolites can be used to separate chronic cervicitis, CIN, and cervical cancer, which are shown in Table 1. The main metabolites contributing to these discriminations were formate, histidine, tyrosine, unsaturated lipid, $\beta$-glucose, glycine, inositol, choline, $\alpha$-glucose, creatine, carnitine, acetone, glutamine, acetylcysteine, acetate, alanine, lactate, valine, isoleucine, and VLDL.

When the data were subjected to classical statistical analysis using SPSS, the statistically significant results were chosen by $p<0.05$. Then, there were 12 metabolites were considered to indicate statistically significant results, which were formate, tyrosine, $\beta$-glucose, inositol, glycine, carnitine, glutamine, acetate, alanine, valine, isoleucine, VLDL.

Compared with chronic cervicitis and CIN, the levels of VLDL, alanine and glycine were increased in the samples of patients with cervical cancer, whereas the levels of isoleucine, valine, glutamine, carnitine, inositol, $\beta$-glucose, tyrosine and formate were reduced. However, compared with cervical cancer, acetate had higher concentration in CIN, and lower concentration in chronic cervicitis.

Because alanine, glutamine, carnitine, inositol, $\beta$-glucose and formate were both significant different between cervical cancer and its precancerous diseases (CIN and chronic cervicitis), the altered expressions of these six metabolites were calculated and illustrated in Figure 3. And VLDL, inositol and $\beta$-glucose were much more different as their $p<0.01$.

Table 1. — Serum metabolites of patients with chronic cervicitis, CIN, and cervical cancer.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Chemical shift</th>
<th>CIN $p&lt;0.005$</th>
<th>chronic cervicitis $p&lt;0.005$</th>
<th>Possible biochemical origin(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>0.81(m), 0.88(m), 1.26(m), 1.57(m), 2.09(m)</td>
<td>—</td>
<td>+1.049 0.001</td>
<td>+2.470 lipid metabolism</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.93(t), 1.00(d)</td>
<td>—</td>
<td>-1.025 0.026</td>
<td>-1.364 energy metabolism</td>
</tr>
<tr>
<td>Valine</td>
<td>0.98(d), 1.04(d)</td>
<td>—</td>
<td>-1.043 0.013</td>
<td>-1.355 TCA cycle</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.33(d), 4.11(q)</td>
<td>—</td>
<td>+1.172 —</td>
<td>-1.026 energy metabolism</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.47(d), 3.77(q)</td>
<td>0.024</td>
<td>+1.803 0.036</td>
<td>+1.635 energy metabolism</td>
</tr>
<tr>
<td>Acetate</td>
<td>1.94(s)</td>
<td>—</td>
<td>-1.580 0.016</td>
<td>+1.888 energy metabolism; pyrimidine and amino acid degradation</td>
</tr>
<tr>
<td>Acetylcysteine</td>
<td>2.07(s)</td>
<td>—</td>
<td>-1.089 —</td>
<td>-1.086 energy metabolism</td>
</tr>
<tr>
<td>Glutamate</td>
<td>2.10(m), 2.13(m)</td>
<td>0.030</td>
<td>-1.620 0.023</td>
<td>-1.433 TCA cycle</td>
</tr>
<tr>
<td>Acetone</td>
<td>2.22(s)</td>
<td>—</td>
<td>-4.055 —</td>
<td>+1.362 energy metabolism; lipid metabolism</td>
</tr>
<tr>
<td>Carnitine</td>
<td>2.46(dd)</td>
<td>0.015</td>
<td>-1.640 0.018</td>
<td>-1.462 lipid metabolism</td>
</tr>
<tr>
<td>Creatine</td>
<td>3.03(s), 3.92(s)</td>
<td>—</td>
<td>+1.180 —</td>
<td>+1.207 serinolysis</td>
</tr>
<tr>
<td>$\alpha$-glucose</td>
<td>3.24(dd), 3.49(t), 4.59(d)</td>
<td>—</td>
<td>+1.199 —</td>
<td>-1.057 glycolysis</td>
</tr>
<tr>
<td>Choline</td>
<td>3.25(s)</td>
<td>—</td>
<td>-1.022 —</td>
<td>+1.144 serinolysis; phospholipid metabolism</td>
</tr>
<tr>
<td>Inositol</td>
<td>3.27(t), 3.56(dd), 3.65(dd)</td>
<td>0.028</td>
<td>-1.552 0.002</td>
<td>-1.421 lipid metabolism</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.58(s)</td>
<td>—</td>
<td>+1.474 0.041</td>
<td>+2.059 serinolysis</td>
</tr>
<tr>
<td>$\beta$-glucose</td>
<td>3.72(dd), 5.23(d)</td>
<td>0.039</td>
<td>-1.429 0.002</td>
<td>-1.729 glycolysis</td>
</tr>
<tr>
<td>Unsaturated lipid</td>
<td>5.26(m), 5.32(m), 5.37(m)</td>
<td>—</td>
<td>+1.036 —</td>
<td>+1.329 lipid metabolism</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>6.88(d), 7.18(d)</td>
<td>—</td>
<td>-1.561 0.023</td>
<td>-1.075 energy metabolism; TCA cycle</td>
</tr>
<tr>
<td>Histidine</td>
<td>7.06(s), 7.73(s)</td>
<td>—</td>
<td>+1.204 —</td>
<td>-1.217 TCA cycle</td>
</tr>
<tr>
<td>Formate</td>
<td>8.43(s)</td>
<td>0.012</td>
<td>-1.250 0.019</td>
<td>-1.276 energy metabolism; pyrimidine and amino acid degradation</td>
</tr>
</tbody>
</table>

* FC: fold change between cervical cancer and its precancerous diseases. Positive sign indicates a higher level in cancer group and negative sign indicates a lower level.

Discussion

Cervical cancer patients were characterized as possessing relatively lower abundance of isoleucine, valine, acetate, glutamine, carnitine, inositol, $\beta$-glucose, tyrosine and formate.
mate, as well as higher abundance of very-low-density lipoprotein (VLDL), alanine, and glycine in their serum compared with the CIN. Compared with cervical cancer, there was only the abundance of acetate in a patient with chronic cervicitis, differing from the CIN, which had a higher level in cancer. Nevertheless, changes in the concentrations of serum metabolite with cervical cancer, and CIN patients clearly pointed to an altered energy metabolism. The relatively lower level of glucose was the same with cervical cancer tissue [30], in which the decreased glucose levels in tissue was due to elevated energy requirement. Potential precursors of glucose in gluconeogenesis, such as alanine and glycine, were found at higher levels in patients with cervical cancer. Levels of alanine and glycine in cervical cancer were different with oral cancer [24]; these may be caused by its hypoxia and ischemia. However, the increasing energy expenditure and metabolism may be followed by the development of these diseases, which may lead these precursors at higher concentrations and glucose as major energy source. These could be the reason of such changes of metabolites from CIN to cervical cancer. Furthermore, several intermediates of tricarboxylic acid (TCA) cycle were found at a lower concentration in patients with cervical cancer, such as glutamine, tyrosine, valine, and formate, which suggest a suppressed TCA cycle. Formate, as an intermediate of TCA cycle and pyrimidine and amino acid degradation, was less concentrated in patients with cervical cancer. As required by cells as an amino donor, glutamine was the synthetic precursor of α-ketoglutarate, tyrosine was precursor of fumarate, and valine and isoleucine were the precursor of succinate, which may be affected by TCA cycle. Furthermore, glutamine can

Figure 3. — A significant difference for the metabolites which have both p<0.05 between cervical cancer and its precancerous diseases, p values are marked in the figure.
Conclusion

In this study, metabolomics analysis of cervical cancer, CIN, and chronic cervicitis were characterized by ‘H NMR, and analyzed by PLS-DA. The results showed that the sample points of these three female diseases had achieved discrimination. It indicated that 20 metabolites had contributed significance for the classification of diseases and 12 of them had statistical significance. Moreover, this method allowed distinct differentiations among the three diseases. Six metabolites, alanine, glutamine, carnitine, inositol, β-glucose, and formate, had significant differences between cervical cancer and its precancerous diseases, which may be identified as potential biomarkers and used for discrimination of cervical cancer.

Acknowledgement

This study was supported by the National Natural Science Foundation of China (No. 21005050), the Beijing Natural Science Foundation (No. 2133061), and the Funding Project for Academic Human Resources Development in Institutions of Higher Learning under the Jurisdiction of Beijing Municipality (No. PHR201108147).

References


Expression of PKCα, PKCε, and P-gp in epithelial ovarian carcinoma and the clinical significance

X. Lili, T. Xiaoyu
Department of Gynecology and Obstetrics, the First Affiliated Hospital of Henan University of Science and Technology, Luoyang (China)

Summary

Aim: To inspect the expression of two protein kinase PKC isozyme hypotype PKCα and PKCε in the epithelial ovarian carcinoma tissue, and investigate their relation with multi-drug resistance with P-glycoprotein (P-gp) medium. Materials and Methods: Adopted immunohistochemistry SP method to determine expression of PKCα, PKCε, and P-gp in 64 cases of epithelial ovarian carcinoma, 18 cases of epithelial borderline ovarian carcinoma, 15 cases of epithelial ovarian benign tumor, and 15 cases of normal ovarian tissue. Results: The expression of PKCα, PKCε, and P-gp in the epithelial ovarian carcinoma is obviously higher than expression in the normal, benign, and borderline epithelial ovarian carcinoma; the expression of PKCα, PKCε, and P-gp in the recurrent carcinoma tissue is obviously higher than that in the person with initial treatment; the expression of above-mentioned three indicators in epithelial ovarian carcinoma is unrelated with the pathological type, pathological grade, and clinical stage during initial treatment of the carcinoma; there is a close relation among PKCα, PKCε, and P-gp in epithelial ovarian carcinoma (p < 0.01). It is indicated through research that PKCα, PKCε, and P-gp is related with the survival time and poor prognosis of the patient of epithelial ovarian carcinoma, i.e., the positive expression rate of PKCα, PKCε, and P-gp of the person with recurrent carcinoma is higher than that of the person without recurrent carcinoma (p < 0.05). However, the survival rate of the patients with positive expression of three indicators is remarkably lower than those with negative expression (p < 0.05). Conclusion: There is a consistency between expression of PKCα, PKCε, and P-gp in the epithelial ovarian carcinoma, which indicates that the expression of both plays an important role in generation of drug resistance in chemotherapy of ovarian carcinoma with P-gp medium. Joint detection of three indicators has an active guiding role in judgment of the therapeutic effect of clinical chemotherapy and prognosis estimation of the patient.

Key words: Epithelial ovarian carcinoma; PKCα; PKCε; P-gp; Immunohistochemistry; Multi-drug resistance.

Introduction

Ovarian carcinoma is one of three major malignancies of female, the five-year survival rate is low, and chemotherapy is the main adjuvant therapy means. However, later clinical stage at definite diagnosis and chemotherapy resistance are the main reasons for poor prognosis and short survival time of the patients. It is indicated through research that the chemotherapy resistance mechanism of the tumor cells is quite complicated. The overexpression of P-glycoprotein (P-gp) coded by the multi-drug resistance gene is the most important. It is indicated through recent research that there is a certain relation and correlation between protein kinase C (PKC) and P-gp in tumor chemotherapy resistance mechanism and expression of multi-drug resistance gene. In the research, the immunohistochemistry method is adopted to determine the expression situation of PKCα, PKCε, and P-gp in benign and malignant tumors and normal tissues, and it is found that PKCα and PKCε can participate in formation of ovarian carcinoma multidrug resistance (MDR) through adjustment of P-gp expression, which provides the theoretical basis for reversion of ovarian carcinoma MDR and judgment of chemotherapy effect and prognosis.

Materials and Methods

Patients and specimens

The specimens were randomly selected from the Pathology Department of the First Affiliated Hospital of Henan University of Science and Technology, Lou Yang, China, including 112 cases of paraffin embedding filed from 2005 to 2012 and 64 cases of ovarian carcinoma. They included 41 cases of tissue specimens through initial treatment operation without chemotherapy and 23 cases of tissue specimen through secondary cytoreductive surgery with tumor recurrence after chemotherapy. 28 cases with serous cystadenocarcinoma, 13 cases with mucinous cystadenocarcinoma, 19 cases with endometrioid carcinoma, and four cases of adenosarcoma; for pathological grade, 12 cases were Grade I, 27 cases Grade II, and 25 cases Grade III; according to FIGO staging standard, eight cases of Stage I, seven cases of Stage II, 41 cases of Stage III, and eight cases of Stage IV; 41 cases of patients underwent four to ten chemotherapy treatment courses after initial operation treatment of ovarian carcinoma, including 30 cases with PAC (cisplatin, Adriamycin, cyclophosphamide) scheme and 11 cases with TP (cisplatin, palclitaxel) scheme. All 23 cases of recurrent ovarian carcinoma were within six months from discontinuation of chemotherapy. Fifteen cases were benign epithelial ovarian carcinoma, 18 cases of epithelial ovarian borderline carcinoma, and 15 cases of normal ovarian tissue. The ages of all cases were from 74 to 28 years, with the average age of 59 years.
Immunohistochemistry
All specimens were fixed in 10% formaldehyde solution and underwent conventional paraffin embedding. Serial four sections were placed on glass slides and pretreated with TEN g/l polyphosphate lysine. Antigen retrieval was performed by microwaving sections in 0.01 mol/l citrate buffer for ten minutes and then cooled to room temperature. Endogenous peroxidase activity was inhibited by incubation with 3% hydrogen peroxide in methanol for 20 minutes at room temperature and non-specific binding was blocked by incubation with 5% bovine serum albumin in phosphate-buffered saline (PBS) at room temperature. After PBS washing for three times, the specimens were reacted overnight at 4°C with P-gp or PKCε monoclonal antibody. The main reaction procedures of immunohistochemistry were conducted in accordance with instructions of the test kit. In each test, the known positive film was the positive control and PBS was the negative control instead of the primary antibody.

Result judgment
Five high power fields were randomly selected and counted over 500 tumor cells. PKCα and PKCε were judged as positive cells with the membrane or cytoplasm color and P-gp was judged as positive cells with the membrane color. The semi-quantitative was conducted in accordance with the number of positive cells and cell staining intensity [2]: no positive cell: 0 points, positive cells: 1% ~30%: 1 point, 31% ~70%: 2 points, 71%~100%: 3 points; 0, 1, 2, and 3 points were counted in accordance with the positive staining intensity (no color: 0 points, light brown: 1 point, brown yellow: 2 points, and dark brown: 3 points). Two scores of each section were accumulated. 0 points: negative, 1~2 points: weakly positive (+), 3~4 points: positive (++), and 5~6 points: strongly positive (+++). The negative in the experiment referred to weakly positive to strongly positive (+ ~ +++).

Results
Expression of PKCα, PKCε, and P-gp in various ovarian tissues and their relation with parameters of clinical pathology
The positive staining of PKCα, PKCε, and P-gp in ovarian carcinoma is brown, and is positioned in the cell membrane or cytoplasm (Figures 1-3). The PKCα positive rates in the borderline and malignant ovarian tissue were 6% and 64%, respectively, and there was no expression in the normal and benign ovarian tissues ($X^2 = 45.383, p < 0.01$). The expression rates of PKCε in benign, borderline, and malignant tissues were 7%, 11%, and 73%, respectively, and there was no expression in the normal tissue ($X^2 = 53.364, p < 0.01$). The expression rates of P-gp in borderline and malignant tissues were 17% and 55%, respectively, and there was no expression in the normal and benign ovarian tissues ($X^2 = 28.777, p < 0.01$). The above-mentioned three indicators included P-gp membrane expression and PKCα and PKCε cytoplasm expression. Through $X^2$ inspection, the discrepancy in the tissue type, pathological grade, and clinical stage of negative and positive groups of PKCα, PKCε, and P-gp in ovarian carcinoma was not significant ($p > 0.05$).

Expression of P-gp, PKCα, and PKCε in initial treatment and recurrent ovarian carcinoma tissue is shown in Table 1. It can be seen that the positive expression rate of above-mentioned three indicators in the patient with recurrent ovarian carcinoma tissue was obviously higher than that in the patient with initial treatment, which is of difference significance.

<table>
<thead>
<tr>
<th>Item</th>
<th>Number of cases</th>
<th>P-gp %</th>
<th>PKCα %</th>
<th>PKCε %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial treatment</td>
<td>41</td>
<td>15</td>
<td>36.6</td>
<td>18</td>
</tr>
<tr>
<td>Recurrent</td>
<td>23</td>
<td>20</td>
<td>87.0</td>
<td>19</td>
</tr>
</tbody>
</table>

$X^2$  

$p$ <0.01 <0.01 <0.05
Expression of PKCα, PKCε, and P-gp in epithelial ovarian carcinoma and the clinical significance

Relation among PKCα, PKCε, and P-gp expression

The Kappa internal consistency coefficient was used to inspect the relation among PKCα, PKCε, and P-gp expression and the results showed that there was consistency between expression of PKCα and P-gp, and Kappa coefficient was 0.511, \( p < 0.01 \). Similarly, there was good consistency between PKCε and P-gp expression, and Kappa coefficient was 0.461, \( p < 0.01 \) (Table 2).

Expression of P-gp, PKCα, and PKCε and relation with recurrence and prognosis

The follow-up survey conducted in 64 cases of epithelial ovarian carcinoma for an average 37.5 months (three to 72 months), with 23 cases of recurrence, included nine cases of P-gp (+)/PKCα (+)/PKCε (+), five cases of P-gp (+)/PKCα (-)/PKCε (+), seven cases of P-gp (+)/PKCα (+)/PKCε (-), and two cases of P-gp (-)/PKCα (+)/PKCε (+). The positive expression rate of P-gp, PKCα, and PKCε of the patient with recurrence was obviously higher than that of the patient without recurrence \( (p < 0.05) \). Kaplan-Meier method was adopted to analyze the survival time of 64 cases of ovarian carcinoma patients and expression of P-gp, PKCα, and PKCε, and the survival rate of the patients with positive expression of P-gp, PKCα, and PKCε was lower than those with negative expression \( (p < 0.05) \) (Table 3, Figures 4-6).

Discussion

Ovarian malignancy is one of three major malignancies of female genitals and the mortality ranks the first place among the gynecological malignancies. Epithelial ovarian carcinoma accounts for about 90%, and the principle treatment is surgical, aided with chemotherapy and radiotherapy. The chemical drug treatment is the main adjuvant therapy for ovarian carcinoma. Furthermore, it is found through research that compared with other solid carcinomas, the overall response rate to chemotherapy of epithelial ovarian carcinoma is up to 60% to

Table 2. — Relation among PKCα, PKCε, and P-gp expression.

<table>
<thead>
<tr>
<th>P-gp</th>
<th>PKCα</th>
<th>PKCε</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>Positive</td>
<td>7</td>
<td>31</td>
</tr>
</tbody>
</table>

Table 3. — Expression of P-gp, PKCα, and PKCε and relation with survival rate with ovarian carcinoma.

<table>
<thead>
<tr>
<th>P-gp</th>
<th>PKCα</th>
<th>PKCε</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>5.7</td>
<td>48.3</td>
</tr>
<tr>
<td>( p )</td>
<td>0.021</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Figure 4. — Kaplan-Meier survival curve of patients with positive and negative P-gp expression.

Figure 5. — Kaplan-Meier survival curve of patients with positive and negative PKCα expression.

Figure 6. — Kaplan-Meier survival curve of patients with positive and negative PKCε expression.
70%. However, with increase of the treatment course of chemotherapy, at least 80% of chemotherapy patients have acquired MDR, causing failure of chemotherapy, which is the major barrier for a successful ovarian cancer therapy. P-gp, a transmembrane ATP-dependent efflux pump, is encoded by the mdr-1 gene, which can recognize endogenous metabolites and xenobiotics as substrates, including anticancer drugs such as anthracyclines, epipodophyllotoxins, and Vinca alkaloids. Moreover, the overexpression of P-gp in tumor cells is the main mechanism of multi-drug resistance [3]. It is found in the present experiment that there was no positive expression found in the normal ovarian and benign ovarian tumors, which further confirms that the ovary is not the enrichment organ of P-gp [4]. The expression of P-gp in ovarian carcinoma is higher than that in the normal ovarian tissue, benign, and borderline epithelial ovarian carcinoma tissue. Furthermore, the expression in the recurrent case of ovarian carcinoma within half a year after chemotherapy for numerous times was obviously higher than that in the patient with initial treatment, generating the acquired drug resistance. The result is consistent with the research at home and abroad. With the in-depth investigation of the chemotherapy resistance mechanism in recent years, P-gp has been recognized as the drug resistance index of malignancy. In research, P-gp is considered as the drug resistance index for ovarian carcinoma, to investigate the influence of PKC, on the ovarian carcinoma drug resistance, and possible approach.

