Contents

LETTER TO EDITOR
Emerging etiopathogenic connections between Arg399Gln polymorphism and systemic malignancies besides their etiological role in endometrial carcinomas
S. Kapoor - Mechanicsville, VA (USA)
A commentary of an article that deals with Arg399Gln polymorphism as probable etiological factor in endometrial cancer is made.

REVIEW ARTICLE
Brain metastases from cervical carcinoma: overview of pertinent literature
E. Piura, B. Piura - Beer-Sheva, ISRAEL
Overview of the articles published in the literature addressing brain metastases from cervical carcinoma.

ORIGINAL ARTICLES
Retromesenteric para-aortic lymphadenectomy in gynecologic malignancy
Retromesenteric preparation offers a safe access for lymphadenectomy and is quick to learn.

Prognosis of high-grade endometrial cancer: a comparison of serous-type and clear cell type to grade 3 endometrioid-type
Worse prognosis and chemo-response in serous type than endometroid grade 3 endometrial cancer are shown.

Octreotide is the favorable alternative for cisplatin resistance reversal of ovarian cancer in vitro and in nude mice in vivo
Y. Shen, M.L. Ren, Y.H. Shi, Y.X. Zhang, Y.L. Cai - Nanjing, CHINA
Octreotide somatostatin analogs acting on chemoresistence may improve response to chemotherapy in ovarian cancer.

HPV at the time of vaccine: has screening reached its goal?
E. Tartaglia, D. Iafusco, A. Cocca, S. Palomba, M. Rotondi, P. Mastrantonio - Catanzaro, ITALY
The HPV prevalence, namely HPV-16, is well-represented in Molise but recognizes a strict geographic distribution.

Immunotherapy with dendritic cells for gynecological neoplasias: a new therapeutic approach?
M.A. Michelin, E.F.C. Murta - Uberaba, MG (BRAZIL)
The stimulation and differentiation of dendritic cells may be a novel approach in treating gynecological neoplasies.

The influence of interval between conization and laparoscopic radical hysterectomy on the morbidity of patients with cervical cancer
H. Li, J.Y. Jang, H. Li, K. Chen, X.G. Shao - LiaoNing, CHINA
The correlation between postoperative sequelae and the interval between conization and subsequent laparoscopic radical hysterectomy and pelvic lymphadenectomy in cervical cancer patients are evaluated.

Management of ASCUS findings in Papanicolaou smears. A retrospective study
C. Iavazzo, I. Boutas, C. Grigoriadiis, N. Vrachnis, N. Salakos - Athens, GREECE
The dilemma in the management of ASCUS patients is due to the uncertain clinical significance of this diagnosis.

Prognostic factors determining recurrence in early-stage endometrial cancer
Age and clinicopathological parameters of tumors are significant predictors for recurrence in early-stage endometrial cancer.

Ten years survival of FIGO Stage IIIC epithelial ovarian cancer cases due to lymph node metastases only
E. Grossi, S. Noli, G. Scarfone, A. Villa, F. Parazzini, S. Cipriani, G. Bolis - Milan, ITALY
Women with ovarian cancer Stage IIIC due to nodal involvement have a five-year survival of about 80% and ten-year survival of about 50%.
Epidermoid or dermoid cysts of the ovary? Clinicopathological characteristics of 28 cases and a new pathologic classification of an old entity
A. Kondi-Pafiti, A. Filippidou-Giannopoulou, E. Papakonstantinou, C. Kleanthis, C. Iavazzo, C. Grigoriadis - Athens, GREECE
Clinicopathological study of 28 cases of epidermoid ovarian cysts with literature review.

A retrospective analysis of endometrial carcinoma cases surgically treated with or without para-aortic lymph node dissection followed by adjuvant chemotherapy
Para-aortic node dissection may not improve prognosis of endometrial carcinomas in which adjuvant chemotherapy using platinum, anthracycline, and taxane is performed.

Overexpression of c-Met in cervical intraepithelial neoplasia
C. Comunoglu, K. Boynukalin, M.G. Ugur, R. Al, G. Mocan Kuzey, C. Baykal - Istanbul, TURKEY
The significance of the C-Met expression in cervical intraepithelial neoplasia was evaluated.