PKC is a kind of widespread phospholipid-dependent enzyme, which can mediate the oncogene signal, participate in regulation of cell cycle and play a role in regulating cell growth and apoptosis. Therefore, it is related with occurrence and development of the tumor in multiple links. It is reported by Prevostel et al. [5] that the transposition of PKC in the cell can make PKC close to different protein substrates in the subcellular region, so that the cell will cause different external reactions. Therefore, PKC activity of multiple tumor tissue cells is high, and the hypotype expression is changed. It is prompted in the research that the expression of two hypotypes of PKCa and PKCe in ovarian carcinoma is higher than that in the normal tissue as well as benign and borderline epithelial ovarian carcinoma, which further reflects that PKC may participate in occurrence and development of ovarian carcinoma. PKC is closely interrelated to signal transduction of cell proliferation, differentiation, and apoptosis. On the one hand, the activated PKC can enable phosphorylation of some tumor proteins or enzymes in the cells, and the phosphorylated proteins enter the core to activate the specific gene, and increase transcription and expression; on the other hand, a portion of PKC hypotype or hydrolyzed PKC enters the core to directly combine DNA, which influences gene expression based on transcription, causing disorders in the cellular pathway. Therefore, PKC may participate in MDR formation through above-mentioned mechanism [6]. Zhan et al. [7] proposed that PKC directly influences expression of different drug resistance proteins and the phosphorylation degree. The overexpression of PKC or activity increase can enable phosphorylation of P-gp coded by MDR gene and generate MDR phenotype. Lee et al. [8] used a kind of inhibitor Go6976 of PKCa, which can reduce MDR expression, and increase doxorubicin-induced apoptosis. Bellrao et al. [9] used the inhibitor of PKC to act on the breast cancer cell which was resistant to doxorubicin. The result is that the inhibitor retards PKC activity, reduces P-gp phosphorylation, and increases intracellular drug accumulation. Therefore, the P-gp mediated MDR is blocked. On the other hand, it is reported that PKC activators were transfected with full-length PKC-alpha genes driven by the ecdysone promoter imported through transfection in MCF7 cells which enabled the stable expression of PKCa [10]. It is believed that PKCa may participate in P-gp transcription and regulate the expression. In addition, Sun et al. [11] found that QA3 was a derivative of the substituted 1,3-dimethyl-1H-quinoxalin-2-ones, which can suppress expression of P-gp in MDR cancer cells. Its mechanism is that QA3 significantly decreases the intracellular level of ATP, stimulates ATPase activity in membrane microsomes, and decreases PKC activity. High expression of PKC and P-gp in chemotherapy resistance of recurrent ovarian carcinoma was found in the present research, and there was a positive correlation between PKC and P-gp.

At present, it has been found that PKC has 12 hypotypes in mammalian cells. PKC hypotypes have the heterogeneity, and different extracellular stimuli will activate different PKC hypotypes, and the expressions of PKC hypotypes in various cells are different. In different PKC isozymes, PKCa is the main modifier of MDR phenotype. It is found in many drug resistant cell lines (for example, KB, MCF7, P388, rat 180 and UV -2237M cells) that the rising level of PKCα, which can reduce MDR expression, and increase doxorubicin-induced apoptosis. Bellrao et al. [13] analyzed the cancer cells of five cases of patients with ascites ovarian carcinoma, and found that the levels of MDRI and PKCe mRNA in four cases were relatively increased, one case was remarkably increased after chemotherapy, which may have been caused by PKCe that activated the drug resistance gene. Through research of prostate cancer cell line LNCaP, Flescher et al. [14] believe that PKCe can be the signal molecule for generation of P-gp. It is also prompted in the present research that there is a consistency between PKCe and P-gp. Therefore, it is indicated that PKCe and PKCe is related with ovarian carcinoma P-gp mediated MDR, and the function is exerted in two aspects of increasing P-gp phosphorylation and adjusting transcription.
During establishment of drug resistance cell line, the acquisition of MDR process is always related with MDR1 gene amplification. However, with in-depth research of MDR mechanism of tumor patients, it is believed that the overexpression of P-gp in malignant tumor cells can be determined, but whether it is caused due to MDR1 gene amplification cannot be determined. On the contrary, it is believed that a rise of MDR1mRNA and P-gp is activated by the external stimuli, therefore rising the MDR1 gene. Therefore, it is inferred that blockage of MDR1 gene activation mechanism can suppress MDR phenomenon in malignancy. Some scholars suppress the PKC signal pathway, to eliminate activation of MDR1 gene [15]. Therefore, the present authors believe that it is more effective to look for the signal pathway of MDR1 gene than direct use of reversal agent to suppress P-gp, if the acquired MDR of ovarian carcinoma is blocked in the upstream of P-gp. High expression of PKCa, PKCe and P-gp in chemotherapy resistance of recurrent ovarian carcinoma is found in the research, and there is a consistency between PKCa, PKCe, and P-gp. Therefore, in the future research, we can apply the antisense oligonucleotide, monoclonal antibody and transfected wild-type P35 of PKCa and PKCe in the molecule level to reverse the ovarian carcinoma MDR, and apply it in clinical trials. In addition, in recent years, with increase of cisplatin and doxorubicin resistance in the ovarian carcinoma chemotherapy drugs, paclitaxel is applied more widely as the first-line drug. However, scholars found through research that the drug resistance mechanism of Paclitaxel includes expression of P-gp, PKC expression change and so on [16]. The patients with ovarian carcinoma in this paper underwent TP or PAC treatment, which is the common first-line scheme for ovarian carcinoma. The high expression of PKCe and P-gp is also prompted in the generated chemotherapy resistance mechanism, which is consistent with the aforementioned research.

Through analysis with Kaplan-Meier survival curve, it was shown that the survival time of the patient with positive expression of PKCa, PKCe, and P-gp was obviously shorter than patient with negative expression of PKCa, PKCe, and P-gp. It is prompted that whether PKCa, PKCe, and P-gp are expressed may become the important indicator of tumor prognosis. In summary, PKCa and PKCe expression is obviously related with ovarian carcinoma tissue chemotherapy resistance, which may play an important role in P-gp mediated ovarian carcinoma MDR. Therefore, in order to improve ovarian carcinoma chemotherapy effects, the combined drugs should be used, and the reversal agent in connection with various hypotyopes of PKC can also be applied, which will remarkably improve the sensitivity of malignancy to chemotherapy. In addition, patients with ovarian carcinoma will be subject to determination of PKCa, PKCe, and P-gp, which is helpful for prediction of the chemotherapy results and prognosis judgment, and also helpful for the clinician to reasonably select the chemotherapy drugs based on the expression, with the goal to guide clinical treatment.

References


Address reprint requests to:
T. XIAOYU, M.D.
Department of Gynecology, the First Affiliated Hospital of Henan University of Science and Technology, No. 24 Jinghua Road, Luoyang 471003, Henan Province (China)
e-mail: xiong-ly144@126.com
A diagnostic dilemma for solid ovarian masses: the clinical and radiological aspects with differential diagnosis of 23 cases

M. Genç1, A. Solak2, B. Genç2, O.N. Sivrikoz3, S. Kurtulmuş4, A. Turan1, N. Şahin1, E.B. Gür1

1 Department of Obstetrics and Gynecology, Sifa University School of Medicine, İzmir; 2 Department of Radiology, Sifa University School of Medicine, İzmir; 3 Department of Pathology, Sifa University School of Medicine, İzmir; 4 Department of Obstetrics and Gynecology, Eagean Maternity and Women’s Health Training Hospital, İzmir (Turkey)

Summary

Objective: This study aimed to analyze the clinical characteristics and diagnostic features of ovarian fibromatous masses. Materials and Methods: The authors reviewed the records of 23 women who underwent laparotomic surgeries and whose final histopathological diagnoses were ovarian fibroma, cellular fibroma, or fibrothecoma from January 2005 to January 2013. The clinical, ultrasonographic, magnetic resonance imaging, tumor marker, therapeutic, and histologic data were analyzed. Results: The mean age of the patients was 50.9 years. Sixteen patients were menopausal. The preoperative ultrasonography examination incorrectly diagnosed seven lesions as uterine fibromas, and the magnetic resonance imaging examination incorrectly labeled three lesions as pedunculated subserous uterine fibromas. The cancer antigen-125 levels of 17 cases were measured, with four being abnormal. Twenty-three patients underwent a laparotomy. Twenty patients underwent a total hysterectomy with bilateral salpingo-oophorectomy, and three underwent a tumorectomy. The histologic diagnosis was fibrothecoma in 21 cases, fibroma in one case, and cellular fibroma in one case. Histopathologic examination of the endometrium of seven of the 20 patients who underwent hysterectomy revealed simple endometrial hyperplasia without atypia. Conclusion: Ovarian fibromas and fibrothecomas are often misdiagnosed as uterine fibromas and occasionally mistaken for malignant tumors of the ovary preoperatively. As these tumors originate from ovarian stroma, they may be hormone-active tumors. Therefore, they may lead to premalignant changes in the endometrium. The preoperative evaluation of the endometrium is recommended.

Key words: Cellular Fibroma; Fibroma; Fibrothecoma; Magnetic Resonance Imaging; Ultrasonography.

Introduction

Ovarian fibromas and fibrothecomas, which comprise 1-4% of all ovarian neoplasias, are the most common benign tumors of the ovarian stroma [1, 2]. They are included in the sex cord stromal tumors of ovaries and may present at any age. Ovarian fibromas/fibrothecomas are usually confused with uterine fibromas and rarely diagnosed preoperatively as they appear solid upon ultrasonography (USG) [3].

Ovarian fibromas are occasionally accompanied by ascites and cancer antigen 125 (CA-125) elevation in the serum; the clinical picture in that case may resemble that of a malignant ovarian tumor [4, 5]. The risk of Meig’s syndrome, characterized by ascites and hydrothorax, increases, especially in tumors larger than 1 cm. These lesions are generally asymptomatic and detected during routine gynecologic examinations [4]. As they originate from the stroma, they may secrete hormones (estrogen and rarely, androgens) and cause some clinical signs that depend on estrogen release.

This study examined 23 patients with ovarian fibromas or fibrothecomas with respect to their preoperative symptoms and USG and magnetic resonance imaging (MRI) results. As these tumors may be hormone-active, they may induce histopathological alterations in the endometrium.

Materials and Methods

This retrospective study was approved by our hospital’s local ethics committee. Informed consent forms were not needed. The study included 23 female patients who underwent operations for various gynecologic indications and were diagnosed with ovarian fibroma, fibrothecoma, or cellular fibroma by pathological examination between January 2005 and January 2013. The demographic data (age, height-weight index, parity, mode of delivery [spontaneous vaginal vs. caesarean section]), menopausal state (patients with amenorrhea for longer than one year after the last menstrual cycle, were accepted as menopausal), indications for operation, USG and MRI imaging findings, and tumor markers including serum CA-125 and carbohydrate antigen 19-9 (CA19-9) were analyzed. Two patients who presented to the present hospital with menometrorrhagia underwent endometrial biopsy before the operation, and one patient presenting with postmenopausal bleeding underwent fractional curettage before the operation.

Data collection and image interpretation

The medical records and images of the patients were accessed via the hospital registry (Hospital Information System: HIS, Picture archiving computed system: PACS). All patients were evaluated using B-scale USG, and 20 patients were also evaluated with color Doppler US. Fifteen patients underwent a pelvic MRI examination. The MRI examinations were performed using a 1.5 T device and an eight-spiral body coil. Intravenous contrast material...
(0.1 mmol/kg gadolinium diethylenetriaminepentaacetic acid) was administered to all patients during the examination. The images were consensually evaluated by two radiologists (A.S., B.G.) experienced in abdominal radiology. The mass’s location, size (the two largest orthogonal diameters were evaluated in terms of millimeters), configuration, morphology, echogenicity relative to neighboring structures, and vascularization upon color Doppler ultrasonography (CDUS) (vascularization absent, avascular, vascularity present and close to the uterine myometrium; moderately vascular, vascularization less than the myometrium; hypovascular, more than myometrium; and hypervascular) were assessed.

Parameters similar to those examined in the ultrasonographic examination were investigated in the MRIs. In addition, the lesion’s signal intensity relative to the uterus was examined in the T1- and T2-weighted examinations, and the contrast uptake pattern relative to the uterus and the capsule and the presence of necrosis were analyzed in the post-contrast images. Contrast uptake equal to, less than, and more than the uterus were interpreted as isovascular, hypovascular, and hypervascular enhancements, respectively. Both the USG and MRI were used to search for intra-abdominal fluid and pathologies of the uterus and other pelvic structures. Preoperative chest X-rays were re-evaluated particularly for the presence of pleural fluid.

Pathological examination

Operational materials of the patients were fixed with 10% formalin, and four-micron sections were obtained from paraffin-embedded blocks. Each slide was stained with hematoxylin and eosin (H&E), examined under a light microscope, and diagnosed based on the pathological properties of the tumors.

Statistical analyses

Statistical analyses were performed to examine the relationship of tumor size with age, the presence of ascites, and CA-125 levels. The data are expressed as the median and range. Frequency distributions were compared using rank correlation and the Chi-square test, and median values were compared using the Student t test or Mann-Whitney U test. A two-sided p value less than 0.05 was considered statistically significant.

Results

Clinical findings

A total of 28 lesions were detected in the 23 female patients included in the study (lesions were bilaterally located in five patients). The age range of the patients was between 31 and 67 years. The mean presenting age was 50.9 years. Sixteen (69.6%) of the patients were considered to be in menopause. Twenty-one patients were multiparous, and two were nulliparous. The main symptoms were nonspecific abdominal pain (n=9), abdominal bloating (n=5), menometrorrhagia (n=2), and menopausal bleeding (n=1). Three patients presented with acute abdominal pain and underwent an emergency operation with the initial diagnosis of ovarian torsion. Three patients presenting with acute abdomen were in menopause. The largest tumor diameter was 16 cm and the smallest was 11 cm. One (6.25%) menopausal patient complained of postmenopausal bleeding.

The mean tumor diameter was 13.6 cm. There was no significant relationship between the tumor size and patient age (p > 0.05). However, three patients were asymptomatic and underwent an operation after the detection of a mass in the ovary during a routine gynecologic check-up.

Radiological findings

A total of 22 (22/28, 88%) lesions were detected by USG in 20 of 23 patients. Both lesions were observed on USG in two of the five patients with bilateral lesions, whereas only a single lesion was detected in the remaining three patients with bilateral lesions. Three patients were free of any pathology in the ovaries upon USG. The USG examination revealed that 11 of the lesions had a solid internal structure, seven had a mixed solid-cystic internal structural pattern, and two had hyperechogenic components suggestive of fatty tissue. Two tumors, in contrast, had a cystic appearance containing a dense, viscous fluid, due to prominent necrosis. On the Doppler scan, one of the hypervascular lesions (n=4) was found to have a low-impedance arterial flow suggestive of a malignancy. Three of these with an avascular masses (n=5) had ascites in the abdomen; the presence of severe abdominal pain suggested the presence of ovarian torsion. Seven lesions were reported as pedunculated subserous uterine myomas because they showed an echogenicity close to the uterine internal echo and a blood flow close to that of uterus. Six masses, however, exhibited much lower blood flow compared with the uterine parenchyma. A total of 16 masses were localized by MRI in 15 patients. The majority of the lesions (10/16, 62.5%) were iso- or hypo-intense in the T1-weighted imaging and hypo-intense in the T2-weighted imaging. The majority were homogenous in T1 (9/16, 56%) and heterogeneous in T2 (9/16, 56%). On the MRI examination, masses close to the midline and hypovascular relative to the uterus (n=3) were reported, with the initial diagnosis of a pedunculated myoma (Figure 1). In the other patients, the masses stained iso- (n=8) or hyper-intense (n=4) relative to the uterus were reported as ovarian benign solid tumoral masses. The MRI examination of the patient who had a suspicious malignancy on the Doppler scan showed similar contrast enhancement as the papillary projections, and thus the mass was reported as a borderline or malignant ovarian mass. Four (4/16, 25%) patients had areas of necrosis appearing hypointense in the T1-weighted images and hyperintense in the T2-weighted images. Two of these patients had ascites in the abdomen. Twelve (12/16, 75%) patients had a capsule with contrast uptake that surrounded the lesion and appeared isointense in the T1-weighted images and hypointense in the T2-weighted images. All patients had a normal preoperative chest X-ray, and no pleural effusion was evident. The patients’ ages, symptoms, and radiological findings are summarized in Table 1.

Laboratory findings

The CA-125 levels were measured in 17 patients and were in the normal range (< 35 IU/ml) in 13 (13/17, 76.5%) patients. These levels were higher than the upper limit of
normal (65, 68, 73, and 80 IU/ml) in only four patients (4/17, 23.5%). There was no relationship between the tumor size and CA-125 elevation ($p > 0.05$).

All five patients in whom the CA 19-9 levels were measured had normal CA 19-9 levels (< 40 IU/ml). Three of the four patients with elevated CA-125 levels had abdominal ascites both on USG and during the operation. In contrast, the patient with a CA-125 level of 64 IU/ml had no free fluid in the abdomen in both preoperative imaging examinations and during the operation. The largest diameter of the tumor was measured as eight cm.

**Operative findings**

Twenty of the patients who were operated on underwent a total abdominal hysterectomy and bilateral oophorectomy, and simple mass resection was performed in three patients. Five patients had bilateral lesions, and the remaining patients had a unilateral lesion (left ovary: eight, right ovary: ten). The tumor size ranged between two and 17 cm, and the mean diameter was 8.7 cm. Nine (39.1%) patients were diagnosed with abdominal ascites. The tumor size ranged from eight to 17 cm (mean 11.8 cm) in the patients who had ascites in the abdomen. Five of the patients

---

Figure 1. — A 51-year-old female patient with abdominal pain. The ultrasonographic examination revealed an 8x7 cm solid avascular mass with a myoma-like internal structure and diffuse ascites in the abdomen. (A) A subsequent MRI showed an 8x7x6 cm hyperintense mass-like formation (white arrow) at the midline, which had a close relationship with the uterus. There is also free fluid in the abdomen (black arrows). (B) In post-contrast fat suppression axial sections, the lesion shows no contrast uptake (white arrow), and (C) a normal contrast uptake is evident in the uterus (star). (D) The lesion was reported to be a torsioned myoma, but the histopathological examination revealed a torsioned fibrothecoma. The microscopic specimen shows dense, spindle-shaped cellular structures with round nuclei that do not contain cellular atypia and mitosis. The presence of neighboring foci of necrosis and hemorrhage suggested torsion (H&E x40).
with abdominal ascites had a left ovarian tumor, and four had a right ovarian tumor. None of the patients with bilateral fibrothecomas had abdominal ascites.

**Histopathological findings**

In seven patients, the masses of ovarian origin were sent for frozen examination during the operation, and the results were negative. One of the two patients presenting with menometrorrhagia had a preoperative endometrial curetage result consistent with simple endometrial hyperplasia without atypia, whereas the other had a result consistent with an irregular proliferative endometrium. The patient who presented with postmenopausal bleeding had a preoperative curettage that showed endometrial superficial epithelium. The results of the pathological examination of the cases revealed fibrothecoma in 21 patients, cellular fibroma in one patient, and fibroma in one patient. In the patients, five fibrothecomas originated from both ovaries, six originated from the left ovary, and ten originated from the right ovary. Cellular fibroma and fibroma originated from the left ovary. Five of the 16 patients who were in menopause and underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy had an endometrial histopathology consistent with simple endometrial hyperplasia without atypia (Table 2). Two of the four patients who were premenopausal and underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy had an endometrial histopathology consistent with simple endometrial hyperplasia without atypia. Out of the seven patients with an endometrial pathology consistent with simple endometrial hyperplasia without atypia, six were diagnosed with a fibrothecoma in the ovary, and one had a fibroma in the ovary (Table 3). Ovarian torsion was observed in three women, all of whom were menopausal (Figure 1D).

**USG examinations three months and one year after the operation did not show abdominal ascites in any patient. The follow-up laboratory results showed normal CA-125 levels. The present authors continue to follow-up these patients in an asymptomatic state.**

**Discussion**

Ovarian fibromas/fibrothecomas are the most common benign tumors of the ovary, which are included in the group of sex cord stromal tumors of the ovary. They are almost always benign and curable by surgical excision. This tumor is usually seen in postmenopausal women [2, 6]. The mean age of the women in the present study was 50.9 years, and 16 (69.6%) of the patients were in menopause.

Microscopically, ovarian fibromas are solid tumors originating from fibroblastic cells [2, 7, 8]. Ovarian fibromas are solid tumors consisting of cellular bundles and intersecting strips of hyaline-appearing collagen and fibrous tissue. Fibroblastic cells are spindle-shaped and do not possess signs of atypia. Fibrothecomas comprise lipid-
laden theca cells forming thecomas and some other cells forming fibromas; this terminology is used when the distinction between these two forms is not clear [8]. Cellular fibromas, which are a rare form of ovarian fibromas, can also occur. A tumor containing cells with closely packed nuclei with absent or minimal nuclear atypia in addition to one to three mitoses per ten high-powered fields is classified as a cellular fibroma [9]. These tumors are benign despite reports of local recurrence after surgery. A tumor showing moderate nuclear atypia and three mitoses per ten high-powered fields has malignant potential and is designated as a fibrosarcoma.

The symptoms are generally non-specific and include pelvic pain and menometrorrhagia [4]. However, three of the patients in this series presented with acute abdomen and underwent an operation with the diagnosis of ovarian torsion; the histopathological examination revealed a fibrothecoma in the ovary. The largest diameter of the fibrothecomas in the torsioned ovaries was 16 cm, and the smallest was 11 cm; the mean diameter was 13.6 cm. Son et al. reported torsions with a rate of 6.4% [6]. This study also detected a mean tumor diameter of 11 cm. Vijayaraghavan et al. reported a patient presenting to the hospital with acute abdominal pain who was diagnosed with right ovarian torsion; after undergoing a laparotomy, this patient was found to have a fibroma with a diameter of 16 cm accompanied by a torsioned ovary [10].

Misdiagnosing ovarian fibromas as uterine fibromas in the preoperative period is common [3, 11]. The present authors also operated six patients with an initial diagnosis of uterine fibroma as a result of imaging examinations during the preoperative workup. Due to their fibrous content, ovarian fibromatous masses can be confused in the radiological examinations with subserous, pedunculated uterine fibroids that extend especially into a broad ligament. In a study assessing 35 patients with ovarian fibromas and fibrothecomas by MRI, Shinagore et al. compared their findings with nine uterine fibroids in the same patient group and stressed that neither the signal properties of the mass in the T1- and T2-weighted sequences nor the contrast uptake were radiological distinguishing features from uterine fibroids [12]. Fibromas and fibrothecomas can be distinguished from uterine fibroids only by demonstrating the relationship of the lesion with the ipsilateral ovary. The presence of a follicle or capsule surrounding the lesion increases the possibility of a fibroid. Ovarian fibroids actually lack a capsule, but they considerably slenderize the ovarian stroma they originate from, forming the pattern of a capsule. Oh et al. observed a capsule surrounding the lesion on MRI with a rate of 67% in a 24-patient series [13]. The present authors also noticed a capsule surrounding the mass in 75% of the patients; however, they did not encounter any cystic pattern consistent with an ovarian follicle in the area surrounding the mass.

The present authors observed a serous cystadenoma in addition to a fibrothecoma in the right ovary of one of the patients who had bilateral fibrothecomas and a serous cystadenoma in the same ovary as the fibroma in another patient. In a 38-year-old woman with bilateral fibrothecomas, the right ovary had changes consistent with endometriosis. The histopathological examination of the uteri of 20 patients who underwent a total abdominal hysterectomy revealed adenomyosis in two patients, uterine fibroma and proliferative endometrium in 11 patients, and simple endometrial hyperplasia without atypia in seven patients. The authors’ search of the English literature did not reveal any studies reporting the development of endometrial hyperplasia in patients with fibrothecoma/fibroma as frequently as in this study (7/20, 35%).

Five (21.7%) women in the present study had bilateral fibrothecomas. Son et al. [6] reported a rate of 4.3% for bilateral tumors, and Paladini et al. [3] reported a rate of 6%. Previous studies reported a bilateral tumor incidence of zero to 11.76% [1, 2, 4, 11]. The present authors found a bilateral tumor rate of 21.7%, which was higher than in previous studies. Ten of the patients had right ovarian tumors, six had left ovarian tumors, and five had bilateral fibrothecomas. Cellular fibromas and fibromas were of left ovarian origin. They also found that, unlike Sivanesaratnam et al. and Leung et al. [4, 11] but in agreement with Son et al. [6], the possibility of having a right ovarian fibroma was higher.

In the present study, the tumor diameter in nine patients (39.1%) with abdominal ascites ranged between eight and 17 cm. The average tumor diameter was 11.8 cm in patients with abdominal ascites. However, those patients with no ascites had a tumor diameter ranging from two to 11 cm, with an average size of 6.6 cm. In the present study, the tumor size was not proportional to ascites (< 0.05). The relationship between fibromas and ascites is explained by the observations showing that the cortex layer of the ovary, the origin of the fibroma, does not have lymph vessels, making the tumor form a transudate [2, 6, 7].

Abdominal ascites disappear after tumor resection [2, 14]. Nine patients in the present study who had abdominal ascites at the preoperative period also had no signs of ascites at the three-month and one-year follow-ups. The CA-125 elevation and presence of abdominal ascites were correlated with each other in the present study (p < 0.05). Three of nine (33.3%; 3/9) patients with abdominal ascites had CA-125 elevation, whereas six (66.7%; 6/9) had subnormal CA-125 levels (CA-125 35 IU/ml). The present results are consistent with those reported by Pastnerand et al. and Son et al. [5, 6].

The CA-125 levels normalized three months later in the patients with a preoperative elevation of these levels. Previous studies also reported rare CA-125 elevation that normalized after the operation [14]. Surgery is the recommended mode of treatment in fibromas/fibrothecomas. Although radical surgery has been recommended in perimenopausal and postmenopausal women, simple excision of the mass has been recommended in younger patients [2].
Ovarian fibromas/fibrothecomas may be confused with uterine fibromas due to their echogenicity, which is similar to the internal echo of the uterus, and due to a similar blood flow as the uterus. They may also be confused with malignant ovarian tumors due to the accompanying free abdominal fluid, elevated tumor marker level, and solid tissue pattern [4, 5]. The initial diagnosis of ovarian fibromas may be misleading until the surgical therapy is performed and the diagnosis is confirmed by definitive histopathological examination. As fibromas/fibrothecomas originate from the ovarian stroma, they may actively secrete hormones, including estrogen and androgens. They may also cause premalignant changes in the endometrium, such as endometrial hyperplasia due to unopposed estrogen release at the premenopausal and postmenopausal periods [15, 16]. Therefore, evaluating the endometrium for estrogen exposure with ultrasonography and histopathological examination is beneficial, especially in premenopausal and postmenopausal patients scheduled to undergo salpingo-oophorectomy or tumor excision. Myoma uteri, irregular proliferative endometrium, and simple endometrial hyperplasia without atypia, which the present authors observed in the histopathological examination of the patients who underwent hysterectomy in this study, are conditions that are observed when the endometrium is exposed to estrogen. Therefore, evaluating the uterus using ultrasonography with regard to endometrial thickness and using endometrial biopsy with regard to histopathological diagnosis at the preoperative period will be useful to guide the selection of the operation type.

The main limitations of this study were its retrospective nature and the small sample size. The authors believe that future studies with larger sample sizes examining various pathologies (e.g., torsioned uterine fibroids) are clearly needed.

**Conclusion**

Ovarian fibromas and fibrothecomas are common solid tumors of the ovaries. Due to diagnostic challenges, gynecologists and radiologists should consider ovarian fibromas in differential diagnoses, especially in patients with ovarian solid masses at a postmenopausal age. This tumor may be confused with uterine fibromas in radiological imaging studies. In postmenopausal patients presenting with acute abdominal torsion, ovarian fibromas/fibrothecomas should be considered as possibilities among the potential gynecological etiologies. These tumors may occasionally be confused with malignant ovarian tumors due to abdominal ascites, elevated CA-125 levels, and their solid structure. However, it should be noted that these tumors are benign and may be completely cured with surgical therapy. They may also be hormone-active and thus may lead to premenopausal changes in the endometrium due to unopposed estrogen release. The endometrium should be thoroughly evaluated before and after the operation. A more radical therapy would be appropriate in patients who have a preoperative diagnosis suggestive of fibroma or fibrothecoma and no concerns regarding fertility.

**References**


Address reprint requests to:
M. GENÇ, M.D.
Department of Obstetrics and Gynecology, Şifa University School of Medicine, İzmir, Türkiye
Fevziipaşa Bulvan No: 172/2, 35240, Basmane/İzmir (Turkey)
e-mail: doktorminegenc@gmail.com
Introduction

Uterine cervical cancer is the most common gynecological cancer. This disease affects 15 women per 100,000 women annually in Japan, and its incidence has recently been increasing. Almost all cases of cervical cancer and its precursor lesion, cervical intraepithelial neoplasia (CIN), are caused by the human papillomavirus (HPV), and prolonged infection with high-risk HPV (HR-HPV) is particularly likely to cause cervical cancer [1, 2]. Most women experience one or more infections by HPV in their lifetime; however, more than 90% of these infections are transient because the human immune system can eradicate the HPV within two years [3]. However, in a small percentage of HPV infections, the virus persists in the cervical epithelium and is integrated into the host DNA, leading to the formation of a cervical lesion.

HPV is a DNA virus, and the viral genome consists of 7900 bp that encode eight open reading frames (ORFs). The E6 and E7 genes in these ORFs have a great influence on the formation of cervical lesions. The products of these two genes induce uncontrollable cell proliferation by inactivating p53 and pRb [4, 5]. The cervical epithelial cells express E6/E7 mRNA at a constant high level due to prolonged HPV infection and integration of HPV into their DNA.

Brush cytology has been widely used for the detection of cervical lesions worldwide. The HPV DNA test has recently been used in cases where an abnormality is detected by brush cytology. In Western countries, the HPV DNA test has recently been widely adopted for cervical examination, and because the HPV DNA test is more sensitive than brush cytology, the simultaneous use of both tests is recommended for detecting early cervical lesions [6-8]. A study from the Netherlands showed that screening with the HPV test prior to cytology also improves the effectiveness and decreases the costs associated with cervical cancer examination [9]. However, the DNA HPV test has a low specificity and does not entirely reflect the progression of the cervical lesion since it detects transient HPV infection [8, 10].

The use of HPV tests that detect HPV mRNA has recently been increasing. The APTIMA HPV Assay, one such HPV test, has been approved by Food and Drug Administration (FDA) and is also currently available for sale in more than ten European Union countries.

In the present study, the authors aimed to evaluate the clinical performance of the APTIMA test for the detection of cervical lesions in Japan, and compared it with the HPV DNA tests that are already in use in Japan.

Evaluation of the Human Papillomavirus mRNA Test for the detection of cervical lesions in Japan

Y. Nakayama1,3, M. Yamada1, A. Kurata1, H. Kiseki2, K. Isaka4,5, M. Kuroda1

1 Department of Molecular Pathology, Tokyo Medical University, Tokyo; 2 Kosei Chuo General Hospital, Tokyo
3 Tsujimaru International Patent Office, Kyoto 4 Department of Obstetrics and Gynecology, Tokyo Medical University, Tokyo
5 Hitachi City Endowment for Community Healthcare of Obstetrics Gynecology, Ibaraki (Japan)

Summary

Aims. For the screening of cervical abnormalities, human papillomavirus (HPV) DNA testing is widely used along with Papanicolaou (Pap) testing. Although the sensitivity of the HPV DNA testing is good, its specificity is relatively low. In the present study, the authors evaluated the use of the Gen-Probe APTIMA HPV Assay for the detection of HPV mRNA and compared it with HPV DNA testing. Materials and Methods. Liquid cervical Pap specimens collected from 410 women were assessed using the APTIMA test, the QiaGen Hybrid Capture 2 HPV DNA (HC2) Test, and the AMPLICOR HPV Test. Results. The sensitivity and specificity for the detection of high-risk HPV were 85.6% and 99.2% for the APTIMA test, 94.1% and 98.4% for the HC2 test, and 90.2% and 95.7% for the AMPLICOR test, respectively. As the severity of the cervical lesion progressed, the positive rate of the three tests indicated a similar increase. The clinical sensitivity and specificity for the detection of squamous intraepithelial lesion (SIL) were 91.2% and 84.2% for the APTIMA test, 94.5% and 80.4% for the HC2 test, and 87.9% and 78.2% for the AMPLICOR test, respectively. Conclusion. The APTIMA is sensitive and specific for the detection of high-risk HPV. In the specimens with SIL, the APTIMA test is more specific than the HC2 and the AMPLICOR tests. This indicates that the APTIMA test may improve patient management and reduce the cost of screening.

Key words: Cytology; Human papillomavirus; mRNA; Specificity; Squamous intraepithelial lesion.
Population and sampling
A total of 410 cervical specimens were acquired from four hospitals in Japan (Kosei Chuo General Hospital, Kamata General Hospital, Sanno Medical Center, and Kosugi Clinic) between November 2011 and April 2012. Specimens were obtained from women who underwent a cervical cytology examination for the following reasons: (1) cervical cancer screening; (2) cervical cytology testing as cancer was suspected; (3) need for a re-examination based on the result of a previous cytology examination; and (4) undergoing treatment for cervical cancer. The mean age of the women was 39 ± 9.3 years (range, 20–76). In all the cases, cervical cytological examinations were performed using the Cervex-Brush and the cervical samples were preserved in PreservCyt solution. The cytologic specimens were prepared from this solution according to the liquid-based cytology (LBC) methods, and HPV tests were simultaneously performed. Woman who provided written informed consent were enrolled. The Ethics committee of Tokyo Kosei Chuo General Hospital approved all protocols.

Cytology
Cytological diagnosis was made by cytotechnologists and cytopathologists according to the Bethesda system. The potential diagnoses included negative for intraepithelial lesion or malignancy (NILM), low-grade squamous intraepithelial lesion (LSIL), high-grade intraepithelial lesion (HSIL), atypical squamous cells of unknown significance (ASC-US), atypical squamous cells–cannot exclude HSIL (ASC-H), or squamous cell carcinoma (SCC).

HPV mRNA test
The Gen-Probe APTIMA HPV Assay was used for HPV mRNA testing. A one-ml sample from the preserved solution was placed in a test tube containing a buffer solution to lyse the cells and extract their mRNA. The test tube was then loaded onto a fully automated TIGRIS DTS system and the solution was analyzed according to the manufacturer’s instructions. The APTIMA test can detect the E6/E7 mRNA of 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Aliquots of four ml and 250 μl from the preserved solution were used for HC2 and AMPLICOR testing, respectively.

Evaluation of the HPV tests
Three cases using the APTIMA, HC2, and AMPLICOR tests were conducted. The first case indicated a unanimous HR-HPV positive result. The second case indicated a unanimous HR-HPV negative result. However, deviations were noted during the testing of the third case, and therefore, a Linear Array HPV Genotyping Test (LA) was used. The sample in which HR-HPV was detected on LA testing was considered to be HR-HPV positive.

Statistical analysis
The authors calculated the sensitivity, specificity, and positive and negative predictive values (PPV/NPV) by using two × two tables, and the results are described with 95% confidence intervals. The probabilities were compared using McNemar’s test. Data analysis was performed using the SPSS software.

Results
Of the 410 women who provided cervical specimens, 153 cases were found to be HR-HPV positive, whereas 257 were found to be HR-HPV negative (Table 1). Of the 153 HR-HPV-positive cases, 121 were found to be positive by all three tests and the other 32 HR-HPV cases were diagnosed by LA HPV typing. In the HR-HPV-positive group, 131 cases (85.6%) were found to be HR-HPV positive and 22 (14.4%) were found to be HR-HPV negative by APTIMA testing. Of the 22 cases that were HR-HPV positive but were negative by APTIMA testing, the cytological diagnosis was NILM in 12, ASCUS in eight, and LSIL in two.

Table 1. — Comparison of the APTIMA, HC2, and AMPLICOR tests with regard to the high-risk HPV status.

<table>
<thead>
<tr>
<th></th>
<th>Cases of high-risk HPV</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>APTIMA</td>
<td>131</td>
<td>2</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>22</td>
<td>255</td>
<td>277</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>257</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC2</td>
<td>144</td>
<td>4</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
<td>253</td>
<td>262</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>257</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMPLICOR</td>
<td>138</td>
<td>11</td>
<td>149</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>246</td>
<td>261</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>257</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

APTIMA: Gen-Probe APTIMA HPV Assay; HC2: Hybrid Capture 2 HPV DNA Test; AMPLICOR: AMPLICOR HPV Test; HPV: human papillomavirus.
cases. Of the HR-HPV-negative cases, 255 (99.2%) were found to be negative and two (0.8%) were found to be positive by APTIMA testing. Of the two cases that were HR-HPV negative but positive by APTIMA testing, the cytological diagnosis was NILM in one and ASCUS in the other case.

Of the HR-HPV-positive cases, 144 (94.1%) were found to be positive and nine (5.8%) were found to be negative by HC2 testing. Of the nine cases that were HR-HPV positive but were negative by HC2 testing, the cytological diagnosis was NILM in eight and ASCUS in one case. Of the HR-HPV-negative cases, 253 (98.4%) were found to be negative and four were found to be positive on HC2 testing. Of the four cases that were HR-HPV negative and were also negative on HC2 testing, the cytological diagnosis was ASCUS in two, ASC-H in one, and HSIL in one case. Of the HR-HPV-positive cases, 138 (90.2%) were found to be positive and 15 (9.8%) were found to be negative by AMPLICOR testing. Of the 15 cases that were HR-HPV positive but negative on AMPLICOR testing, the cytological diagnosis was NILM in four, ASCUS in five, LSIL in five, and HSIL in one case. Of the cases that were HR-HPV negative, 246 (95.7%) were found to be negative and 11 (4.3%) were found to be positive on AMPLICOR testing. Of the 11 cases that were HR-HPV negative but were positive on AMPLICOR testing, the cytological diagnosis was NILM in seven, ASCUS in two, ASC-H in one, and HSIL in one case.

Subsequently, the authors examined the HR-HPV positive rate of all the three tests according to the cytological diagnosis. The positive rate of the three tests showed a similar pattern of increase as the lesion progressed from NILM to HSIL (Figure 1). The sensitivity for HR-HPV was 85.6% for the APTIMA test and 94.1% for the HC2 test (Table 2). The sensitivity of the HC2 test was significantly higher than that of the APTIMA test ($p < 0.01$); however, no significant difference in specificity was noted between these tests. The sensitivity for HR-HPV was 85.6% for the APTIMA test and 90.2% for the AMPLICOR test; however, no difference in sensitivity was noted between these tests (Table 2). In contrast, the specificity for the APTIMA test (99.2%) was significantly greater than that of the AMPLICOR test (95.7%) ($p < 0.05$).

The authors then compared the results of the three tests in the 91 cases with a cytological diagnosis of SIL (comprising LSIL and HSIL). In these cases, the sensitivity was 91.2% for the APTIMA test and 94.5% for the HC2 test; however, no significant difference was noted in the sensitivity between these tests (Table 3). In contrast, the specificity of the APTIMA test (99.2%) was significantly greater than that of the HC2 test (80.4%) ($p < 0.01$).

Although no significant difference was noted in the sensitivity between the APTIMA and the AMPLICOR tests (Table 3), the specificity of the APTIMA test (84.2%) was greater than that of the AMPLICOR test (78.2%) ($p < 0.05$).
Among the 50 cases diagnosed as HSIL, the sensitivity was 96.0% for the APTIMA test and 98.0% for the HC2 test; however, no significant difference in the sensitivity was noted between the tests (Table 4). In contrast, the specificity was 76.3% for the APTIMA test and 72.4% for the HC2 test \((p < 0.01)\). Although no significant difference in sensitivity was noted between the two tests (Table 4), the specificity of the APTIMA test (76.3%) was significantly greater than that of the AMPLICOR test (71.2%) \((p < 0.05)\).

**Discussion**

Uterine cervical cancer is an important cancer as almost all types of cervical cancer are caused by an HR-HPV infection [11]. Because many adult women have been infected with HR-HPV at least once during their lifetime, HPV testing is used worldwide for cervical cancer examination. DNA and mRNA tests are currently available for HPV detection [12]. Although the HPV DNA test has been widely used to detect the presence of the HPV genome, this method also detects the presence of a transient HPV infection that may never cause a cervical lesion, resulting in a relatively low specificity [10]. The HPV mRNA test has recently attracted attention as a novel method to replace the HPV DNA test. Because the development of cervical lesions requires high levels of expression of the HPV-derived E6/E7 genes [4], the HPV mRNA test is believed to more accurately reflect the onset and progression of cervical lesions. In the present study, the APTIMA test showed high sensitivity (85.6%) and specificity (94.1%) for HR-HPV detection in cases with cervical lesions. Previous studies comparing the APTIMA and HPV DNA tests showed that the APTIMA test had similar sensitivity but better specificity as compared to the HPV DNA tests [13-15]. However, in the present study, the APTIMA test was more specific than the AMPLICOR test, but the sensitivity of the APTIMA test was significantly lower than that of the HC2 test. Moreover, the APTIMA test did not indicate a superior sensitivity or specificity in any of the HPV-infected specimens.

HPV 66 is one of many HPV types that cause cervical cancer [16]. As HPV 66 can only be detected by the APTIMA test, the authors expected the sensitivity of the APTIMA test to be higher than that of the HC2 and AMPLICOR tests. However, both the APTIMA and HC2 tests showed positive results in all the five cases wherein HPV 66 was detected by LA HPV typing. The authors believe that this one of the reasons why the APTIMA test was not found to be superior to the HC2 test in the present study. This unexpected detection of HPV 66 by the HC2 test may represent a type of cross reaction, which is a phenomenon that has been reported in prior studies [15, 17].

mRNA, the target of the APTIMA test, is less stable than DNA, and this instability is believed to cause a decline in sensitivity. However, in the present study, the authors used a proteolytic enzyme in the preserved solution (the LBC method), which has been shown to preserve RNA in a stable form for two to five weeks. Therefore, the authors believe that mRNA instability did not significantly affect the present results [18-20].