Analysis of epidermal growth factor receptor (EGFR) status in endometrial stromal sarcoma
EGFR immunohistochemical expression is quite common in endometrial stromal sarcoma, while concurrent genetic anomalies have not yet been established.

Correlated expression of Fas, NF-κB, and VEGF-C in infiltrating ductal carcinoma of the breast
X.L. Dai, S.L. Zhou, J. Qiu, Y.F. Liu, H. Hua - Jiangsu Province, P.R. CHINA
In infiltrating ductal breast carcinoma, Fas expression was negatively correlated with the expression of both NF-κB and VEGF-C.

Prognostic value of lymph node status and number of removed nodes in patients with squamous cell carcinoma of the vulva treated with modified radical vulvectomy and inguinal-femoral lymphadenectomy
A. Gadducci, A. Ferrero, R. Tana, M.G. Fabrini, P. Modaffari, A. Fanucchi, C. Vignati, P. Zola - Pisa, ITALY
Stage and node status are the most important prognostic variables for vulvar cancer. There is a trend favoring a better groin control following extensive lymphadenectomy.

Factors affecting response of chemotherapy in women with ovarian cancer
J. Lubin, A. Markowska, P. Knapp - Poznan, POLAND
Modulation of interleukin-8 expression may be a promising method in sensitizing cells to chemotherapy, in drug-resistant ovarian cancers.

CASE REPORTS

A case of granulosa cell tumor of the ovary detected from metastatic foci
A case of granulosa cell tumor of the ovary, where the primary focus did not appear as a mass is reported.

Micro-metastases into the uterine leiomyoma from invasive ductal breast cancer under adjuvant tamoxifen therapy: case report
A case of uterine micro-metastases in a patient “treated” with tamoxifen for breast cancer is reported.

Leiomyosarcoma after hysteroscopic myomectomy: a case report
G. Carta, P. Palermo, R. Di Ramio, V. De Lellis, A. Carta, F. Patacchiola - L'Aquila, ITALY
Uterine sarcomas are rare tumors. Hysteroscopy plays an important role in the evaluation and evolution of the disease.

Aggressive deep angiomyxoma - a case report and review of the literature
P. Kaščák, M. Zámečník - Trenčín, SLOVAK REPUBLIC
A case of a young woman with aggressive deep angiomyxoma locally infiltrative, non-metastasizing, and with risk of highly recurrence is described.
Can malignant transformation in mature cystic teratoma be preoperatively predicted?
M. Futagami, Y. Yokoyama, H. Mizukami, T. Shigeto, H. Mizunuma - Aomori, JAPAN
The authors attempted to determine whether malignant transformation of mature cystic teratoma can be preoperatively predicted.

Synchronous squamous cell carcinoma of the endometrium and endometrioid adenocarcinoma of the ovary
Synchronous squamous cell carcinoma of the endometrium and endometrioid adenocarcinoma of the ovary: a case report and review of the literature.

Aggressive angiomyxoma of the vaginal wall at the initial stage: a case report
Angiomyxoma should be considered in the differential diagnosis of vaginal polypoid lesions.

Carney complex and teratoma maturum ovarii - a case report
L. Nejkovic, V. Pazin, S. Dragojevic-Dikic - Belgrade, SERBIA
A case report of Carney complex and teratoma maturum ovarii in a 17-year-old patient treated by surgery is presented.
Emerging etiopathogenetic connections between Arg399Gln polymorphism and systemic malignancies besides their etiological role in endometrial carcinomas

S. Kapoor

Private practice, Mechanicsville, VA (USA)

I read with great interest the recent article by Samulak et al. in a recent issue of your esteemed Journal [1]. The article is thought to be highly provocative. Interestingly, over the past few years, Arg399Gln polymorphism has been demonstrated to have a significant etiologic role in the development of other systemic malignancies besides endometrial carcinomas.

A close relationship exists between the Arg399Gln polymorphism and certain gynecological cancers. For example, an increased incidence of cervical cancer has been noted in females with the Arg399Gln polymorphism [2]. Female patients with the Gln/Gln genotype are 1.7 times more likely to develop cervical cancer in comparison to those exhibiting the Arg/Arg genotype. In addition, the presence of the Arg399Gln polymorphism predisposes African and Asian females to an increased risk of developing breast carcinomas [3].