The cases diagnosed with SIL (including LSIL or HSIL) by cytological testing showed equal sensitivity and higher specificity compared to HPV DNA tests, which is consistent with a study on APTIMA tests in Western countries [15, 19, 21, 22]. Although the authors did not perform histological examination in the current study, some studies has shown that the HPV E6/E7 mRNA test using the RT-PCR method is more specific than the HPV DNA test for the diagnosis of CIN [20, 23]. HPV DNA tests may be less specific than HPV mRNA tests because, as stated above, DNA tests can detect transient HPV infection and also exhibit cross reactivity with certain low-risk HPV types [24]. Since HR-HPV cases detected by the HPV mRNA test have a greater tendency to progress to CIN over a long period than those detected by HPV DNA testing [25], the higher specificity of the APTIMA test may only become apparent during the follow-up of the cases in the present study.

The combined use of cytological examination and the HPV test is currently recommended for the prevention and early detection of cervical lesions, and this recommendation is being increasingly followed in Japan. However, only 5% of the patients who are infected with HR-HPV eventually develop cervical cancer [26]. Therefore, a more specific detection method is desirable in order to reduce examination costs. Since the APTIMA test has a higher specificity compared with the HPV DNA tests, the authors believe that this test could replace the HPV DNA tests in Japan.

**References**


Address reprint requests to:
M. YAMADA, M.D.
Department of Molecular Pathology,
Tokyo Medical University
6-1-1 Shinjuku, Shinjuku Ward,
Tokyo 160-8402 (Japan)
e-mail: yamapath@tokyo-med.ac.jp
Protective and sensitive effects of melatonin combined with adriamycin on ER+ (estrogen receptor) breast cancer

C. Ma1, L.X. Li2, Y. Zhang1, C. Xiang1, T. Ma1, Z.Q. Ma1, Z.P. Zhang1

1 Department of Oncology, the First Hospital of Shijiazhuang, Shijiazhuang
2 Department of Clinical Laboratory, the First Hospital of Shijiazhuang, Shijiazhuang (P.R. China)

Summary
Objective: This study aims to investigate the protective and sensitive effects of melatonin (MLT) in the treatment of breast cancer.

Materials and Methods: ER+ breast cancer rat model was established and then rats were randomly divided into five different groups as follows: control group, Diss group, adriamycin (ADM) group, MLT group, and MLT combined with adriamycin (M+A) group. Tumor weights and one month survival rate were compared among these groups. In addition, changes of tumor tissues and expression of E-cadherin were observed under optical microscopy or electro-microscopy.

Results: Tumor weights were significantly lighter in M+A group than those in ADM group \( (p < 0.05) \). Under optical and electro-microscopy, tumor cell apoptosis was obviously increased in MLT group, and tumor cell injury was more severe in M+A group than that in ADM group; additionally, expression of E-cadherin was higher in MLT group and M+A group than that in other groups. Moreover, MLT group had the highest one month survival rate (100%), there was the poorest life quality in ADM group, but the best life quality in MLT.

Conclusion: MLT could enhance the sensitivity of tumor to ADM in vivo and improve patient’s life quality.

Key words: Melatonin; Breast cancer; Adriamycin; Metastasis.

Introduction
Breast cancer is a malignant tumor with a high incidence in females from more developed Western countries. Although historically the incidence of breast cancer in women in China was low, it has been on the increase in the past 20 years. In large cities, such as Beijing and Shanghai, breast cancer is the most common type of malignant tumor diagnosed in women. As the country with the highest population in the world, there are a large total number of cases annually. Anthracycline chemotherapeutic agents, including adriamycin (ADM), are among the main agents used for breast cancer chemotherapy. ADM has been essential in breast cancer therapy, particularly in prolonging the survival of patients with advanced and metastatic breast cancer. However, due to the dose-limiting cardiotoxicity of ADM, it is not suitable for use in patients with cardiac disorders. The administration of ADM may cause varying degrees of myocardial toxicity, seriously affecting the patients’ quality of life, and in certain cases has caused toxicity-related mortality [1-3].

In recent years, studies of myocardial toxicity have been reported, but no drug has been satisfactorily applied in a clinical environment [4-6]. With developments in the treatment of malignant tumors, tumor reduction and disease relief in the short term are no longer sufficient and steps to improve quality of life and prolong the survival of patients are drawing an increasing amount of attention. There has been a strong clinical demand for cardioprotective drugs for use in tumor patients [7]. There is a need for a drug that is not only helpful in the treatment of breast cancer, but also alleviates ADM-induced cardiotoxicity.

Melatonin, produced in the pineal gland which is outside of the blood-brain barrier, acts as an endocrine hormone and is widely distributed in different organs. In animals, melatonin (MLT) is associated with several biological functions. Some studies have indicated that MLT inhibited the growth of many malignant tumors, such as ER+ breast cancer [8-11]. The authors’ previous study also demonstrated that exogenous MLT suppressed the proliferation of breast carcinoma cell lines and enhanced the sensitivity of ER+ breast carcinoma cell line MCF-7 to ADM [12, 13], but we did not perform further study in animal model. In this study, the authors established rat model with ER+ breast cancer and further confirmed the effect and mechanism of MLT on proliferation and resistant to ADM in breast cancer rat model in order to provide basic evidences for developing safer and more effective drugs.
Materials and Methods

Animals

The study included 140 Sprague-Dawley (SD) female rats, about 200-250 gram weight, were purchased from animal center of Hebei medical university. All rats were fed under light-dark conditions that was 12 hours light time (6:00-18:00) and 12 hours dark time (18:00-6:00) by turns. Room temperature was 25 ± 2°C and the humidity in the air was 45% - 50%. All rats underwent experiments after being fed for three weeks. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the First Hospital of Shijiazhuang.

Establishing breast cancer rat model

According to Russo methods [14], 130 of SD female rats were injected with N-nitroso-N-methylurea (NMU) to induce breast cancer. Ten SD female rats were injected with Diss as control.

All rats were weighed and numbered through Dagar method. Rats were treated with 50 mg/kg NMU through intraperitoneal injection. Then rats were weighed again after two weeks, and then treated once again. Rat breast were checked once a week to observe tumor growth.

Groups

Rats were divided into five groups: blank group; Diss group; MLT group that rats were treated with 10 mg/kg/day MLT for 15 days; ADM group with rats that were treated with 2.5 mg/ml ADM, qod, for seven times; M+A group that rats were treated with 10 mg/kg/d MLT for 15 days and 2.5 mg/ml ADM were administered from the third day, qod, for seven times. MLT was injected before ADM treatment. Some rats were sacrificed at 18 days after tumors were taken out and weighed, and others were observed while still alive.

Electro-microscopy observation

The tumor tissue was placed in a 10 ml flasket, then 4% glutaraldehyde was added for immersion. After fixation at 4°C for one hour, the sample was washed with phosphate buffer solution (PBS) for three times within ten minutes, followed by fixation using 1% osmic acid at 4°C for two hours. Then PBS was used for washing for three times within ten minutes. The dehydration was conducted using ethanol with gradient concentration (50%, 70%, 80%, 90%, 100%, and 100%; ten minutes for each step), followed by dehydration using acetone for two times (ten minutes for each time). The sample was immersed with mixture liquid of acetone and resin (3:1, 1:1, and 1:3, respectively) for 15 minutes, followed by immersion with pure resin for 30 minutes. Then the sample was embedded in epoxy resin (type 812, 815), followed by polymerization (successive 37°C, 45°C, and 60°C; 24 hours for each temperature). The ultrathin sections were prepared in a ultramicrotome, followed by double electron staining using lead citrate for 30 minutes. Finally, the sections were observed and photographed using H-7500 transmission electron microscope.

Immunohistochemistry assay

Four-μm tumor tissues were sliced and then dewaxed with dimethylbenzene, absolute alcohol, 95% alcohol, 80% alcohol, 70% alcohol, respectively, and then added ethylenediamine tetracetic acid (EDTA) solution, after treatment of primary antibodies, tissues were incubated overnight at 4°C, then washed with PBS, followed by treated with EnVision reagent to incubate for 30 seconds at room temperature, then stained with 3,3-dimetobenzidine (DAB) which was stopped through observation under light microscopy, and then dehydrated with 70% alcohol, 80% alcohol, 95% alcohol, absolute alcohol, and dimethylbenzene, respectively. Finally, sections were observed under light microscopy.

Results of immunohistochemistry were judged as follows: most of positive primrose or brown-yellow particles were located in cytoplasm; few positive particles were located in nucleus. According to counting positive cells and color depth to score under five different fields: 0 score: proportions of positive cells were less than 5%; 1 score: proportions were 5%-24%, 2 scores: proportions were 25%-49%; 3 scores: proportions were 50%-74%; 4 scores: proportions were more than 75%. In addition, according the color depth, no-staining color was 0 score, light yellow presented 1 score, yellow presented 2 scores, and brown and yellow presented 3 scores. If positive cells scores × color scores < 1 score, this case were a negative expression of ER or E-cadherin, others were judged as positive expression of ER or E-cadherin.

Recording of survival condition

Rats from every group were recorded and observed feeding condition and mental status. One month survival rate was also recorded.

Statistical analysis

Statistical analysis was performed by SPSS 13.0 software and all results were presented as mean±SD. One-way analysis of variance was used to compare with different groups and χ² test was used for comparisons of one month survival rate. A p value < 0.05 denoted a significant statistical difference.

Results

Tumor formation

Only one rat died without any reason in vehicle group; seven rats died during injection or before dividing into groups in treatment groups. Seven rats failed to establish breast tumor model. Therefore, 116 rats were randomly divided into treatment groups. Tumor formation rate was 91.5%. Eight rats from every group were sacrificed and then developed tumors; others were observed at one month survival rate.

Tumor weight

The tumor weights of blank group, Diss group, MLT group, ADM group, and M+A group were 23.45 ± 5.08, 27.52 ± 4.93, 22.51 ± 4.26, 14.84 ± 2.99, and 10.06 ± 3.13 grams, respectively, and there was no significant difference among blank group and Diss group (p > 0.05). Moreover, there was also no significant difference between MLT group and blank group (p > 0.05), but tumor weights from ADM
Protective and sensitive effects of melatonin combined with Adriamycin on ER+(estrogen receptor) breast cancer

Group and M+A group had an obvious difference from those from blank group ($p < 0.01$), while, tumor weights of M+A group was lighter than those of ADM group ($p < 0.01$) (Table 1).

**General changes**

In MLT group and M+A group, tumors had relative integrity and smooth membrane envelopes and were easily stripped off, but there was reverse phenomenon in blank group, Diss group, and ADM group, in which the tumors adhered with adjacent tissues and grew aggressively, and some tumors boundary were not clear.

**Changes of tumor tissues under electro-microscopy**

Tumor tissues in blank group and Diss group were not observed obvious abnormal changes; tumor cell apoptosis

Figure 1. — A: The damage of tumor cell in ADM group by high power under electro-microscopy ($\times 20.0KX$). B: The damage of tumor cell in M+A group by high power under electro-microscopy ($\times 20.0KX$).

Figure 2. — A: The expression of E-cadherin of tumor in blank group ($\times 400$). B: The expression of E-cadherin of tumor in MLT group ($\times 400$). C: The expression of E-cadherin of tumor in M+A group ($\times 400$).
was dramatically increased in MLT group; loss of most iliac crests in mitochondria and reduction of rough endoplasmic reticulums and free ribosomes were observed (Figure 1A).

Tumor tissues were severely injured in M+T group, and many iliac crests of mitochondria disappeared and many cellular organs were decreased (Figure 1B).

Expression of E-cadherin in tumor tissues among groups

Immunohistochemistry assay confirmed that expression of E-cadherin was not obvious in blank group, Diss group, and ADM group. Light yellow area was seen by survivin staining, and overall scores were ranged from 0-2 (Figure 2A). In MLT group and M+T group, there were many brown cells around tubules and proportion of positive expression of E-cadherin area was more than 50%, in which overall scores were 3-6, thus indicating that there was a strong positive expression of E-cadherin (Figure 2B, 2C).

Comparisons of one month survival condition among groups

One month survival conditions of blank group and Diss group were 4/16 and 6/16, respectively; there was no significant difference between two groups (p = 0.35). All rats were alive in MLT group (14/14); moreover, one month survival rate was significantly higher in M+T group (11/14) than that in ADM group (5/14) (p = 0.012). There was no obvious difference between MLT group and M+T group (p = 0.11) (Table 2).

Comparisons of general conditions

In blank group and Diss group, weight, fair diet, and response of rat was worsened with the enlargement of tumor size. Although tumor was obviously small in ADM group, general condition was still weak and ADM group had the highest mortality rate, tumor size was not significantly decreased in MLT group, but had good general condition. The M+T group had the best conditions.

Discussion

Previous studies have confirmed that MLT inhibited the growth of breast cancer cell lines and had a reverse effect on MCF-7/ADM resistant to ADM in vitro [12, 13]. However, there are complicated factors in vivo, as it is necessary to systemically evaluate anti-tumor effect of MLT and sensitivity of MLT to ADM in vivo.

According to references protocol [14] and considering immune function and endocrine factors, breast cancer SD rat model with positive estrogen receptor induced by NMU peritoneal injection method was applied instead of the xenograft in nude rat model. On the one hand, one of anti-tumor mechanisms of MLT is to regulate the immune and endocrine function [15, 16], and the other hand, breast cancer SD rat model, established by injecting twice 50 mg/kg, had a high tumor formation rate (91.5%), was valuable, and had a clinical significance. This method had some advantages, such as high tumor formation rate and similar tumor formation time, but also had disadvantages, such as long modeling period. Results from pathology and immunohistochemical analyses showed a positive expression of estrogen receptor, which confirmed that the model was established successfully.

Previous study found that MLT had anti-tumor effect through direct or indirect complex mechanisms, which made a great part of tumor cells in non-proliferation state, with even apoptosis [17, 18]. The present study also observed similar results. Tumor size became smaller in SD rat model after treatment of exogenous MLT, but there was no significant difference between control group and treatment group, which was relevant to short observing time. Additionally, some researches also reported that MLT enhanced the sensitivity to chemotherapy [19]. The present results confirmed that tumor weight was lighter in ADM group than that in MLT group, control and vehicle group (p < 0.01), but heavier in ADM than that in ADM combined with MLT group (p < 0.01). This result further demonstrated that MLT enhanced the sensitivity of tumor cells to ADM. Attentively, one month survival rate was highest and no death in MLT group. Many studies indicated that MLT seemed to have a fundamental role as a system regulator in haemopoiesis and immune-enhancement [20, 21], moreover, MLT protected against stress damage [22-25]. The present authors also reported that MLT had a protective role in the myocardium by reducing ADM-induced myocardial oxidative damage [26]. These protective effects made tumor animal maintain good survival condition, which complied with therapeutic aim for late cancer patients. The rats presented poor conditions in the ADM group; however the same group did not show a significant difference with regards to the survival rate when compared to the control group due to a strong toxic reaction. Although ADM had some advantage in anti-tumor effects, its cyto-toxicity was also considered in clinic. A balance point should be found that not only suppresses the growth of tumor, but also improves life quality of patients with cancer. Interestingly, the authors observed that tumor weight and one month survival rate were better in MLT combined with ADM group than those in ADM group. Obviously, MLT enhances the toler-

Table 2. — Comparisons of one month survival rate among groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>One month survival number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Diss</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>MLT</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>ADM</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>M+T</td>
<td>14</td>
<td>11</td>
</tr>
</tbody>
</table>

Blank and Diss: x² = 1.45, p = 0.35. Blank and MLT: x² = 17.5, p = 0.000. Blank and ADM: x² = 0.00, p = 1.00. Blank and M+T: x² = 8.57, p = 0.005. MLT and M+T: x² = 3.36, p = 0.11. ADM and M+T: x² = 6.71, p = 0.012.
formance of rate to chemotherapy. This provides a new thought that MLT combined with high-dose ADM increases complete remission rate for good condition patients, but MLT combined with low-dose ADM for poor condition patients also reduces tumor load and improved life quality. Thus, MLT combined with ADM had many obvious advantages, for example, increasing the sensitivity of tumor to ADM and complete remission rate, moreover, toxicity is evidently reduced in MLT combined with ADM group, compared with ADM group. In other words, tumor is suppressed at the utmost; meanwhile, patients never pay dearly for anti-tumoral chemotherapy. This regimen has with no doubt a significant meaning in clinical treatment.

The metastasis of carcinoma is a major life-threatening factor. Recent studies found that the reduction of adhesive capability of tumor cells led to metastasis, and E-cadherin is the well-studied member of the cadherin family and in epithelial cells. E-cadherin-containing cell-to-cell junctions are often adjacent to actin-containing filaments of the cytoskeleton. Furthermore, loss of E-cadherin function or expression has been implicated in cancer progression and metastasis. Additionally, E-cadherin, a suppressor gene, down-regulation decreases the strength of cellular adhesion with a tissue, resulting in promoting the development of tumor. Previous studies confirmed that MLT induced the expression of E-cadherin and increased cell-to-cell junctions [27, 28]. In this study, the authors obviously observed that both in MLT group and MLT combined ADM group, membrane of tumor cell was clear and tumor tissue had obvious boundary with normal tissues. These results indicated that tumor cells had weak metastasis capacity. Conversely, in control group and ADM group, tumor tissues adhered with normal tissues and tumor cell membranes were unclear, moreover, there were few expression of E-cadherin. These imply that MLT might reduce the incidence of tumor metastas

In conclusion, the authors demonstrated that MLT could inhibit the proliferation and metastasis of tumor, enhance the sensitivity of tumor to ADM, and have a protective effect against ADM-induced cardiotoxicity in ER+ breast cancer rat model. Although many studies have reported that MLT selectively inhibited tumor formation and protected normal tissues, the exact mechanisms are still to be elucidated [29]. Nonetheless, the present results indicate that MLT probably becomes one of important ancillary drugs to treat breast cancer. The authors also believe that with the deeper basic study and large-scale randomized controlled studies, MLT will be widely used in clinic.

References


Address reprint requests to:
Y. ZHANG, M.D.
Department of Oncology,
The First Hospital of Shijiazhuang,
No 36 Fanxi Road,
Shijiazhuang 050010 (P.R. China)
e-mail: yanzhangdoc@163.com
A large ovarian leiomyoma discovered incidentally in a 76-year-old woman: case report

S. Ichigo¹, H. Takagi¹, K. Matsunami¹, T. Murase², T. Ikeda², A. Imai¹

¹ Departments of Obstetrics and Gynecology and ² Pathology, Matsunami General Hospital, Gifu (Japan)

Summary

Background: Ovarian leiomyoma is a very rare type of ovarian tumor. This benign tumor is seen in the pediatric age group to premenopausal women. Case: A 76-year-old woman had a huge leiomyoma (19 x 11 x 10 cm) of the right ovary. The preoperative diagnosis was difficult to distinguish from a broad ligament leiomyoma or ovarian cancer. Conclusions: Although these tumors are benign, its extreme rarity led us to report an additional and rather unusual case of ovarian leiomyoma, and to focus some attention on this type of tumor.

Key words: Leiomyoma; Ovary; Solid tumor.

Introduction

Leiomyoma is one of the rarest solid tumors of the ovary and less that 60 cases have been reported in the literature. Those tumors are only occasionally seen in the pediatric age group [1-3] to premenopausal women [4-10]. A majority of such tumors are small sized and they are discovered incidentally. It therefore appears justified to report an additional and rather unusual case of ovarian leiomyoma, and to focus some attention on this type of neoplasm.

In this report, we present a case of a huge primary ovarian leiomyoma found in a 76-year-old woman whose preoperative diagnosis was difficult to distinguish from a broad ligament leiomyoma.

Case Report

A 76-year-old woman (gravida 2, para 2) who had been in menopause for 25 years visited the local physician because of abdominal discomfort. A huge mass was palpated in the pelvis extended to the umbilicus, and ultrasonography of her abdomen and pelvis revealed the presence of a huge solid tumor which arose out of the pelvis. This huge mass seemed to be originated from the one side ovary. She was introduced to us to have a gynecologic examination.

The pelvic examination and transvaginal ultrasonography revealed the presence of a huge solid tumor with hypoechoic irregular area. The tumor was hard and easily palpated through the abdominal wall, of which movability was limited. These findings suggested the possible malignant potential of this tumor. However, this tumor did not accompany with large amount of ascites and laboratory tests showed that the levels of serum tumor markers or LDH were all within the normal range, except slight increase of CA125 level 49.9 U/ml (normal < 37). The magnetic resonance image (MRI) revealed that the tumor had an irregular shape with a smooth border and consisted of both solid and cystic part (Fig.1). It occupied almost whole the pelvis and grew out to the extrapelvic space. However, the little enlarged appearance of uterine corpus was also seen. Additionally, no sign of distant metastasis or pelvic lymphadenopathy were observed.

The differential diagnosis of this huge tumor included broad ligament leiomyoma, left ovarian solid tumor, and ovarian cancer. On laparotomy, the huge tumor occupied almost the entire pelvic cavity. Only small amount of ascites was observed and the parietal and visceral peritoneum was smooth. The tumor was found to only slightly adhere to the omentum and mesentery. It was originated from right ovary, extending to the retroperitoneal space under the broad ligament. She underwent bilateral salpingo-oophorectomy and total hysterectomy. We did not perform the extirpation of the omentum, upper abdomen, and retroperitoneal lymph nodes. Because of low malignant possibility, based on the findings, it would be better to perform further operation later after obtaining the final precise histological diagnosis of this ovarian tumor.

The tumor measured 19 x 11 x 10 cm (Fig.1). Its surface was smooth, and the cut surface was firm and white. No capsule rupture or adhesion observed. The microscopic examination showed irregular bundles and whirling of the spindle-shaped cells with elongated blunt nuclei (Fig.2). There was no atypia or pleomorphism and there were 2-3 mitotic figures per 10 high-power fields. Cystic areas with varying degrees of hyaline degeneration and myxomatous changes were also present. There was no trace of normal ovarian tissue.

The postoperative period was uneventful.

Discussion

Ovarian leiomyoma, a rare benign tumor, is most often seen in premenopausal women (up to 85% of cases) [4-10] and accounts for 0.5-1% of all benign ovarian neo-
There are a number of theories of the origin of these tumors. They most likely arise from smooth muscle cells in the ovarian hilar blood vessels, but other possible origins include cells in the ovarian ligament, smooth muscle cells or multipotential cells in the ovarian stroma, undifferentiated germ cells, or cortical smooth muscle metaplasia.

These tumors may be found by chance during routine physical examination or incidentally at surgery of autopsy [1, 13]. Clinically, many patients are asymptomatic. When symptoms are present, a variety of clinical presentations has been described: abdominal pain, a palpable mass, hydrothorax and/or ascites, hydronephrosis, slight elevated tumor marker CA125 [2, 3, 14-18]. Case reports show a unilateral predominance, with no predilection for left or right ovary.

Other smooth muscle processes that can involve the ovary include parasitic uterine leiomyomas, ovarian smooth

Figure 1. — Saggital (a) and axial (b) T2-weighted MR images showing a huge pelvic heterogenous mass associated with little enlarged uterus (arrow).

Figure 2. — (a) Macroscopic findings for the extirpated tumor. The external surface was smooth. The arrow shows infundibulopelvic ligament edge. (b) The tumor is composed of fascicles of spindle-shaped cells (H&E, x100).
muscle metaplasia, intravenous leiomyomatosis, and leiomyomatosis peritonealis disseminata [1, 19]. Leiomyoma can also arise adjacent to the ovary in the broad ligament. Primary ovarian leiomyoma must also be differentiated from a pedunculated subserosal uterine leiomyoma, which may have lost its original attachment and has instead become attached to the ovary, thus deriving its blood supply. The co-existence of an ovarian leiomyoma with a uterine leiomyoma has been reported by several authors [5, 6]. There was no associated uterine leiomyomatosis in our case. The tumor in the present case was of ovarian origin, because it replaced nearly the entire ovary and the normal ovarian tissue could not be discerned.

Once the smooth muscle nature of the tumor is known, leiomyoma must be differentiated from leiomyosarcoma. The histological features of malignancy have not been well defined due to the rarity of these tumors. Pathologists have traditionally utilized criteria that emphasized the level of mitotic activity [11]. However, Prayson and Hart [19] have described two cases of mitotically active ovarian leiomyoma which had a subsequent clinically benign course. Clearly, the significance of other factors such as necrosis and cellular atypia, which are now used in the evaluation of uterine smooth muscle tumors [20], needs to be determined.

In summary, primary ovarian leiomyoma, although extremely rare, must be considered in differential diagnosis of pelvic solid tumor; it clinically and radiologically mimicked a broad ligament leiomyoma. The accurate diagnosis may be most frequently made at the time of exploratory laparotomy.

References


Address reprint requests to:
A. IMAI, M.D.
Institute of Endocrine-Related Cancer,
Matsunami General Hospital,
185-1 Dendai
Kasamatsu, Gifu 501-6062 (Japan)
e-mail: aimai@matsunami-hsp.org.jp
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. Wincewicz¹,², P. Lewitowicz¹,², O. Adamczyk-Gruszka³, S. Sulkowski⁴, L. Kanczuga-Koda⁵, M. Koda⁴

¹ Departments of Anatomy and Pathology, Faculty of Health Sciences, Jan Kochanowski Memorial University of Kielce, Kielce
² NZOZ Zakład Patologii, Department of Pathology, Kielce
³ Department of Gynaecology, Faculty of Health Sciences, Jan Kochanowski Memorial University of Kielce, Kielce
⁴ Department of General Pathomorphology, Medical University of Białystok, Białystok
⁵ Department of Pathology, Białystok Oncology Centre, Białystok (Poland)

Summary
Combined ovarian tumors are found in common pathologic practice due to amazing potential of ovarian tissue to copy almost every tissue of human body and imitate many neoplasms of various other organs in a very flexible way. A multicystic tumor is presented in this case report of 35-year-old woman. It consisted of a cyst with sebum and hair and cavities with papillomatous projections and mucus. The ovarian tumor was diagnosed a mature cystic teratoma presenting mainly as dermoid cyst and mucinous adenocarcinoma in situ, arising within atypical proliferative mucinous tumor. This report demonstrates how histoformatory properties are reflected in ovarian tumorigenesis. Such a stunning histoformativitiy makes ovaries the possible site of primary origin for malignant tumors that mimic extra ovarian differentiation. In the authors’ point of view, the diagnosis of primary ovarian mucinous tumor within cystic teratoma is firm, whenever simultaneous extraovarian involvement by mucinous neoplasm is excluded.

Key words: Adenocarcinoma in situ; Atypical proliferative mucinous tumor; Dermoid cyst; Mature cystic teratoma.

Introduction
Combined ovarian tumors are found in common pathologic practice due to amazing potential of ovarian tissue to copy almost every tissue of human body and imitate many neoplasms of various other organs in a very flexible way. Among them, teratomas are the most educative examples of neoplastic histoformativity. They can form many histological structures that develop from all three germ layers and could appear as unique for certain organs that are distinct from ovary. Teratomas can coexist with mucinous neoplasm of ovary [1]. Although mature cystic teratomas (MCTs) are the most frequent ovarian germ cell tumors, such a malignant transformation is a rare event in their course [2]. It is estimated that adenocarcinoma comprises approximately 5.8% of all cases of malignant transformation of ovarian teratoma, while large majority of such malignancies are squamous cell carcinomas [3]. Indeed, squamous cell carcinoma is the most common malignancy growing from cystic teratoma with a poor prognosis affected by histopathological grading and extend of invasion [4]. Mucinous adenocarcinomas are described as uncommon transformation of mature cystic teratoma. Nevertheless, they have quite favorable prognosis with one reported case of woman who survived five years after operation without recurrence of the disease [2] and the other report of female patient whose three-year-long follow-up was free of disease [5].

Here the authors present a case of dermoid cyst and adenocarcinoma in situ within atypical proliferative mucinous tumour of 35-year-old woman.

Materials and Methods
A 35-year-old woman underwent unilateral salpingo-oopherectomy and excision of subserosal uterine leiomyoma due to abdominal pain and discomfort in the pelvic region. Right adnexa with tumor and a conventional subserous leiomyoma were sent to histopathological examination. Tissues were fixed in 10% buffered formalin solution. The representative samples were dissected and embedded in paraffin blocks at 56°C according to standard procedures. The material was sliced into three-µm thick specimens that were routinely stained with hematoxylin-eosin. Histopathological examination was done by two independent pathologists with determination of conventional histopathological parameters of diagnosed entities.
Results

Clinical data and macroscopic findings
No other tumor masses were detected in the body of the patient. The ovarian tumor measured 7 x 5 x 4 cm. On cut surface it was composed of a few cysts which were filled with sebum, hair, and semi-fluid viscous content. Internal surfaces of the cysts were mostly smooth but focally there were prominent papillomatous projections into cystic lumen. The adjacent fallopian tube was four cm long and accompanied by perisalpingeal cysts up to one cm in diameter. On endoscopic investigation and imaging there was no neoplastic involvement of intestines and lungs of the patient. There was no trace of neoplasm in material that was obtained from uterine abrasion as well.

Microscopic findings
In samples from a cyst with sebum and hair, there were quite a few histological structures and tissues that originated from all three germ layers. There was classical pattern of dermoid cyst that was lined by epidermis with well developed skin adnexa. Within the cyst there was a focal growth of ciliated pseudostratified epithelium of respiratory tract as well (Figure 1A). In close vicinity of these elements of mature cystic teratoma, there were cysts lined by relatively tall mucinous epithelial cells that varied in a degree of atypia from none to prominent (Figure 1B). This neoplastic counterpart was diagnosed as atypical proliferative mucinous tumor (APMT) with morphological features of intestinal type of epithelium. The cuboidal glandular mucinous epithelium with atypia presented with epithelial stratification, micropapillary projections, and a compact assembly of carcinomatous tubes that coalesced into cribriform pattern in papillomatous excrescences of cysts with viscous, mucinous content (Figures 1B, 1C). There was also budding of this epithelium into cyst wall but no evident stromal invasion of ovary. These foci were consistent with adenocarcinoma in situ. The cancer cells were relatively tall with basally oriented highly atypical nuclei. Moreover goblet cells were present in better differentiated areas of this atypical mucinous tumor (Figure 1D). Careful analysis revealed that walls of teratoma and APMT showed distinct borders with teratoma tissues and fibrous wall of APMT. No extension through the ovarian capsule was noted corresponding with pT1a (FIGO Stage 1A). No recurrence was noted after surgical resection. The ovarian tumor was diagnosed a MCT with atypical proliferative mucinous tumour with foci of adenocarcinoma in situ.

Discussion
Whenever adenocarcinoma and cystadenoma is encountered within teratoma, some questions could appear as follows: Are adenocarcinoma, cystadenoma, and teratoma synchronous tumors or are they parts of one combined entity? What is the origin of malignant component? Is the prognosis different for adenocarcinoma arising from intestinal type epithelium in comparison to adenocarcinoma growing from ciliated epithelium of respiratory type? and so on.
The prognosis of adenocarcinoma arising within teratoma is still not precisely estimated but staging and grading seem to affect prognosis in the similar way as in other adenocarcinomas. In the present authors’ opinion the mode of spreading is going to be different for adenocarcinoma no matter from which teratomatous epithelium such a malignancy comes from. Not only the epithelial origin of teratomatous adenocarcinoma could play a role, but also the primary location certainly affects prognosis, for example adenocarcinoma arising from respiratory or intestinal epithelium of teratoma. In such a case, mode of spreading and prognosis could be totally different from conventional lung adenocarcinoma or colon adenocarcinoma which grow at quite different sites and face different histological barriers at their primary sites of origin.

Immunohistochemistry sometimes helps to elucidate an origin of malignant tumors that are found in ovarian dermoid cysts [6]. For example p63 and CK5/6 stainings were strongly positive in squamous carcinoma and this immunoreactivity was preserved in stratum of basal cells that covered the inner surface of the mucinous cyst in a case of monodermal teratoma. That distribution of immunostain suggested that these basal cells could be a site of origin of diagnosed malignancy [6]. Moreover, such a basal-cell pattern of staining was thought to be quite characteristic for mucinous cysts of mature teratomas and was completely lost in ovarian benign and borderline cystic mucinous cystadenomas, giving a clue for eventual differential diagnosis in doubtful cases [6]. MCT-derived mucinous borderline-like tumor was predominantly positive for cytokeratin 20 [7]. However, immunohistochemistry results were not straightforward because there was also a little intriguing partial immunoreactivity for cytokeratin 7. Besides this, MUC5AC was also partially positive with only residual immunoreactivity for MUC2 and MUC6. This mixed pattern of staining was a ground to support an idea that mucinous borderline tumor was originating from gastrointestinal epithelium of teratoma [7].

However, sometimes nor immunohistochemistry nor molecular biology methods fail to explain a coexistence of various ovarian tumors as in case of synchronous rhabdomyosarcoma arising in a MCT and contralateral serous carcinoma [8]. Other types of sarcomas were also reported in association with MCT as rhabdomyosarcomatous transformation and contralateral serous carcinoma [8].

Carcinomatous and sarcomatous component can arise not only on the basis of mature teratoma but also from immature teratomas [9]. Namely, a malignant mixed Müllerian tumor (MMMT) of nasopharyngeal teratoid carcinomasarcoma type was accompanied with malignant neuroectodermal foci that resembled ganglioneuroblastoma with pronounced immunoreactivity for synaptophysin, S-100 and neuron-specific enolase [9]. Mucinous adenocarcinoma and teratoma were reported in quite unusual settings as in coexistence with a large cell neuroendocrine carcinoma [10].

If both appendix and ovary are involved with borderline mucinous tumor, the determination of primary site of the tumor is quite challenging, particularly if mucinous tumors give a clinical picture of pseudomyxoma peritonei (PMP) and morphology and immunoprofile overlap with characteristics of the secondary neoplasms of appendiceal origin [11]. If adenocarcinoma is encountered within ovarian cystic teratoma, both respiratory and gastrointestinal epithelium could be considered as a potential tissue of neoplastic origin [12, 13]. If epithelium that lines cysts of teratoma is a ciliated, pseudostratified columnar epithelium that contains ciliated cells, goblet cells and basal cells its respiratory type could be unequivocally elucidated [12, 13]. The adenocarcinoma in multicystic teratoma gives a quite mixed pattern of staining. The crucial cytokeratins are CK7 that is predominantly positive in case of primary origin from gynecological tract and CK20 that is strong in tumor of gastrointestinal type [14]. To examine utility of immunohistochemistry, CK7, CK20, CDX2, and villin stains were studied in a largest so far group of 44 ovarian mucinous adenomas and adenocarcinomas ex-MCTs without extravarian presentation of mucinous tumor [14]. In this report, 15 cystadenomas without pseudomyxoma ovarii showed the same intensity both for CK7 and CK20 [14]. If so, whenever appendix is involved, it is truly impossible to judge what the origin of the mucinous neoplasm is only on the ground of immunohistochemistry. Eight proliferative mucinous cystadenomas without pseudomyxoma ovarii exhibited much stronger CK7 staining than CK20 immunoreactivity, while all cystadenomas with Meigs syndrome lacked CK7 and CK20 immunorexpression. To make immunohistochemistry more puzzling, adenocarcinomas manifested presence or lack of CK7 and CK20 expression in all three following combinations (CK7-/CK20+, CK7+/CK20+, or CK7+/CK20-) [14]. Meigs syndrome was accompanied with CK7-/CK20+ immunophenotype with CDX2 and villin expression in adenocarcinoma [14]. Such a mixed immunohistochemistry with positive immunoreactivity to CK20 suggested teratomatous origin of at least a subgroup of described ovarian tumors [14]. In the present authors’ point of view, the diagnosis of primary ovarian mucinous tumor within cystic teratoma is firm, if only simultaneous extravarian involvement by mucinous neoplasm is excluded, as in the presented case. Indeed in described example immunohistochemistry would not add anything essential to diagnosis in perspective of prognosis of this patient especially because the authors did not diagnose invasive adenocarcinoma. We conclude that although this mucinous tumor presents morphologic features of intestinal type epithelium, it is still primary tumor of ovary. In this case, careful clinical information
was more useful than additional performance of immunohistochemistry. Namely any suspected extraovarian masses were not present on clinical inspection that included imaging, bronchial, and gastrointestinal endoscopy and uterine abrasion. Thus, a successful cooperation with clinician always goes first before performance of any additional staining in routine pathologist’s practice.

To sum up, the present report is one of few that present quite a rare coexistence of dermoid cyst and adenocarcinoma in situ within atypical proliferative mucinous tumour of 35-year-old woman with emphasis that in such cases clinical data are so useful that limit the need of performance of additional histopathological stainings.

Acknowledgement

This publication is institutionally affiliated to Medical University of Białystok, Poland.

References


Address reprint requests to:
A. Wincewicz FEBP, M.D., PhD,
Department of Anatomy,
Faculty of Health Sciences,
Jan Kochanowski Memorial University,
Kielce IX Wieków Kielce St 19,
25-317 Kielce (Poland)
e-mail: ruahpolin@yahoo.com
andwinc@gmail.com
Angioleiomyoma of the uterus: report of a distinctive benign leiomyoma variant

A. Zizi-Sermpetzoglou¹, D. Myoteri¹, E. Arkoumani¹, K. Koulia¹, A. Tsavari¹, E. Alamanou², E. Moustou¹

¹Department of Surgical Pathology, Tzaneion General Hospital, Piraeus
²Department of Gynecology, Tzaneion General Hospital, Piraeus (Greece)

Summary

Angioleiomyoma is a relatively rare type of leiomyoma of the uterus that originates from smooth muscle cells and contains thick-walled vessels. Angioleiomyoma is usually found in the skin of the lower extremities. Uterine angioleiomyoma has similar morphological features to that of the skin. The authors present a case of a 50-year-old woman who was admitted to the present hospital with the complaint of lower abdominal pain. On clinical examination, she was found to have a palpable lower central abdominal mass. Pelvic ultrasound revealed uterine enlargement, multiple small leiomyomas, and a large mass in the myometrium. The patient underwent total hysterectomy and bilateral salpingo-oophorectomy. On histological examination, the mass was diagnosed as angioleiomyoma. Hemangioma, angiofibroma or angiomyofibroblastoma were also included in the differential diagnosis. The treatment of choice for angioleiomyoma is surgical excision, and either angiomyomectomy or simple hysterectomy are proven to be equally effective; the decision depends on the patient's symptoms and her desire to preserve fertility.

Key words: Angioleiomyoma; Uterus; Immunohistochemistry.

Introduction

Angioleiomyoma or vascular leiomyoma is a benign mesenchymal neoplasm that is composed of smooth muscle cells and thick-walled vessels [1]. Angioleiomyomas are usually found in the subcutis of the lower extremities, head and trunk, and to the best of our knowledge, only a few cases of uterine angioleiomyomas have been reported so far [2-5]. Clinical diagnosis may be difficult but microscopically it can be easy recognized as a specific type of leiomyoma. The authors describe a case of angioleiomyoma of the uterus presenting with lower abdominal pain.

Case Report

A 50-year-old multipara premenopausal woman presented to the gynecology outpatient department with a one-month history of lower abdominal pain and abnormal uterine bleeding for the last three weeks. She mentioned that she was on medication with ramipril/hydrochlorothiazide for hypertension and that she had undergone right saphenectomy 20 years ago. Clinical examination revealed an irregularly enlarged uterus but no other masses in the pelvis. Pelvic ultrasound scan (US) revealed uterine enlargement, multiple uterine leiomyomas, and a large mass in the myometrium (Figure 1). The latter exhibited abnormally increased vascularization in the color Doppler ultrasonography. The endometrium appeared unremarkable. Both ovaries and fallopian tubes were normal. Histological examination of the mass showed a moderately cellular neoplasm composed of interlacing smooth muscle bundles with interspersed abundant thick-walled vessels (Figures 3a and 3b). Mitoses were absent and neither pleomorphism nor necrosis was observed. In the immunohistochemical study, the spindle cell component showed positivity for smooth muscle actin (SMA) (Figure 4a) and vimentin while the vascular component was immunoreactive for CD34 (Figure 4b). No reactivity to desmin and S100 was found. These findings led to the diagnosis of angioleiomyoma. Histological examination of the endometrium, bilateral tubes, and ovaries revealed no significant pathology. Multiple leiomyomas and foci of adenomyosis were found in the myometrium.

During follow-up, the patient has had no complications or further symptoms and remains disease-free 2.5 years after surgery.

Discussion

Angioleiomyomas are benign, relatively common neoplasms described in the lower extremities, head, and trunk [1]. They differ from other types of leiomyomas in that they are encapsulated and contain numerous vessels. The numerous veins that are present vary in size and have muscular walls of varying thickness. On this basis, three histological subtypes have been recognized: solid, cav-
Figure 1. — US revealing a large mass in the myometrium.

Figure 2. — Gross appearance of a large hemorrhagic mass in the myometrium.

Figure 3. — A) Dilated vascular channels with small amounts of smooth muscle (H-E x100). B) Thick-walled vessels interspersed between smooth muscle cells (H-E x400).

Figure 4. — A) Spindle cell component positive for smooth muscle actin (SMA x200). B) Vascular component immunoreactive for CD34 (CD34 x200).
ernous, and venous. In the solid type, the vascular channels are numerous but small while tumors of the cavernous type are composed of dilated vascular channels with small amounts of smooth muscle. Tumors of the venous type exhibit veins with thick muscular walls with smooth muscle cells that extend tangentially from the peripheries of the veins merging with the intravascular tumor substance [6].

Only very few cases of angioleiomyomas have been described at sites other than the extremities and head. These unusual sites include the oral cavity [7], the palate and tonsils [8], the scrotum [9], and the female genital tract [10]. According to Hsieh et al., the number of uterine angioleiomyomas (UAL) reported in the literature was around six [2].

Similar to angioleiomyomas elsewhere, UALs are composed of smooth muscle bundles with prominent thick-walled vessels and represent a subtype of the uterine leiomyomas. The study on vascular system of intramural leiomyomas by Walocha et al. revealed that usual leiomyomas contained vascular network with density similar to or lower than that of normal myometrium [11]. These are predominantly capillaries along with a few arterioles and small arteries. In contrast, UALs have abundant thick-walled vessels with intersecting smooth muscle bundles. They occur usually between the fourth and sixth decades of life and as in the presented case, pain is their dominant clinical feature. This symptom can be explained by local ischemia due to vessel contraction. However, the exact pathogenetic mechanism of the pain remains unclear. Uterine angioleiomyomas can reach a large size, presenting as an abdominal mass or can be multiple, resulting in severe menorrhagia [2]. Abnormal bleeding has also been attributed to local dysregulation of the vascular structures in the uterus.