Duman et al. have recently reported that certain chronic lymphoid leukemias may result because of Arg399Gln polymorphisms [4]. Similarly, the Gln399Gln genotype almost doubles the risk of developing childhood acute lymphoblastic leukemia especially in female populations [5]. In addition, the Arg399Gln polymorphism exhibits synergistic activity and increases the risk of developing childhood acute lymphoblastic leukemia by nearly 3.7 times when associated with the CYP2E1*1B polymorphism.

A similar etiopathogenetic role exists between gastric carcinomas and the Arg399Gln polymorphism [6]. Similarly, the Arg399Gln polymorphism significantly influences the prognosis in lung carcinoma patients [7]. Interestingly, in Asian populations, the presence of the Arg399Gln polymorphism confers an increased risk of developing prostatic malignancies [8]. The risk is increased almost by 43% in Asian men demonstrating developing prostatic malignancies [8]. The risk is increased almost by 43% in Asian men demonstrating developing prostatic malignancies [8].

The editors in chief were not able to obtain a comment to the letter from Dr. Samulek et al. which ever is appropriate.

Revised manuscript accepted for publication September 12, 2012

References


Address reprint requests to:
S. KAPOOR, M.D.
7487 Sherwood Crossing place # 302
Mechanicsville, VA 23111 (USA)
e-mail: shailendrakapoor@yahoo.com
Brain metastases from cervical carcinoma:
overview of pertinent literature

E. Piura¹, B. Piura²

¹Department of Obstetrics and Gynecology, Sapir Medical Center, Kfar-Saba, Sackler School of Medicine, University of Tel-Aviv
²Unit of Gynecologic Oncology, Department of Obstetrics and Gynecology, Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva (Israel)

Summary

Brain metastasis from cervical carcinoma is rare with only about 100 cases documented in the literature and an incidence among cervical carcinoma patients of 0.6%. The median interval between diagnosis of cervical carcinoma and brain metastases is 18 months. The brain can be the only site of distant metastasis of cervical carcinoma (“isolated brain metastases”) (46.8%) or brain metastasis can be part of a disseminated cervical carcinoma involving also other sites of the body (53.2%). Brain metastasis of cervical carcinoma affects most often the cerebrum (73%) and can be either single (one metastasis) (50.6%) or multiple (≥ two metastases) (49.4%). Treatment of brain metastases has evolved over the years from whole brain radiotherapy (WBRT) alone to multimodal therapy including surgical resection (craniotomy) or stereotactic radiosurgery (SRS) followed by WBRT ± chemotherapy. The median overall survival after diagnosis of brain metastases is four months; however, a better survival is achieved with multimodal therapy (craniotomy followed by WBRT) compared to craniotomy alone or WBRT alone. The worst survival is observed in patients with no treatment. Although based on a very small number of patients, the best survival is noticed in patients having SRS either alone or in combination with other treatment modality.

Key words: Cervical carcinoma; Brain metastases; Craniotomy; Whole brain radiotherapy; Stereotactic radiosurgery.

Introduction

Cervical carcinoma is the second most common cancer in women worldwide, after breast carcinoma, with > 500,000 new cases diagnosed each year and an incidence of 15/100,000 women/year [1, 2]. Cervical carcinoma may spread by (1) direct extension to surrounding tissues and organs: parametria, vagina, urinary bladder, and rectum; (2) lymphatic drainage of the uterine cervix with the pelvic lymph nodes being first involved and then the para-aortic lymph nodes being the first extra-pelvic lymph nodes involved; (3) hematogenous route with the blood circulation carrying blood-borne cervical tumor cells from the uterine cervix to distant sites [3]. The most common sites of distant metastasis of cervical carcinoma are the lung, bone, and liver and it has been estimated that 15% of cervical carcinoma patients develop distant metastasis during the course of their disease [4, 5]. It has been suggested that the evolution of therapy for cervical carcinoma over the years, especially the introduction of modern radiotherapy machines and techniques, has achieved better local control of the tumor in the pelvis and has allowed more patients to survive longer, which, in turn, has provided sufficient time for distant metastasis to develop and become clinically apparent [4].