Grossly, UALs present as circumscribed, gray-white nodules with blood-filled cystic spaces. Sometimes, the tumoral mass may contain dilated vessels that can be mistaken for multiloculated and multiseptated ovarian tumor or adenomyosis. Microscopic examination reveals abundant thick-walled vessels, separated by whorled, anastomosing fascicles of uniform, fusiform smooth muscle cells. Areas of myxoid changes, hyalinization, calcification and fat may be seen. As a rule, mitotic activity and coagulative necrosis are usually absent [3].

Clinical diagnosis may be difficult and the patients’ age and symptoms very often lead to the suspicion of a malignant gynecological tumor, as occurred in the present case. Microscopically, when the vascular component predominates, angioleiomyoma needs to be differentiated from hemangioma or arteriovenous malformation. As a rule, angioleiomyomas are well-circumscribed neoplasms that contain at least foci of typical spindled smooth muscle cells. Hemangiomas are rare in the uterus, and tend to be ill-demarcated grossly and microscopically. The differential diagnosis from other neoplasms, such as angiofibroma, fibroma, angiomyolipoma or angiofibroblastoma is based on immunohistochemical stains for smooth muscle cells (actin) and vessel markers (CD34, CD31).

Complete surgical excision is the treatment of choice, and either angiomyomectomy or simple hysterectomy are both proven to be effective; the decision depends on the patient’s symptoms and her desire to preserve fertility.

In conclusion, angioleiomyoma should be included in the differential diagnosis of a multicystic hypervascular mass located in the pelvis. However, clinical diagnosis of UAL is difficult and it seems impossible to be differentiated preoperatively from a malignant gynecological tumor. Surgical excision is the treatment of choice, either by angiomyomectomy or by simple hysterectomy.

References


Address reprint requests to:
A. ZIZI-SERMETZOGLOU, M.D.
3143, Alikos
19400 Ag. Marina, Koropi, Athens (Greece)
e-mail: adserbet@yahoo.gr
Adenocarcinoma of the cervix associated with a neuroendocrine small cell carcinoma of the cervix in the spectrum of Muir-Torre syndrome

P. Donati1, G. Paolino2, M. Donati1, C. Panetta1

1Dermatopathological Laboratory “San Gallicano Institute of Rome”, Rome
2Clinica Dermatologica, La Sapienza University of Rome, Rome (Italy)

Summary
Muir-Torre syndrome (MTS) is an autosomal genodermatosis that is diagnosed by the presence of at least one sebaceous gland tumor and at least one visceral malignancy. The most frequent visceral malignancies reported in literature are low-grade colon-rectal and genitourinary cancers, with prolonged survival. The authors report the case of a 52-year-old female, with a positive familial history for MTS, who developed a cutaneous sebaceous carcinoma, a synchronous colon-rectal adenocarcinoma, and a metachronous endocervical adenocarcinoma associated with a neuroendocrine small cell carcinoma of the cervix (SCNC), with lymph node metastasis. The rare occurrence in literature of the cervical SCNC and the rarest occurrence of a neuroendocrine carcinoma in the context of a MTS deviate from the usual and low-grade types of cancers normally described with MTS. It should be always appropriate to assess any symptoms that might reveal an underlying malignancy, although not within the spectrum of neoplasms most associated with this rare syndrome.

Key words: Muir-Torre syndrome; Sebaceous carcinoma; Adenocarcinoma; Neuroendocrine small cell carcinoma of the cervix.

Introduction
Muir-Torre syndrome (MTS) is an autosomal genodermatosis that is diagnosed by the presence of one sebaceous gland tumor and at least one visceral malignancy [1]. Cutaneous neoplasms diagnostic for MTS include sebaceous adenomas, sebaceous epitheliomas, sebaceous carcinomas, and keratoacanthoma; these tumors, develop often after an internal malignancy presentation [2]. While, the most frequent visceral malignancies, reported in literature, are low-grade colon-rectal and genitourinary cancers [1].

Neuroendocrine small cell carcinoma of the cervix (SCNC) is a rare subtype of cervical cancer with an aggressive behavior, that deviate from the usual low-grade types of cancers normally associated with MTS [1-2].

The authors report the case of a 52-year-old female, with a positive familial history of MTS, who progressively developed synchronous and metachronous malignancies.

Case Report
A 52-year-old Caucasian female, presented to the present Department with a three-month history of a nodule arising in the left nipple; the lesion (1.2 x 1 cm) was firm and showed a central portion slightly ulcerated and did not produce secretions. (Figure 1A)

At the general clinical examination, the patient did not have any other cutaneous lesions, which could require further investigations. Three years prior the patient had removed, in another Institute, a sebaceous adenoma on the frontal region. In the family history, the patient presented two brothers with a positive history of Muir-Torre syndrome (MTS), diagnosed two years prior.

A surgical excision of the cutaneous lesion of the nipple was performed. Histologic examination of the skin biopsy showed a poorly circumscribed epidermoid-follicular proliferation (Figures 1A-C); the keratinocytes showed variably sebaceous differentiation, characterized by remarkably vacuolated neoplastic cells. The nuclei were large with visible nucleoli and scattered mitoses. (Figure 1D) Based on these histopathological findings, a final diagnosis of sebaceous carcinoma was made.

One month after, during a colonoscopy, an asymptomatic polyloid lesion of the transverse colon was removed. At the histological examination, the lesion was compatible with an adenocarcinoma (Figure 2A) with focal aspects of mucinosis (G2). The lesion infiltrated the muscle layer thickness, with a little desmoplastic reaction and a poor inflammatory infiltrate (pT2N0). For this reason, a partial colectomy was performed.

According to the patient’s history and to the familial history, the authors decided to perform a molecular evaluation, which showed a mutation in the exon7- gene MSH2; this mutation was the same detected also in her two brothers. A final diagnosis of sebaceous carcinoma was made.

Seven months later, the patient experienced metrorrhagia; during the gynecological visit, a biopsy of a friable mass in the proximal endocervical canal was performed. Histological examination revealed a poorly differentiated endocervical adenocarcinoma (Figure 2B). However, at higher magnification, in the lower limit of the adenocarcinomatous tumor, there was an atypical bluish epithelioid small cell population, arranged as cords, with trabecular pattern (Figure 2C). Cytologically, these cells were oval to polygonal with iperchromatic nuclei (Figure 2C). This cell pop-
population was positive for chromogranin (Figure 2D), synaptophysins, CD10 and CD56; while it was negative to cytokeratin 20 (CK 20) and TTF-1. A final diagnosis of an adenocarcinoma associated with a neuroendocrine small cell carcinoma (SCNC) of the endocervix was made.

A total hysterectomy associated with a lymphadenectomy of left and right pelvic lymph nodes and of the lumbar-aortic lymph nodes was performed; one lymph node was found to be metastatic by SCNC component (pT1b1, pN1; FIGO Stage IIIB).

Currently the patient is carrying out chemotherapy treatments with periodic imaging studies.

Discussion

MTS is a dominant condition characterized by the simultaneous presence of visceral malignancies and skin tumors (particularly sebaceous gland tumors). The principal muta-
Adenocarcinoma of the cervix associated with a neuroendocrine small cell carcinoma of the cervix in the spectrum of Muir-Torre syndrome

The authors think that this report is further evidence of how more new malignancies can be found to be associated with MTS and how it should be always be appropriate to analyze any symptoms that might reveal an underlying malignancy, although not within the spectrum of neoplasms most associated with this rare syndrome.

References


Address reprint requests to: G. PAOLINO, M.D.
Clinica Dermatologica,
La Sapienza University of Rome,
Viale del Policlinico 15, 00186 Rome (Italy)
e-mail: gio8519@libero.it
Three synchronous primary pelvic cancers – a case report

M.E. Căpilna1, S.C. Rusu1, C. Laczko1, B. Szabo1, C. Marian2

1 First Clinic of Obstetrics and Gynecology, University of Medicine and Pharmacy, Târgu-Mureș
2 Department of Pathology, University of Medicine and Pharmacy, Târgu-Mureș (Romania)

Summary
The occurrence of synchronous primary gynaecologic malignancies is a relatively common event. However, the occurrence of three different pelvic cancers is very rare. In this report, the authors describe the clinical, surgical, and pathological findings of a patient with synchronous primary malignancies of the fallopian tube, endometrium, and sigmoid colon. To the authors’ knowledge, it is the first case described in the literature with such an association of primary synchronous cancers.

Key words: Synchronous cancer; Fallopian tube cancer; Endometrial cancer; Sigmoid colon cancer.

Introduction
Synchronous occurrence of endometrial and adnexal (ovarian or tubal) cancer in female genital tract is a well known event in gynaecological oncology. They may indicate either metastatic or independent neoplasms and the clinical or therapeutic implications and prognosis are very different in each occasion. Compared to metastatic dual cancer, two simultaneous primary cancers are relatively rare and can be easily recognized if the histologic types of each cancer are different. Furthermore, the occurrence of a pelvic third cancer is an extremely rare event.

The aetiology of synchronous malignancy is uncertain but it has been postulated that embryologically similar tissues of the female genital tract may develop synchronous neoplasms when simultaneously subjected to carcinogens [1, 2].

Recently, the authors experienced three primary cancers occurring in both tubes, endometrium, and sigmoid colon with three different histologic patterns. This case, along with the diagnosis and the treatment of synchronous female genital malignancy, will be briefly reviewed.

Case Report
In April 2013, a 61-year-old white female, gravida 1, para 0, was referred to the present clinic with the diagnosis of endometrial cancer and a possible synchronous ovarian cancer. She was menopausal since age of 50 and she described a vaginal bleeding that had begun three months prior. Her past medical and surgical histories were relatively unremarkable, but her family history revealed a duodenum cancer of her mother, a colon cancer of her grand-father. A clinical and ultrasound examination in March 2013 revealed the uterine origin of bleeding, a normal volume uterus, but with a 18-mm thick endometrium with a polyp-like image without apparently myometrial invasion and multiseptated, 111 x 97 x 90-mm mass having both solid and cystic components in the right ovary, suggesting malignancy; the left ovary appeared normally. A pelvic computed tomographic scan confirmed the existence of the previously mentioned multiseptated cystic right ovarian mass and minimal ascites in the pelvic cavity. In addition, there were no abnormal findings in the abdominal cavity and thorax, with no extraperitoneal enlarged lymph nodes. She underwent in April 2013 a diagnostic uterine curettage under anesthesia in a private hospital, revealing a grade 3 endometriod adenocarcinoma of the endometrium. The CA-125 was elevated 141.8 U/ml. Routine blood test investigations were normal. The clinical diagnosis was Stage IA, grade 3 endometrial carcinoma and a synchronous (primary or metastatic) ovarian cancer.

At laparotomy, a right adnexal tumour involving both tube and ovary, of 11 x 10 x 9 cm, but mobile, with smooth surface and both solid and cystic parts was discovered. The frozen section revealed malignant tissue. There was a small amount of ascites in the abdomen. The left ovary and the uterus appeared normal. On the sigmoid colon, a tumour producing bowel stenosis and a retraction of the serosa, very suggestive for malignancy, was discovered. The authors performed a total abdominal hysterectomy with bilateral adnexectomy, pelvic, and para-aortal lymphadenectomy, total omentectomy, appendectomy, recto-sigmoid colon resection (about 20 cm), and peritoneal biopsies. The whole procedure lasted 275 minutes. There were no intraoperative complications. The postoperative recovery was uneventful and she was discharged home after hospitalisation for nine days.

The final pathology report described a high-grade (MD Anderson grading system) serous adenocarcinoma of right adnexa, involving both tube and ovary; its origin could not be detected, but contained different microscopic patterns: solid, papillary, cystic, micropapillary, and glands (Figure 1). In the left tube, a high-grade serous adenocarcinoma involving the mucosal and muscular layer, but without serosal involvement was found. The left ovary was microscopically normal. For these reasons, the authors considered also the right adnexal tumour of tubal origin. The cytology of the ascites revealed malignant cells. There were no metastases into the appendix, peritoneum, omentum, and in the 25 from the right and 35 from the left side pelvic and from the
55 para-aortal lymph nodes. Final pathological staging was a high-grade tubal cancer stage pT1cN0.

In the endometrium, an endometrioid type adenocarcinoma grade 3 with mucosal invasion only was found (pT1a grade 3N0) (Figure 2).

The third synchronous cancer was a moderate differentiated adenocarcinoma of the recto-sigmoid junction with subserosal invasion without metastases in the 17 regional lymph nodes (pT3N0 Dukes-MACB2) (Figure 3).

The oncology commission in the present hospital decided to begin adjuvant treatment focusing on the tubal cancer. The patient underwent carboplatin/paclitaxel chemotherapy and she is doing well after four courses.

**Discussion**

The most commonly reported synchronous malignancies are the coexistence of ovarian and endometrial cancers, but genital tract malignancies can arise from more than two anatomical sites, as primary neoplasia. Although the etiology of synchronous malignancies remains unclear, it has been postulated that the extended Müllerian system, comprising ovarian epithelium, fallopian tube, uterus, and cervix respond as a single morphological unit to produce primary cancers in different sites. Another theory, which could explain even other sites, suggests that these neoplasms originate in metaplasia occurring in different tissues [4].

Until now, limited cases of synchronous primary genital cancers have been reported in the literature, and even less for a third pelvic cancer with an extra-genital origin.

Taking into considerations only two cancers out of three of the present patient (fallopian tube and endometrium), Eisner et al. [1] described also two synchronous primary cancers of fallopian tube and endometrium, and Atasaver et al. [3] another five sites synchronous cancers involving ovary, both tubes, endometrium, and cervix. The present search did not find a similar case in the literature, comprising three synchronous malignancies involving two genital sites (tube and endometrium), and another pelvic extra-genital one with different embryologic origin (sigmoid colon).

The present pathologic findings fulfilled the conditions described previously for identification of primary synchronous cancers, such as different histologic types (major criterion) or all the following minor criteria: [1] both tumours confined to primary sites; [2] no direct extension between tumours; [3] no lymphovascular tumour emboli; [4] no or only superficial myometrial invasion; and [5] distant metastases [5-7].

Simultaneous detection of malignancy in different organs challenges the clinicians and pathologists to make correct diagnosis and arrange proper management [8]. Appropriate therapy for synchronous cancers must be planned indi-
Three synchronous primary pelvic cancers – a case report

vidually. Different parameters such stage, grade, extension, tumour resection margins, etc, should be taken into consideration. As a consequence, the present oncology staff decided for this special case that most aggressive tumour necessitating first line adjuvant treatment is the high-grade serous adnexal adenocarcinoma.

References


Address reprint requests to:
M.E. CAPILNA, M.D.
First Clinic of Obstetrics and Gynecology, University of Medicine and Pharmacy, str. Gheorghe Marinescu no. 50, 540136, Târgu-Mureș (Romania)
e-mail: mcapilna@gmail.com
Metastases of renal clear cell carcinoma to ovary – case report and review of the literature

M. Kostrzewa, M. Żyła, J. Władziński, T. Stetkiewicz, G. Stachowiak, J.R.Wilczyński
Department of Gynecology and Oncological Gynecology, Polish Mother’s Memorial Hospital Research Institute of Lodz, Lodz (Poland)

Summary
In the literature the renal-ovarian axis has been demonstrated. Although, kidney and ovary are in a very distant anatomic position, they are supposed to have a lot of in common. This unusual connection begins from embryology, vascularization, and metastasizing tumors to each other. In the present systemic review the authors showed 24 case reports published in the literature, describing the metastases of primary renal cancer to ovary and only four cases reporting primary ovarian cancer metastases to kidney. Finding primary origin of the tumor is crucial in diagnostic process and subsequent therapy. The present case is a 25th case of renal cell carcinoma (RCC) metastasizing to ovary. The authors report the case of 51-year old woman with a four-year history of metastatic renal clear cell carcinoma (MRCC) presented in the present hospital with contralateral metastasis in right ovary.

Key words: Renal clear cell carcinoma; Ovarian metastases.

Introduction
Renal cell carcinomas (RCCs) most often metastasize to bones, lungs, central nervous system, liver, lymph nodes, adrenal glands, and contralateral kidney. Other sites of metastases are rare [1, 2]. Moreover, ovarian cancer metastasize mainly to contralateral ovary, lymph nodes in minor pelvis, para-aortal nodes, distant mediastinal or supraclavicular nodes, and characteristic intraperitoneal seeding. Hematogenous metastases are seen in lungs, bones or vagina [1, 2].

About 6% of ovarian cancers found at laparotomy are secondary from other sites like stomach, breast, fallopian tube, bowel cancer or contralateral ovarian cancer [2, 3]. Kidney cancer rarely disseminates to ovary, which may cause a misdiagnosis of the cancer’s primary origin and lead to inappropriate subsequent adjuvant treatment.

In the literature there were published 24 case reports describing the metastases of primary renal cancer to the ovary and only four cases reporting primary ovarian cancer metastases to kidney. The present is the 25th case of RCC metastasizing to the ovary.

Case Report
A 51-year old woman with a four-year history of metastatic RCC was admitted to Department of Gynecology and Oncological Gynecology, because of suspected tumor in right ovary which was revealed during follow-up positron emission tomography computed tomography (PET/CT) examination. In March 2013 PET examination showed a hyperactive, 15-mm diameter focus in pelvis SUV 8.2mm, which corresponded to right ovary (Figure 1). Inguinal right-sided lymph nodes were also hyperactive SUV 2.7 and enlarged to 14 mm. The renal clear cell carcinoma was primary diagnosed in September 2009, after left nephrectomy. The patient was admitted to hospital that time with a short history of back pain and irregular, unreactive for typical treatment arterial hypertension for six years. Histopathologic examination revealed tumor in left kidney 85 x 75 x 70 mm in size, pTx Nx Mx. A year after that, the patient was complaining of headaches and progressive narrowing right-sided field of view. MRI examination in July 2010 showed a tumor in left occipital lobe. Left occipital craniectomy confirmed metastasis of clear cell carcinoma from kidney. The adjuvant radiotherapy was performed. In December 2012 the biopsy of thyroid gland demonstrated the metastasis. Thyroidectomy revealed the metastasis of clear cell carcinoma from kidney. (CKAE1/AE3+, CD10+).

On admission to the present hospital, transvaginal ultrasonography was performed and confirmed the presence of hyperechoic, well vascularised (RI-0.51 PI=0.75), 16 x 14 mm in size focus in right ovary. Because of the patient’s medical history and postmenopausal age, she qualified to undergo laparotomy with panhysterectomy.

The initial result of extemporaneous histological examination of right ovary was not conclusive. Detailed microscopic examination demonstrated the clear cell carcinoma metastasis from kidney. The patient was discharged in good general condition. Control follow-up transvaginal ultrasonography in two months after laparotomy did not reveal any pathological lesions in the pelvis minor.

Discussion
Our patient and most of patients reported in the literature (Table 1) presented multifocal metastasizing renal cell carcinoma (MRCC) and was treated by nephrectomy and...
metastasectomy. Typical treatment for RCCs is nephrectomy. In the past several years, immunotherapy consisting of recombinant interleukin-2 (rIL-2) and recombinant interferon-alpha (rIFN-alpha) has been considered standard first-line treatment for patients with MRCC. Moreover, following metastasectomy is nowadays recommended in MRCC [4, 5]. Metastasectomy (even incomplete) is thought to be an independent prognostic factor for survival. (P1/4.01, HR 0.297) [4]. Alt et al. examined 887 patients with MRCCs treated by nephrectomy and revealed that complete metastasectomy was associated with a significant prolongation of median cancer-specific survival (4.8 years vs 1.3 years; P < 0.001). A survival advantage from complete metastasectomy was also observed among patients with multiple, non-lung-only metastases, who had a five-year cancer-specific survival rate of 32.5% with complete resection vs 12.4% without complete resection (p < 0.001) [5].

Autopsy studies revealed that less than 1% of RCC metastases are directed to ovaries. However, up to 4.2% of secondary ovarian tumors are of renal origin [6]. Metastatic tumors of the ovary are a significant diagnostic problem in the identification of ovarian tumors. It is very confusing in differential diagnosis especially when metastasis occurs from tumors which are histologically similar to primary tumors of the ovary.