The brain, along with the bone, liver and lung, is one of the most common sites of metastasis from various cancers with about 170,000 patients newly diagnosed with brain metastases each year in the USA [6-8]. Common sources of brain metastases are lung, breast, renal, and colorectal carcinoma and malignant melanoma; about 15% of patients with these cancers develop brain metastases during the course of their disease [6, 8-10]. Nevertheless, brain metastasis from female genital tract cancers, apart from choriocarcinoma, is rare, with only about 1% of patients with female genital tract malignancy developing brain metastasis during the course of their disease [11, 12]. The first report of brain metastasis of cervical carcinoma is attributed to Henriksen [13] who in 1949 reviewed 125 autopsies of cervical carcinoma patients and revealed one (0.8%) patient with brain metastases. Andrew [14] in 1953 was presumably the first to document brain metastasis in a living cervical carcinoma patient. Since then, 34 papers (single case reports and series of patients) on brain metastasis of cervical carcinoma in living patients have been published in the literature, totaling 96 patients [14-47]. This review summarizes these 34 papers and focuses on the following topics: pathway of metastatic spread from cervical carcinoma to the brain, incidence of brain metastases from cervical carcinoma, characteristics of the cervical carcinoma, interval between diagnosis of cervical carcinoma and brain metastases, characteristics of brain metastases from cervical carcinoma, treatment of brain metastases from cervical carcinoma, and survival after diagnosis of brain metastases from cervical carcinoma.
Pathway of metastatic spread from cervical carcinoma to the brain

The primary route of spread of blood-borne tumor cells from cervical carcinoma to the brain is through the cervical veins, internal iliac veins, common iliac veins, inferior vena cava, right atrium, right ventricle, pulmonary artery, lungs, pulmonary veins, left atrium, left ventricle, aorta, carotid arteries, and brain arteries into the brain parenchyma [41]. The spread of cervical carcinoma to the brain via the arterial circulation of the brain is supported by the fact that about two-thirds of patients with brain metastases from cervical carcinoma have also lung metastases and nearly one-tenth of patients with lung metastases from cervical carcinoma have also brain metastases [21]. Another possible route is from the veins of the pelvis to the paravertebral venous plexus (Batson’s plexus) into the venous sinuses of the brain and then to the brain parenchyma. It has been speculated that pressure increases in the thorax or abdomen due to coughing or straining and are presumed to induce a retrograde flow of venous blood from the pelvis into the paravertebral venous plexus [8, 14, 15, 48].

Incidence of brain metastases from cervical carcinoma

Incidence of brain metastases at autopsy of cervical carcinoma patients

Of 812 postmortem examinations performed in cervical carcinoma patients collected from reviews by Henriksen (1949) [13], de Alvarez (1953) [49], Holzaepfel and Ezell (1955) [50] and Badib et al. (1968) [51], 15 (1.85%) showed brain metastases. Interestingly, while Henriksen [13] in 1949 found one (0.8%) case of brain metastases among 125 necropsies of cervical carcinoma patients, Badib et al. [51] in 1968 found nine (3.2%) cases of brain metastases among 278 autopsies of cervical carcinoma patients. This increase in incidence of brain metastases in autopsies of cervical carcinoma patients has been postulated to be a result of better loco-regional control of the cervical carcinoma over the years resulting in prolongation of survival and thus enabling enough time for brain metastases to develop [24].

Incidence of cervical carcinoma as source of brain metastases at autopsy of patients with brain metastases

Kishi et al. [52] surveyed 101 autopsies of patients with brain metastases and found that cervical carcinoma was the source of brain metastases in four (3.9%) patients as opposed to lung carcinoma which was the source of brain metastases in 59 (58.4%) patients. In 80 autopsies of female patients with brain metastases reviewed by Graf et al. [53], cervical carcinoma was the source of the brain metastases in three (3.7%) patients as opposed to lung carcinoma, which was the source of brain metastases in 30 (37.5%) patients, breast carcinoma – 17 (21.2%), malignant melanoma – 13 (16.2%), renal or urinary bladder carcinoma – six (7.5%), thyroid carcinoma – five (6.2%), colorectal carcinoma – three (3.7%), choriocarcinoma – two (2.5%) and endometrial carcinoma – one (1.2%). Thus, cervical carcinoma represents only a small fraction of total metastatic deposits in the brain.

Incidence of brain metastases in living cervical carcinoma patients

In ten clinical reports [17, 18, 21-23, 25, 28, 34, 40, 41] in which the number of cervical carcinoma patients is available, 11,249 cervical carcinoma patients were surveyed and 65 (0.57%) of them were found to have brain metastases (Table 1). The claim that the incidence of brain metastases among cervical carcinoma patients may be increasing over the years because of prolongation of life due to better loco-regional control of the primary disease is not supported by reports published in the literature over the last 40 years, showing that the incidence of brain metastasis among living cervical carcinoma patients has remained steady under 1%.

Characteristics of the cervical carcinoma

The stage of disease at initial diagnosis of the cervical carcinoma was available in 71/96 (73.9%) patients [14-47]. Of these 71 patients, 30 (42.2%) had Stage IB (17 – IB not sub-staged, seven – IB1, six – IB2), 26 (36.6%) – Stage II (two – II not sub-staged, seven – IIA, 17 – IIB), 13 (18.3%) – Stage III (one – IIIA, 12 – IIIB), and two (2.8%) – Stage IVB. Thus, most patients (56/71, 78.8%) with brain metastases from cervical carcinoma documented in the literature had Stage IB (42.2%) or Stage II (36.6%) disease at initial diagnosis of the cervical carcinoma.

Histologic grade of the cervical carcinoma was available in 56/96 (58.3%) patients [14-47]. Of these 56 patients, two (3.6%) had grade 1 (G1), nine (16.1%) – grade 2 (G2), and 45 (80.3%) – grade 3 (G3). Hence, the vast majority of patients (80%) with brain metastases from cervical carcinoma documented in the literature had poorly-differentiated (G3) tumor at initial diagnosis of the cervical carcinoma.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study period</th>
<th>Number of cervical carcinoma patients</th>
<th>Number of patients with brain metastases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peeples et al. [17]</td>
<td>NR</td>
<td>644</td>
<td>2</td>
<td>0.31</td>
</tr>
<tr>
<td>van Nagell et al. [18]</td>
<td>1964-1976</td>
<td>526</td>
<td>4</td>
<td>0.76</td>
</tr>
<tr>
<td>Saphner et al. [21]</td>
<td>1972-1986</td>
<td>1,219</td>
<td>6</td>
<td>0.49</td>
</tr>
<tr>
<td>Kumar et al. [22]</td>
<td>1988-1989</td>
<td>481</td>
<td>2</td>
<td>0.41</td>
</tr>
<tr>
<td>Cormio et al. [23]</td>
<td>1982-1994</td>
<td>1,184</td>
<td>14</td>
<td>1.18</td>
</tr>
<tr>
<td>Ikeda et al. [25]</td>
<td>1974-1994</td>
<td>1,961</td>
<td>8</td>
<td>0.40</td>
</tr>
<tr>
<td>Mahmoud-Ahmed et al. [28]</td>
<td>1982-1999</td>
<td>1,279</td>
<td>6</td>
<td>0.46</td>
</tr>
<tr>
<td>Agrawal et al. [34]</td>
<td>2001-2003</td>
<td>674</td>
<td>4</td>
<td>0.59</td>
</tr>
<tr>
<td>Chura et al. [40]</td>
<td>1995-2006</td>
<td>1,565</td>
<td>12</td>
<td>0.76</td>
</tr>
<tr>
<td>Ogawa et al. [41]</td>
<td>1985-2006</td>
<td>1,716</td>
<td>7</td>
<td>0.40</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>11,249</td>
<td>65</td>
<td>0.57</td>
</tr>
</tbody>
</table>

NR: not recorded.
Histologic type of the cervical carcinoma was available in 83/96 (86.4%) patients [14-47]. Of these 83 patients, 54 (65.1%) had squamous cell carcinoma, 17 (20.5%) - adenocarcinoma, seven (8.4%) - adenosquamous carcinoma, three (3.6%) - neuroendocrine carcinoma, one (1.2%) - clear cell carcinoma, and one (1.2%) - undifferentiated carcinoma. Thus, the most common histologic type of the cervical carcinoma in patients with brain metastases from cervical carcinoma documented in the literature was squamous cell carcinoma.