In presented systemic review (Table 1) 24 out of 25 cases (including the present), metastatic ovarian tumors were clear cell carcinomas from kidney. In 3/25 cases first diagnosed localization of the tumor was ovary and in 7/25 tumors were localized simultaneously in both organs. In the

<table>
<thead>
<tr>
<th>No.</th>
<th>Year</th>
<th>Age</th>
<th>Primary detection</th>
<th>Renal Localisation</th>
<th>Ovarian Localisation</th>
<th>Time to metastases</th>
<th>Other metastases</th>
<th>Histopathology</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1949</td>
<td>57</td>
<td>Kidney</td>
<td>Left</td>
<td>Left</td>
<td>8M</td>
<td>Vagina</td>
<td>RCC</td>
<td>Martzloff et al. [16]</td>
</tr>
<tr>
<td>2</td>
<td>1957</td>
<td>64</td>
<td>Kidney</td>
<td>Right</td>
<td>Bilateral</td>
<td>11Y</td>
<td>Lung</td>
<td>RCC</td>
<td>Vorder Bruegge et al. [10]</td>
</tr>
<tr>
<td>3</td>
<td>1981</td>
<td>68</td>
<td>Kidney</td>
<td>Right</td>
<td>Left</td>
<td>3M</td>
<td>No</td>
<td>RCC</td>
<td>Stefani et al. [17]</td>
</tr>
<tr>
<td>4</td>
<td>1983</td>
<td>52</td>
<td>Simultaneous</td>
<td>Left</td>
<td>Left</td>
<td>No</td>
<td>RCC</td>
<td>Buller et al. [18]</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1992</td>
<td>48</td>
<td>Ovary</td>
<td>Right</td>
<td>Left</td>
<td>8Y</td>
<td>No</td>
<td>RCC</td>
<td>Young et al. [7]</td>
</tr>
<tr>
<td>6</td>
<td>1992</td>
<td>62</td>
<td>Kidney</td>
<td>Left</td>
<td>Right</td>
<td>1Y</td>
<td>Thyroid/Lung</td>
<td>RCC</td>
<td>Young et al. [7]</td>
</tr>
<tr>
<td>7</td>
<td>1992</td>
<td>48</td>
<td>Simultaneous</td>
<td>Left</td>
<td>Left</td>
<td>No</td>
<td>RCC</td>
<td>Young et al. [7]</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1992</td>
<td>28</td>
<td>Kidney</td>
<td>Right</td>
<td>Left</td>
<td>9M</td>
<td>Bone</td>
<td>RCC</td>
<td>Liu et al. [19]</td>
</tr>
<tr>
<td>9</td>
<td>1993</td>
<td>40</td>
<td>Ovary</td>
<td>Left</td>
<td>Bilateral</td>
<td>7M</td>
<td>Skin, parotid, brain</td>
<td>RCC</td>
<td>Spencer et al. [20]</td>
</tr>
<tr>
<td>10</td>
<td>1994</td>
<td>46</td>
<td>Kidney</td>
<td>Left</td>
<td>Bilateral</td>
<td>3Y</td>
<td>No</td>
<td>RCC</td>
<td>Adachi et al. [21]</td>
</tr>
<tr>
<td>11</td>
<td>1996</td>
<td>54</td>
<td>Kidney</td>
<td>Right</td>
<td>Right</td>
<td>3Y</td>
<td>No</td>
<td>RCC</td>
<td>Fields et al. [22]</td>
</tr>
<tr>
<td>12</td>
<td>1996</td>
<td>66</td>
<td>Kidney</td>
<td>Right</td>
<td>Bilateral</td>
<td>11Y</td>
<td>Skin</td>
<td>RCC</td>
<td>Vara et al. [23]</td>
</tr>
<tr>
<td>13</td>
<td>2001</td>
<td>47</td>
<td>Kidney</td>
<td>Left</td>
<td>Left</td>
<td>4Y</td>
<td>No</td>
<td>RCC</td>
<td>Shinojima et al. [24]</td>
</tr>
<tr>
<td>14</td>
<td>2003</td>
<td>50</td>
<td>Kidney</td>
<td>Right</td>
<td>Right</td>
<td>1Y</td>
<td>No</td>
<td>RCC</td>
<td>Insabato et al. [25]</td>
</tr>
<tr>
<td>15</td>
<td>2003</td>
<td>49</td>
<td>Kidney</td>
<td>Right</td>
<td>Not available</td>
<td>14M</td>
<td>Bone, visceral</td>
<td>RCC</td>
<td>Insabato et al. [25]</td>
</tr>
<tr>
<td>16</td>
<td>2003</td>
<td>17</td>
<td>Kidney</td>
<td>Left</td>
<td>Left</td>
<td>2Y</td>
<td>No</td>
<td>RCC</td>
<td>Insabato et al. [25]</td>
</tr>
<tr>
<td>17</td>
<td>2003</td>
<td>48</td>
<td>Simultaneous</td>
<td>Left</td>
<td>Right</td>
<td>Bone</td>
<td>RCC</td>
<td>Hammock et al. [26]</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>2004</td>
<td>61</td>
<td>Kidney</td>
<td>Left</td>
<td>Bilateral</td>
<td>7Y</td>
<td>Skin, omentum, para-aortic</td>
<td>RCC</td>
<td>Valappil et al. [8]</td>
</tr>
<tr>
<td>19</td>
<td>2006</td>
<td>52</td>
<td>Simultaneous</td>
<td>Left</td>
<td>Right</td>
<td>Simultaneous</td>
<td>Bone</td>
<td>RCC</td>
<td>Kato et al. [27]</td>
</tr>
<tr>
<td>20</td>
<td>2007</td>
<td>73</td>
<td>Ovary</td>
<td>No available</td>
<td>Left</td>
<td>No inf.</td>
<td>Bone</td>
<td>PRCC</td>
<td>Stolnicu et al. [28]</td>
</tr>
<tr>
<td>21</td>
<td>2008</td>
<td>52</td>
<td>Simultaneous</td>
<td>Left</td>
<td>Left</td>
<td>No inf.</td>
<td>Lymphatic nodes</td>
<td>RCC</td>
<td>Monzon et al. [29]</td>
</tr>
<tr>
<td>22</td>
<td>2009</td>
<td>54</td>
<td>Simultaneous</td>
<td>Left</td>
<td>Left</td>
<td>6M</td>
<td>Bone, lung</td>
<td>RCC</td>
<td>Toquero et al. [30]</td>
</tr>
<tr>
<td>23</td>
<td>2010</td>
<td>54</td>
<td>Kidney</td>
<td>No data</td>
<td>Bilateral</td>
<td>39M</td>
<td>No</td>
<td>RCC</td>
<td>Guney et al. [31]</td>
</tr>
<tr>
<td>24</td>
<td>2013</td>
<td>50</td>
<td>Simultaneous</td>
<td>Right</td>
<td>Bilateral</td>
<td>No inf.</td>
<td>No</td>
<td>RCC</td>
<td>Holody-Zareba et al. [32]</td>
</tr>
<tr>
<td>25</td>
<td>Our 2013</td>
<td>51</td>
<td>Kidney</td>
<td>Left</td>
<td>Right</td>
<td>10M</td>
<td>Brain/thyroid</td>
<td>RCC</td>
<td>Hołody-Zareba et al. [32]</td>
</tr>
</tbody>
</table>

RCC: renal cell carcinoma; PRCC: papillary cell carcinoma; Y: year; M: month.
rest of reported cases, 10/25 tumors primary localization was in kidney. Although, it is extremely rare for ovarian cancer to metastasize to kidney, the four cases are described in the literature (Table 2). Due to this fact, interpretation of primary origin of the tumor is crucial in diagnostic process and treatment, particularly in simultaneous localization of the lesions. This process may be difficult, because the RCCs predominate in males, in females occur in post-menopausal age when vascular sclerosis is most common in ovaries, and some of metastatic tumors may be mistaken as a primary ovarian tumor [7, 8].

There are some histological differences between RCC and ovarian clear cell carcinoma. The tubules and papillae of primary clear cell carcinoma of the ovary are lined with hobnail cells, positive for Ca 125, and contain intraluminal mucin: findings rarely seen in RCCs. In the present case, Ca 125 blood levels were normal [7-9].

There are some theories about renal-ovarian axis, explaining the etiology of renal-ovarian metastases. One of them bases on anatomic relations between these organs.

Current literature shows that in most of cases 10/25 (40%) metastases arose from left-sided primary renal cancer ipsilaterally to left ovary. In 8/25 (32%) cases, RCCs disseminate to contralateral ovary. According to primary ovarian cancer, probably in all cases ipsilateral left-sided ovarian cancer and left-sided renal metastases occurred. This make a suspicion of hematogenous retrograde venous dissemination by way of left ovarian vein [7,10].

It is known that there is anatomic vessel relation between left kidney and left ovary. The kidney is vascularised by renal artery, ovary by ovarian artery, and ovarian branch from uterine artery. Venous correlation seems to be connected. Left ovary vein goes to left renal vein, and right ovarian vein to vena cava inferior. That is why the anatomy of left renal and ovarian veins may lead incompetent left ovarian vein to retrograde venous flow and enable dissemination of the cancer [11]. Moreover, it is generally known that kidney cancer may disseminate by renal vein forming tumor thrombus (TT). This fact may give rise to the theory that anatomic venous connection between left kidney and left ovary allows metastasizing to ovary. Unfortu-
mors to each other. Even if metastases from kidney cancer to ovary and vice-versa are very rare, it is worth taking this phenomenon into consideration in diagnostic process and identification primary origin of the tumor.

References


Spindle-cell epithelioma of the vagina diagnosed during pregnancy - a case report

S. Pantovic1, A. Stefanovic1,2, J. Stojnic1,2, K. Jeremic1,2, R. Sparic1, S. Kadija1,2, S. Milenkovic3

1 Clinic for Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade; 2 School of Medicine, University of Belgrade, Belgrade
3 Department for Pathology, Clinical Center of Serbia, Belgrade (Serbia)

Summary
Spindle-cell epithelioma or "mixed tumor" of the vagina is an unusual and intriguing vaginal tumor consisting of both epithelial and mesenchymal components. A case of spindle-cell epithelioma of the vagina diagnosed at delivery of a 31-year-old primiparous woman is described. The excision of the mass was performed immediately after the delivery, which was uneventful. The patient was regularly followed up and no evidence of local recurrence or dissemination was found 40 months after surgery. The presentation and the diagnosis of this kind of tumor in pregnancy, and its effect on the pregnancy and delivery are still largely unknown. Since it is unlikely that any institution will have a large number of patients with this rare disease, case reports add further information to this entity. As the number of cases studied is small, close follow-up is recommended although there has been no report in the literature of metastasis so far.

Key words: Spindle-cell epithelioma; Mixed tumor; Vagina; Pregnancy.

Introduction
Spindle-cell epithelioma or intriguing mixed tumor of the vagina refers to a rare neoplasm composed of a proliferation of the spindle cells admixed with epithelial cell islands within. Almost all of the reported tumors were small and well circumscribed, cited in or above the hymen in adult women. Many theories have been given on the origin of these tumors, ranging from possible embryonic remnants being the source, to its origin from the Müllerian ducts or urogenital sinus [1]. Its histogenesis, however, is still unclear.

This condition is most often asymptomatic and discovered accidentally on routine pelvic examination. It usually presents as a nodular mass located near the hymenal ring and may present with vaginal discharge or bleeding [2].

Case Report
A 31-year-old nullipara was referred to the obstetrics department at 41 weeks of gestation for delivery. The antepartum course was unremarkable. Her personal history contained no significant diseases or tumors. On admission, the cervix was three-cm dilated and 0.5 cm long. Vaginal examination also revealed a three-cm, mobile, painless, non-tender pedunculated mass arising from a short (0.2 cm) stalk situated on the left side of the posterior vaginal wall. The tumor was located about two cm behind the hymenal ring, and appeared neither necrotic nor infected. A rectal examination did not reveal any abnormalities, and the patient had no complaints regarding the tumor.

Since the vaginal outlet was not obstructed with the tumor, the authors decided to perform vaginal delivery, which was uneventful and performed through a right mediolateral episiotomy. A male baby of 3,500 grams with an Apgar score of 9 was born. Upon delivery, due to the unknown nature of the tumor, the authors performed the excision. Due to the unknown nature of the tumor, deep dissection was performed and both the placenta and the tumor were submitted for histopathological examination, which did not reveal any abnormalities. Grossly, the excised tumor measured 30×30×25 mm. On section, the cut surface exhibited a pale yellowish submucosal nodular mass of firm consistency.

On microscopic examination, the mass was well circumscribed but unencapsulated, with expansive growth. The tumor was covered with normal vaginal squamous epithelium and consisted of two components: stromal-type spindle cells, which were predominant, and epithelial cells with hyaline globules between them. Neither hemorrhage nor necrosis in the tumor was seen. The mitotic figures were absent (Figures 1A and B).

Immunohistochemistry was performed on paraffin sections, with a broad spectrum of immunohistochemical stains. A positive reaction for pan CK and CD 10 was observed in both components (Figures 2A and B). Vimentin activity was noted only in stromal-type cells. They were uniformly positive for CK7 (cytokeratin) and negative for CK20, smooth muscle actin, desmin, chromogranin, synaptophysin, and S-100 protein. The epithelial component was positive for CK5/6, monoclonal carcinoembryonic antigen and tumor protein 63, while the Ki67 immunopositivity was low (5%). Based on light microscopic appearances along with the immunohistochemical staining, the final diagnosis of spindle-cell epithelioma of the vagina was made.

The postoperative course was uneventful and the patient was discharged on the third postpartum day. At the four-week postpartum visit, the vaginal epithelium was completely healed and free of any lesions or scarring. The patient was regularly followed...
Discussion

Clinical experience with cell epitheliomas of the vagina is limited. The presentation and the diagnosis of this kind of tumor in pregnancy, and its effect on the pregnancy and delivery are still largely unknown. The first reported case of this kind of tumor in the English literature was published in 1953, followed by other case reports, which indicated the average age of occurrence being 40.5 years, with a reported range from 20 to 80 years [2]. In 1993 Branton et al. published a detailed study of 28 cases, still the largest series of its kind [3].

One would consider that a vaginal mass in pregnancy should be excised if obstructing the vaginal outlet. In the present case, the reason for surgical intervention after the delivery was unknown nature of the tumor. Because of the increased vascularisation of the vagina in a term pregnancy, special care is required to minimize the blood loss during surgery in such cases.

up and there was no evidence of local recurrence or dissemination 40 months after the surgery.
Based on the available reports, local excision is considered curative and the prognosis is generally good [2]. Recurrence occurs when the tumor is incompletely excised [3, 4]. In the recurrent cases, no unique features other than apparent incomplete excision were noted [2]. As the number of cases studied is small, close follow-up is recommended, although there has been no report in the literature of metastasis so far [2].

The clinicopathological features of the present case were similar to previously reported cases [5, 6]. A pathological diagnosis of spindle-cell epithelioma of the vagina should always be kept in mind whenever a polyoid mass near the hymenal ring is excised. Spindle-cell epitheliomas are distinct neoplasms and should not be confused with “mixed tumors” occurring at other sites such as the salivary and lacrimal glands, breast, mediastinum, trachea, skin, and vulva [5, 6]. These tumors should be differentiated from other tumor lesions, such as aggressive angiomyxoma, solitary fibrous tumor, malignant mixed tumor, and malignant tumor of the vagina resembling synovial sarcoma [2].

In conclusion, familiarity with these rare tumors among pathologists and gynecologists would perhaps lead to the identification of more of cases and limit misdiagnosis and surgical complications.

References

Address reprint requests to:
J. STOJNIĆ, M.D.,
Medical School, University of Belgrade,
Belgrade, Serbia
Clinic of Gynecology and Obstetrics
Clinical Center of Serbia
Višegradska 26, 11000 Belgrade (Serbia)
e-mail: jelence_01@open.telekom.rs
Introduction

While colorectal carcinoma is the most common malignancy and one of the leading causes of cancer in the western world, it is uncommon before the age of 40 years and has an incidence of 0.002% during pregnancy [1-3]. The first case of rectal carcinoma in a pregnant woman was reported by Cruveilhier in 1842. Since then about 250 cases have been reported in the literature [2]. The disease continues to have a poor prognosis possibly related to delayed diagnosis.

Case Report

A 25-year-old woman (gravida 2, para 1) referred to the present hospital at 15 weeks and four days gestation with complaints of dyspareunia, difficulty to urinate, and painful tumefaction of the perineum. She also experienced diarrhea and pelvic pain for one month. She had a familial background of an uncle with colorectal carcinoma.

The gynecological examination performed under general anesthesia due to severe local pain, revealed a hard and irregular mass in the recto-vaginal septum, confirming the intactness of the vaginal mucosa. A solid, hardly defined, four-cm wide mass invading the recto-vaginal septum was identified immediately above the anal canal on rectal palpation. Ultrasonography confirmed a gestation at 50th percentile, without major abnormalities. The analytical evaluation revealed a carcinoembryonic antigen (CEA) of 0.87ng/ml and a CA-125 of 18 U/ml.

Videorectoscopy demonstrated a zone of mucosal bulging with superficial ulceration, immediately over the anal canal. A vegetating, circumferential, and easily bleeding lesion that did not permit the passage of the colonoscope was detected. Pathological examination of the biopsies demonstrated a moderately differentiated and ulcerated adenocarcinoma.

Magnetic resonance imaging (MRI) revealed a lesion of 12 x 6 x 3 cm, four cm above the anal canal, with serosal invasion, occupying the recto-vaginal septum with multiple implants along the peri-rectal fat and sigmoidal mesentery (T4N1Mx) (Figure 1). No liver metastasis was demonstrated on abdominal ultrasonography.

After consultation with general surgeons and oncologists, a therapeutic proposal of neoadjuvant chemotherapy, radiotherapy, and eventual surgical excision of the tumor was made. The pregnancy was terminated in response to the request of the couple after approval by the ethical committee.

Thoracoabdominopelvic contrast computed tomography (CT) showed disseminated pulmonary micronodules with right hemithorax predominance – possible metastizations. Pelvic MRI performed after receiving neoadjuvant chemotherapy with 5-FU and radiotherapy of 50.4 Gy revealed significant remission of the tumoral mass, mainly replaced by fibrotic tissue, and uncertainty regarding the invasion of the perirectal fat tissue.

The patient was submitted to low anterior resection of rectum with colo-anal anastomosis. The day after she died of multi-organic failure related to fecal peritonitis after dehiscence of the anastomosis suture.

Discussion

Rectal cancer in pregnancy is an uncommon condition with an incidence of 0.002% and has a poor prognosis. In contrast to that observed in the general population, in which colorectal cancers are frequently encountered above the pelvic peritoneal reflection, the majority of them diagnosed during pregnancy are rectal carcinomas [3]. This distribution is believed to reflect a detection bias by a tendency toward rectal examinations during prenatal care [2-4].

Symptoms such as constipation, abdominal pain, nausea, vomiting, and rectal bleeding are common in both pregnancy and colorectal cancer. This may lead to delayed diagnosis of the cancer at a more advanced stage, contributing to poor prognosis of the disease [1, 4, 5]. Pregnancy itself may also have influence in advanced tumors seen in these patients. The elevated levels of circulating...
estrogen and progesterone during pregnancy may stimulate the growth of these tumors [2, 3]. Another factor that may be related to the poor prognosis is the patient’s age. Colorectal cancer is usually a disease that occurs after the fifth decade of life. Some authors report poorer survival for patients younger than 40 years with colorectal carcinoma compared to older patients [6, 7], although others defend a similar overall survival for both groups [3, 8, 9]. Colorectal carcinoma can adversely affect pregnancy, with only 78% resulting in live born infants [10]. The tumor has never been reported metastasize to the fetus, although placental implants were found in one case [2]. The fetus and the placenta were not studied in this patient.

In cases that the disease is suspected, digital rectal examination and sigmoidoscopy should be performed as these methods are expected to reveal more than 80% of colorectal tumors in pregnant patients [11]. MRI has been used for cancer staging. Hepatic ultrasound is a sensitive modality for detecting liver metastasis [4]. CEA may be elevated during pregnancy, therefore it is not useful for diagnosis but may have value during follow-up [2, 4, 5].

Although treatment follows the same general guidelines as for non-pregnant patients [12], the management should be determined on a case-by-case basis by a multidisciplinary team and the patient and her family should be actively engaged in therapeutic decision making. During the first 20 weeks of pregnancy, if the patient wishes to carry her pregnancy to term, primary resection followed by chemotherapy after delivery is advised as management for rectal cancer [1]. Hysterectomy is performed if the mother’s life expectancy is less than the time required for the fetus to reach viability, the uterus is found to be involved at the time of the surgery or if it impedes a complete surgical resection [2,4]. If the patient chooses to terminate the pregnancy, she is managed as a non-pregnant patient after therapeutic abortion [4]. In advanced rectal tumors, the treatment option is usually neoadjuvant radio- or chemotherapy followed by surgery for tumor removal as was done in the present patient. In cases that the tumor is discovered during the second half of the pregnancy, treatment is postponed until after delivery. Vaginal delivery is allowed unless the tumor is located on the anterior wall of the rectum or if it obstructs the birth canal [2, 3]. In case of cesarean section, tumor resection may follow immediately or may be carried out after the uterus has regressed in order to avoid excessive hemorrhage during the surgery and post-operative thromboembolic complications.

Rectal cancer coupled with pregnancy is a challenging combination yielding a poor prognosis [3], despite very early diagnosis during pregnancy as in the present case. Management should involve a multidisciplinary team and the patient and her family should be actively engaged in therapeutic decision making.

Conclusion

Rectal cancer during pregnancy is very rare as these tumors are uncommon before the age of 40 years. Treatment follows the same general guidelines as for non-pregnant patients. Despite the survival data stage-for-stage being the same for pregnant and non-pregnant patients, the disease is associated with a poor prognosis. Whether this poor prognosis reflects, either in whole or in combination, simply a delay in diagnosis, a biologically aggressive tumor in young women or a hormonally driven tumor remains to be determined [3].