Data regarding primary and adjuvant therapy of the cervical carcinoma was available in 74/96 (77.1%) patients [14-47]. Noteworthy is the accrual of these patients occurring over prolonged periods of time during which radiotherapy methods for cervical carcinoma evolved considerably and, in more recent years, radiotherapy for cervical carcinoma (either as primary therapy or as adjuvant therapy) has been given concomitantly with chemotherapy (chemoradiotherapy). Primary therapy in these 74 patients consisted in pelvic radiotherapy - 39 (52.7%) patients (radiotherapy - 23, chemoradiotherapy - 16), radical hysterectomy - 30 (40.5%), simple hysterectomy - one (1.4%), chemotherapy - one (1.4%), and no treatment - three (4%). Adjuvant therapy in these 74 patients consisted in pelvic radiotherapy - 19 (25.7%) patients (radiotherapy - 16, radical hysterectomy and radiotherapy - one, chemoradiotherapy - two), chemotherapy - eight (10.8%), simple hysterectomy - two (2.7%), radical hysterectomy - one (1.4%), and no adjuvant therapy - 44 (59.4%). Thus, in the vast majority of the patients (69/74, 93.2%), primary therapy for the cervical carcinoma consisted in either pelvic radiotherapy or radical hysterectomy. Adjuvant therapy was given to 30/74 (40.5%) patients; in most of the patients, it was pelvic radiotherapy. The surgical technique of radical hysterectomy was compatible generally with a Class III extended hysterectomy as described by Piver et al. [54]. Pelvic lymphadenectomy consisted generally in the removal of lymphatic tissue around the common, external, and internal iliac vessels and anterior to the obturator nerve. Pelvic radiotherapy consisted generally of external megavoltage photonic irradiation employing a high mega electron volt (e.g., 10 MeV) linear accelerator delivering generally 5,000 cGy to the whole pelvis in daily fractions of 200 cGy via an AP-PA opposed fields or four-field box technique. External pelvic radiotherapy was followed generally by brachytherapy using either a Fletcher-Suite applicator (comprised of a uterine tandem and vaginal ovoids) when pelvic radiotherapy was given as primary therapy, or a vaginal cylinder (Delclos) when pelvic radiotherapy was given as adjuvant therapy after radical hysterectomy. Brachytherapy was administered generally by two to three applications usually using Cesium-137 (each application usually 2,000 cGy). Since 1995, in many patients, external pelvic radiotherapy was given concomitantly with intravenous chemotherapy (chemoradiotherapy). The intravenous chemotherapy was composed generally of weekly cisplatin 40 mg/m².

Interval between diagnosis of cervical carcinoma and brain metastases

The interval between diagnosis of cervical carcinoma and brain metastases was available in 81/96 (84.4%) patients [14-47]. It ranged from – 0.25 months to 105 months with a median of 18 months. In 78/81 (96.3%) patients, brain metastasis was diagnosed after diagnosis of cervical carcinoma (range, one week – 8.75 years; median, 18 months). Brain metastasis was diagnosed one week before the diagnosis of cervical carcinoma in 1/81 (1.2%) patients [37] and simultaneously with the diagnosis of cervical carcinoma in 2/81 (2.5%) patients [16, 28]. The relatively wide interval (median, 1.5 years) between the diagnosis of cervical carcinoma and brain metastases strengthens the assumption that prolongation of life due to control of loco-regional disease by radical hysterectomy and/or pelvic radiotherapy (± chemotherapy) provides sufficient time for brain metastasis to develop and become apparent. Age at diagnosis of brain metastases was available in 77/96 (80.2%) patients and ranged from 26 to 73 years (median, 48 years; mean, 48.3 years) [14-47].

Characteristics of brain metastases from cervical carcinoma

Type of brain metastases from cervical carcinoma with respect to whether the metastasis is confined to the brain only (“isolated brain metastases”) or is part of a disseminated disease affecting also other parts of the body was available in 79/96 (82.3%) patients [14-47]. Brain metastasis was an isolated disease confined to the brain in 37/79 (46.8%) patients, whereas brain metastasis was part of a disseminated disease in 42/79 (53.2%) patients.

The amount of brain metastases originating from cervical carcinoma with respect to whether the metastasis was single (“solitary”) brain metastases or multiple brain metastases was available in 79/96 (82.3%) patients [14-47]. Brain metastasis was a single brain metastasis (one metastasis) in 40/79 (50.6%) patients, whereas brain metastasis was multiple brain metastases (two or more metastases) in 39/79 (49.4%) patients.

Site of metastasis in the brain with respect to whether the metastasis is supratentorial (cerebrum) or infratentorial (cerebellum) or both was available in 74/96 (77.1%) patients [14-47]. Brain metastasis was located in the cerebrum in 54/74 (73%) patients, cerebellum in 9/74 (12.2%) patients, and both cerebrum and cerebellum in 11/74 (14.8%) patients.

Thus, brain metastasis from cervical carcinoma is part of a disseminated disease in ~50% of the patients, single in ~50% of the patients, and supratentorial in ~70% of the patients.

Symptoms and signs of brain metastases from cervical carcinoma

Symptoms and signs of brain metastases from cervical carcinoma are not different from symptoms and signs of other space-occupying lesions of the brain. In all 14
patients reported by Cormio et al. [23], the emergence of neurologic symptoms and signs i.e., motor weakness, headache, balance disturbance, seizures, visual disturbance, and confusion led to the diagnosis of brain metastases. In a series of eight patients reported by Ikeda et al. [25], nausea and vomiting due to increased intracranial pressure developed in four patients, headache was present in three patients, convulsion appeared in two patients, and hemiplegia emerged in two patients (evidently some patients had more than one symptom). All six patients reported by Mahmoud-Ahmed et al. [28] developed neurologic symptoms that led to the diagnosis of brain metastases, with headache being the most common symptom. All 12 patients documented by Chura et al. [40] had apparent neurologic symptoms that prompted imaging studies of the brain (computed tomography CT and/or magnetic resonance imaging MRI) leading to the diagnosis of brain metastases. These symptoms included headache – six patients (50%), nausea and vomiting – three (25%), confusion – three (25%), paralysis – two (16.6%) and seizures – two (16.6%) (evidently, some patients had more than one symptom) [40]. In the vast majority of the cases of brain metastases originating from cervical carcinoma reported in the literature, it was the emergence of one or more neurologic symptoms and signs that provoked a search for brain metastases with use of brain imaging studies, most often CT and less often MRI or both CT and MRI. In many cases, the lesion demonstrated in CT and MRI was associated with brain edema. Increased intracranial pressure caused by brain edema associated with the growth of the metastases in the brain parenchyma is the main reason for headache, nausea, vomiting, and development of papilledema in the fundus of the eye.

**Treatment of brain metastases from cervical carcinoma**

Because of the rarity of brain metastases from cervical carcinoma, the accrual of patients occurred over prolonged periods of time during which treatment approaches and modalities changed. Data with respect to treatment modality of brain metastases was available in 81/96 (84.4%) patients [14-47]. Of these 81 patients, 32 (39.5%) had whole brain radiotherapy (WBRT) alone, seven (8.6%) – WBRT and chemotherapy, one (1.2%) – WBRT and stereotactic radiosurgery (SRS), five (6.2%) – surgery (craniotomy) alone, 16 (19.8%) – surgery and WBRT, one (1.2%) – surgery and SRS, two (2.5%) – SRS alone, and 17 (21%) – no treatment (mostly, steroids only). Thus, overall, 56 (69.1%) patients had WBRT, 22 (27.2%) – surgery, seven (8.6%) – chemotherapy, four (4.9%) – SRS, and 17 (21%) – no treatment (evidently, some patients had more than one treatment modality). Thus, the most common unimodal therapy was WBRT alone and the most common multimodal therapy was surgery followed by WBRT.