References


Address reprint requests to:
G. KARAKUS, M.D.
Department of Obstetrics and Gynecology
Hospital Distrital de Santarém
Av. Bernardo Santareno
2005-177 Santarém (Portugal)
e-mail: gunesfigueiredo@gmail.com
Spontaneous intrauterine pregnancy following abdominal radical trachelectomy - a case report

M.E. Căpîlna¹, S.C. Rusu¹, C.I. Puiac², A. Daniilidis³, B. Szabo¹

¹First Clinic of Obstetrics and Gynaecology, University of Medicine and Pharmacy, Târgu-Mureş
²First Clinic of Anaesthesiology and Intensive Care, University of Medicine and Pharmacy, Târgu-Mureş (Romania)
³Second University Clinic of Obstetrics and Gynaecology, Hippokratio General Hospital, Aristotle University of Thessaloniki, Thessaloniki (Greece)

Summary

The authors describe a case report of spontaneous pregnancy after an abdominal radical trachelectomy because of cervical cancer Stage IB2.

Key words: Cervical cancer; Trachelectomy; Pregnancy.

Introduction

The surgical management of cervical cancer patients have changed substantially during the last two decades, especially for young women deserving to preserve their fertility and for advanced or recurrent cases. The excellent prognosis of early-stage cervical cancer, combined with the young age of many patients has led to a focus on maintaining both the survival prognosis and fertility after treatment. Abdominal radical trachelectomy is a fertility sparing procedure that could be offered as an option in suitable situations.

Case Report

A 24-year-old white female was referred to the present clinic with the diagnosis of cervical cancer. She had a high grade squamous intraepithelial lesion (HSIL) smear test three months prior and a following punch biopsy result of glassy cells subtype adenosquamous carcinoma. On clinical examination a bleeding exophytic cervical mass of 5 x 4 cm was discovered, while uterus, ovaries, parametria and rectum were normal. Transvaginal ultrasound demonstrated a cervical tumour of 52 x 48 x 41 mm, with invasion of more than half of the cervix in depth without any pathological findings from the uterine corpus and adnexa. Abdominal computerized tomography (CT) described the same cervical lesion with no extraperitoneal enlarged lymph nodes and normal peritoneal cavity. Radiography of the thorax was normal. The clinical diagnosis was Stage IB2 cervical carcinoma. Routine blood test investigations were normal, except of a urinary infection with group beta streptococcus, for which she received antibiotic treatment. She had not yet had children and it was decided after consultation to proceed with a fertility-sparing treatment.

Under general anaesthesia and with L3-L4 epidural catheter placed on site, she underwent an abdominal radical trachelectomy with pelvic lymphadenectomy, ligation of both uterine arteries, and pararectal fossae drainage. There were no intraoperative complications. The frozen sections of five suspicious lymph nodes from the right side and two from the left side were negative. Also, the frozen section of the upper part of the removed cervix was clear from disease. The whole procedure lasted 260 minutes. The postoperative recovery was uneventful, and she was discharged home after hospitalisation for seven days. The final pathology report described tissues of 35 x 32 x 39 mm cervix and of 25 mm vagina. Adenosquamous carcinoma of glassy type, Broder grade 3, and invaded 10 of 16 mm of the cervical wall (Figure 1). There were no metastases into the parametria (dimensions: right 75 x 25 x 22 mm, left 50 x 31 x 12 mm) and in the 16 from the right and 26 from the left side lymph nodes.

Follow-up was in three months with clinical examination, smear test, ultrasound, squamous cell carcinoma (SCC) marker, and in six months with a CT scan six months postoperatively with no signs of recurrence. She reported normal menstruation after surgery.

She became pregnant on the seven postoperative month spontaneously. Pregnancy proceeded uneventful until 28 gestational weeks, with monthly clinical examination and ultrasound growth measurements of the fetus and of the uterine isthmus. Smear test and colposcopy were performed in the first trimester and were reported as normal. She delivered at 38 weeks of gestation by elective caesarean section a female infant, of 2,550 grams, and 9/1 minute Apgar score. The caesarean section was strait forward and the recovery was uneventful for both mother and infant. She was discharged home on the fifth day postoperative. Seven months after delivery, mother and baby are both fine and well, with normal oncologic follow-up.

Discussion

The abdominal radical trachelectomy technique, imagined and described for the first time by the Romanian gynaecologist Aburel in 1956 [1], has been almost forgotten for four decades and “rediscovered” by Smith et al. in the 1990s [2]. Together with the vaginal radical trachelectomy and laparoscopic pelvic lymphadenectomy described in...
1994 by Dargent et al. [3], it represents a real option for women with cervical cancer Stages IA2-IB2, who desire to preserve their fertility. After analyzing a few hundred cases already published, the abdominal trachelectomy has a shorter learning curve, a higher oncologic radicality, and a slightly better five-year survival compared with the vaginal technique [4]. Another advantage consists on the feasibility to perform it for bulky cervical tumours – Stages IB2 and IIA [5]. Its main disadvantage is considered to be the less favourable obstetrics outcome [4].

In the present series, 11 abdominal radical trachelectomies were performed between May 2010 to July 2013. The present was the first pregnancy in eight patients with normal menstruation after the surgery. Among these eight, only three attempted to conceive until this moment. Three women are amenorrheic: one is postmenopausal now at age 40, one decided to receive adjuvant radiotherapy because of three positive pelvic lymph nodes on final pathology report, and one patient has an amenorrhea, probably due to diminished vascularization of the endometrium. For this reason, in the future, the present authors consider to attempt to preserve at least one of the uterine arteries, in order to provide a better blood supply to the uterus, without compromising the oncologic radicality. They did not consider necessary to perform a cerclage, recommended only by some authors during the abdominal trachelectomy [6], but they considered important to perform a careful pelvic peritoneation at the end of the procedure. As described by Ungar et al. [4], the present authors did not perform a prophylactic cerclage during the pregnancy, because of the alarming tissue resulting at the uterine isthmus – vaginal anastomosis.

The present authors consider abdominal radical trachelectomy as safe as the radical hysterectomy regarding oncologic outcome. It must be offered to patients with early stages of cervical cancer who desire to spare their fertility.

References


Address reprint requests to:
M.E. Căpîlna, M.D.
First Clinic of Obstetrics and Gynecology, University of Medicine and Pharmacy, str. Gheorghe Marinescu no. 50, 540136, Târgu-Mureş (Romania)
e-mail: mcapilna@gmail.com
Uterine extra gastrointestinal stromal tumor presenting as intramural leiomyoma

T. Oge1, D. Arik2, E. Uysal1, O.T. Yalçın1, S. Kabukcuoglu2, S. Ozalp1

1Eskisehir Osmangazi University School of Medicine, Department of Obstetrics and Gynecology, Eskisehir
2Eskisehir Osmangazi University School of Medicine, Department of Pathology, Eskisehir (Turkey)

Summary
Extra gastrointestinal stromal tumors (EGIST) are reported in different sites and organs. This tumors are rare in gynecologic apparatus. Here the authors report an uterine unique tumor represented as intramural leiomyoma. Because of different treatment options, clinicians should be aware of this rare tumor which may be located in uterus and confused with a smooth muscle tumor.

Key words: Uterus; Extra gastrointestinal stroma tumor.

Introduction
Gastrointestinal stromal tumors (GIST) are rare mesenchymal malignancies of the gastrointestinal tract. They usually arise from the muscular layer of the digestive tract and most often express immunoreactivity for CD117, a c-kit proto-oncogene protein [1]. When these tumors develop outside the gastrointestinal tract such as omentum, mesentery, retroperitoneum, and undefined abdominal sites, they are called extra gastrointestinal stromal tumors (EGISTs) [2]. There are unusual sites of EGIST such as uterine, ovarian, tubal, rectovaginal or vulvavaginal spaces presenting as a mass [3-10]. In English literature, there are only two reported cases of uterine EGIST presented as a mass connected to the uterus from the posterior wall [3, 4]. To the best of the authors’ knowledge, they describe the first case of EGIST presenting as a uterine leiomyoma.

Case Report
A 50-year-old woman gravida 4 para 2 was admitted to the hospital because of abdominal pain and constipation. The family history was unremarkable. General examination and laboratory findings were normal. Bimanual pelvic examination revealed enlarged and nodular uterus up to 20 weeks’ gestational size. Transvaginal ultrasound demonstrated the existence of enlarged uterus (15 x 10 x 9 cm) and multiple uterine myomas with bilateral normal ovaries. Laparotomy was planned and patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy. The exposure of abdomen revealed enlarged uterus (15 x 10 x 10 cm) and multiple subserous and intramural myomas. Ovaries and other intraabdominal organs were normal. Postoperative course was uneventful.

Histopathologic evaluation of the specimen revealed cellular lesion with myometrial and lymphatic invasion. In most areas cells were in spindle morphology and pleomorphic epitheloid cells with eccentric hyperchromatic nucleus and large eosinophilic cytoplasm were seen. Some of these epithelioid cells were binuclear (Figure 1). There were tumoral necrotic surrounded with multinuclear osteoclast-like cells. Eosinophilic skenoid fibers were detected (Figure 2). Mitotic figures were more than 10 in 50 high power field (HPF). Immunohistochemistry with CD117 revealed intense membranous and cytoplasmic positivity (Figure 3). Actin was moderately positive while desmin was negative. Histologic activity index with Ki-67 was 30%. According the microscopic and immunohistochemical findings, final histopathological results reported as high grade EGIST.

Discussion
GISTs are mesenchymal tumors of the gastrointestinal tract, approximately 70% occurring in the stomach, 20-30% occurring in the small intestine, and less than 10% in the esophagus, colon, and rectum [1]. GISTs rarely develop outside of the digestive tract especially in the soft tissues of the abdomen and pelvis, designated as EGISTs. EGISTs usually present during adult life as enlarging masses and approximately 80% are located in the omentum and mesentery. In women, rare cases of EGISTs have been reported as an unusual anatomic locations like vaginal, vulvovaginal, ovarian, uterine, and rectouterine pouch of Douglas [3-10]. Most are firm, fleshy, gray-red masses lacking the whorled appearance that is often seen with conventional smooth muscle tumors and may mimic gynecologic malignancies when they appear in the female pelvis. In the present case, the appearance of the tumor resembled intramural and subserous myomas with enlarged uterus. In the two previous cases, the tumor was like a mass attached to the uterus from the posterior wall [3, 4]. There is also one report presenting a case locating at Douglas pouch mimicking uterine tumor [7].
The histologic appearance of EGISTs is variable, but in general there are two patterns: epithelioid and spindle cell. In the epithelioid pattern, cells vary from small uniform cells to large pleomorphic cells with eosinophilic cytoplasm. Another characteristic feature of these tumors is the hyperchromatic nuclei and prominent cytoplasmic vacuole, which in extreme instances result in a signet ring cell appearance. Tumors with a spindled pattern more closely resemble conventional smooth muscle tumors but nonetheless can be distinguished by the fact that their cells usually have a short fusiform shape in contrast to the elongated cells of leiomyomas and leiomyosarcomas. Nuclear palisading is occasionally seen. The stroma is typically composed of mats of fine, hair-like collagen interrupted by a delicate vasculature. Skenoid fibers (extracellular collagen containing eosinophilic globules) were occasionally reported in small intestinal stromal tumors and originally believed to reflect neural differentiation.

Approximately 95% of GISTs and EGISTs have activating somatic mutation of CD117 (c-kit), a tyrosine kinase transmembrane receptor located on chromosome 4 (4q11-q12). CD117 is also expressed in melanoma, angiosarcoma, fibrosarcoma, and liposarcoma. The characteristic morphology with the panel of immunohistochemical markers help to rule out its diagnosis. Tumors with smooth muscle or neurogenic origin have a similar morphology; express desmin or smooth muscle actin (SMA) but do not express CD117. The main differential diagnosis of EGISTs of the pelvic area is leiomyosarcoma. Smooth muscle tumors are immunoreactive for desmin and negative for CD117. In the present case, the tumor stained negative for desmin and strongly positive for CD117, hence the diagnosis of leiomyosarcoma was ruled out.

Because of their rarity, predicting the prognosis of EGIST is not easy. Tumors larger than five cm and more than five mitoses/50 HPFs are considered to be high risk [1]. Although clinicopathological findings are similar, the treatment of GIST and leiomyosarcomas show differences. In GIST, complete surgical resection of primary localized tumor with negative margins remains the best therapeutic option today. In the setting of locally advanced or metastatic disease, imatinib mesylate which is the inhibitor of the tyrosine kinase activity of c-kit has been reported to be effective and safe, however the role of imatinib in the treatment of EGIST is unclear. On the other hand total abdominal hysterectomy and bilateral salpingo-oophorectomy represents the standard treatment of uterine sarcomas. Pelvic and para-aortic lymph...
node dissection in carcinosarcomas is recommended, given their high incidence of lymph node metastases, and may have a role in endometrial stromal sarcomas. Adjuvant therapies, including radiation, chemotherapy, and/or hormonal therapy have limited survival value. Because of these differences pathologists and gynecologists should be careful during the diagnosis and follow-up stage.

As a conclusion, although two previous reports pointed out tumor attached to uterus from the posterior side, this is the first case in which the tumor resembled a uterine myoma during the operation. Histopathological and immunohistochemical findings are important in differential diagnosis of leiomyosarcoma and EGIST, and because of different treatment options, clinician should be aware of this rare tumor which may be located in uterus and confused with leiomyosarcoma.

References


Address reprint requests to:
D. ARIK, M.D.
Eskisehir Osmangazi University School of Medicine, Department of Pathology, Meselik Kampusu, 26480, Eskisehir (Turkey)
e-mail: denarik@hotmail.com
Join us for our 2015 CONGRESSES!

6th International IVI Congress
Reproductive Medicine and Beyond

April 23-25, 2015
Alicante, Spain

www.comtecmed.com/ivi

www.comtecmed.com
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
Chapter 2: Endometrial Cancer and Its Precursor Lesions: Histopathologic and Molecular Aspects
Chapter 3: Molecular Pathologic Aspects of Endometrial Cancer: An Update
Chapter 4: Endometrial Endometrioid Adenocarcinoma: Histology, Precursors and Molecular Alterations.
Chapter 5: Hereditary Endometrial Cancer
Chapter 6: Lynch Syndrome and Endometrial Cancer
Chapter 7: Sentinel Lymph Nodes in Endometrial Cancer
Chapter 8: Lymphatic Mapping for Endometrial Cancer
Chapter 9: Detection of Sentinel Lymph Node in Endometrial Cancer
Chapter 10: Strategies for Treating Patients with Endometrial Cancer
Chapter 11: Surgical Modalities for Treating Patients with Endometrial Cancer
Chapter 12: Management of Advanced Stage Endometrial Cancer

Chapter 13: Clinical Trials Evidence for Efficacy of Postoperative Chemotherapy for Early Endometrial Cancer
Chapter 14: Medical Treatment of Endometrial Cancer
Chapter 15: Chemotherapeutic Agents for Patients with Endometrial Cancer
Chapter 16: Novel Therapeutic Agents for Treatment of Endometrial Cancer
Chapter 17: Targeted Therapies for Endometrial Cancer
Chapter 18: ErbB Targeted Therapy in Endometrial Cancer
Chapter 19: Radiotherapy Treatment for Endometrial Cancer

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.

THERAPEUTIC REVOLUTION
The History of Medical Oncology from Early Days to the Creation of Subspecialty

Pierre R. Band

The challenge in the fight against tumors began in ancient times and still continues today. As referred by the Author of this Book, history is generally written by the winner, however the counterpart, the tumor, unfortunately has not yet been conquered, even if numerous battles have been won while offering surprising results.

Since its debut medical treatment of tumors was utilized as adjuvant therapy for both surgical and radiotherapy and from several decades has become the primary treatment for many of them.

The history of medical treatment of tumors presented in this Book of elevated scientific value enables us to traverse all the phases of its progress. Each chapter discusses different aspects of chemotherapy, the successes obtained, and also the frustrations of the researchers when their expectations were disappointed.

The initial tumor observations, the dawn of new medical drugs, the milestones obtained, the formation of dedicated study groups with the aim to deepen the knowledge of each, to the application of mathematical models for the study of variants that lead to drug resistance, are clearly and carefully described. All of these aspects render this Book useful, not only to the experts, but also to those about to embark in oncological studies.

CONTENTS
Chapter 1: The Author Introduces Himself
Chapter 2: From Dinosaurs to the Dawn of Chemotherapy: A Brief Overview
Chapter 3: World War II’s Legacy to Cancer
Chapter 4: The Immediate Post-World War II Years: Cancer Chemotherapy Spreads its Wings
Chapter 5: The Years of Creativity: 1953-1965. Pre-Clinical
Chapter 6: The Years of Creativity: 1953-1965. Clinical
Chapter 7: The Years of Creativity: 1953-1965. Cooperative Oncology Groups and Clinical Trials
Chapter 8: Combination Chemotherapy: The Road to Cure
Chapter 9: Tumor Growth
Chapter 11: Breast Cancer. 2. A Paradigm for Solid Tumor Chemotherapy
Chapter 12: Successes in Solid Tumor Chemotherapy: Three Key Examples
Chapter 13: From Mathematical Models to Palliative Care and Psycho-Oncology
Chapter 14: October 16, 1973

Study and research are frequently characterized by an overwhelming anxiety to obtain results and there is no time dedicated to reflection. This textbook, however, invites us to stop and reflect on the state of the art of medical oncological therapy and subsequently to resume the challenge right from the very point where previous researchers have led us to, in order to continue with the battle against cancer, which still cannot be considered won.

DOI: 10.2174/97816080581431140101
eISBN: 978-1-60805-814-3
ISBN: 978-1-60805-815-0
See more at: ebooks.benthamscience.com/book/9781608058143/
Foreword

The importance of this book is included in its very theme, as it presents gynecological cancer of the most unfavorable prognosis. In fact, despite the numerous advances in surgery, chemotherapy, and molecular therapies, the survival rates have only slightly improved. Selecting ovarian tumors as the object of study, as assessed by a multi-specialized team, can assist the gynecological oncologists, and also refine the approach to the disease and increase their professional standard.

This book, written by 32 international acknowledged experts, with rich and clear illustrations, offers an expert guide to all aspects of this neoplasia.

From the epidemiology, through risk, management in early and advanced stages, pediatric neoplasia, to the quality of life, the author explores all the possible aspects of this disease and all the implications that affect the outcome.

The chapters are all written very clearly, allowing anyone from the student to the expert to fully benefit from consultation of the manual, and the in-depth information makes it easier to understand its contents.

In conclusion, I believe that the comprehensive text conveys a significant progress in understanding this complex neoplasia.

M. MARCHETTI
A Manual for Cervical Cancer Screening and Control: Principles, Practice and New Perspectives

This book is edited by Margherita Branco, former Director of Cervical Cancer Screening and Cytopathology Unit, National Institute of Heath, Rome (Italy) and by Adhemar Longatto-Filho, of the Laboratory Medical Investigation 14, Faculty of Medicine, Sao Paulo (Brazil).

The topic covered in this book is connected to the prevention and early detection of cervical cancer.

Although cancer of the cervix is a disease that is well-detected and almost eradicated in developed countries that have introduced individual screening programs, it still remains the second or third most common cause of death in developing countries.

The 14 chapters of this textbook thoroughly examine all the “aspects” related to prevention and early detection.

From the general information on this neoplasia, through primary prevention, HIV infection, risk factors, methods of screening, study of biomarkers, organization of training for personnel involved in screening programs, to the general instruction for prevention, this manual offers a complete contribution to improve women’s health.

Contents

Chapter 3: Human Papillomavirus (HPV) infections. M. Branca and A. Longatto-Filho.
Chapter 4: Risk factors for cervical cancer. M. Branca.
Chapter 6: Cancer prevention in developing countries. A. Longatto-Filho.
Chapter 7: Cervical cytology and alternative methods of screening. A. Longatto-Filho.
Chapter 8: Management of women with abnormal cytological results. M. Branca and A. Longatto-Filho.
Chapter 10: Basic concepts of quality and accreditation in Health Care Services. M. Branca.
Chapter 13: Instruction and training of personnel in a cervical cancer screening program. M. Branca and A. Longatto-Filho.
Chapter 14: Universal hygienic measures and precautions for infection prevention in gynecological ambulatory centers and hospitals. M. Branca.

We believe that this book also provides comprehensive coverage and expert guidance of all persons implicated in screening programmes.

Chairman: Péter Bösze (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)
LASZLÓ 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 2015**

Founded in 1974 (ISSN 0390-6663) - Vol. XLII. 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: 600 USD
- Individual: 400 USD
- Single copy: 120 USD

**ONLINE ISSUE**
- Institutional: 450 USD
- Individual: 270 USD
- Single copy: 100 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 2015**

Founded in 1980 (ISSN 0392-2936) - Vol. XXXVI. 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: 600 USD
- Individual: 400 USD
- Single copy: 120 USD

**ONLINE ISSUE**
- Institutional: 450 USD
- Individual: 270 USD
- Single copy: 100 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