Traditionally, patients with isolated (limited to the brain only) and single (solitary) brain metastases would generally undergo resection of the brain lesion by craniotomy followed by WBRT. Patients with multiple brain metastases (with or without extracranial disease) generally would be given WBRT alone (± chemotherapy). WBRT consisted generally in external megavoltage photonic irradiation employing a high mega electron volt (e.g., 10 MeV) linear accelerator delivering 3,000 cGy in ten fractions over two weeks or 4,000 cGy in 20 fractions over four weeks to the whole brain area [25]. Chemotherapy for brain metastases from cervical carcinoma included topotecan, etoposide, taxotere, cisplatin, and ifosfamide [40].

In case reports or series of patients published in literature before 2001, SRS or gamma-knife radiosurgery (GKRS) was not yet included in the treatment of brain metastases from cervical carcinoma. Mahmoud-Ahmed et al. [28] described in 2001 two patients with brain metastases from cervical carcinoma in which therapy for brain metastases included SRS. One patient was diagnosed with isolated multiple brain metastases one week after diagnosis of Stage IB cervical adenocarcinoma. She had pelvic radiotherapy for the cervical carcinoma and SRS for the brain metastases and survived for 22.5 months. Another patient was diagnosed with isolated multiple brain metastases 18.5 months after pelvic radiotherapy for Stage IIB cervical squamous cell carcinoma. She had WBRT and SRS for the brain metastases and survived seven months after diagnosis of brain metastases [28]. Brown et al. [38] described in 2007 a patient that was diagnosed with isolated single cerebral metastases two weeks after diagnosis of Stage IIB2 cervical adenocarcinoma. She had radical hysterectomy for the cervical carcinoma and craniotomy + SRS for the brain metastases and remained alive with disease at the end of five-month follow-up [38]. Ulu et al. [42] described in 2009 a patient who developed isolated single cerebral metastases 24 months after pelvic radiotherapy and chemotherapy for Stage IIA cervical clear cell carcinoma. The cerebral metastatic lesion was operated with SRS and totally excised; nevertheless, there are no details of follow-up [42]. Although the experience of using SRS in the treatment of brain metastases from cervical carcinoma is very limited, it seems that there is an advantage for use of SRS in treating patients who are unable to tolerate craniotomy and for those with surgically inaccessible lesions.

**Survival after diagnosis of brain metastases originating from cervical carcinoma**

Follow-up after diagnosis of brain metastases of the 96 patients documented in the literature ranged from 0.1 to 72 months [14-47]. Data with respect to patient status (alive without disease or alive with disease or dead) at the end of follow-up was available for 87/96 (90.6%) patients. Of these 87 patients, seven (8%) were alive without disease at the end of follow-up ranging from two to 72 months, ten (11.5%) were alive with disease at the end of follow-up ranging from one to seven months, and 70 (80.5%) died of disease from 0.1 to 28.4 months (median,
four months) after diagnosis of brain metastases. Thus, the survival overall after diagnosis of brain metastases of patients with brain metastases from cervical carcinoma ranged from 0.1 to 72 months with a median of four months.

The survival of patients in relation to mode of therapy of brain metastases was assessed in series of more than four patients [21, 23, 25, 28, 40]. All six patients reported by Saphner et al. [21] had WBRT alone for brain metastases from cervical carcinoma and survived two to nine months (median, four months) after diagnosis of brain metastases. Of 14 patients with brain metastases from cervical carcinoma reported by Cormio et al. [23], 11 had steroid treatment only and succumbed within four months after diagnosis due to brain metastases, whereas three patients had WBRT for their brain metastases and survived two, six, and 21 months, respectively, after diagnosis of brain metastases. Of eight patients with brain metastases originating from cervical carcinoma reported by Ikeda et al. [25], three patients had multimodal therapy comprised of craniotomy followed by WBRT and survived 4.1, 7.5, and 10.3 months (median, 7.5 months), respectively, after diagnosis of brain metastases, whereas five patients had WBRT alone and survived 1.8, 1.9, 2, 12.3, and 22.6 months (median, two months), respectively, after diagnosis of brain metastases. Of six patients with brain metastases originating from cervical carcinoma reported by Mahmoud-Ahmed et al. [28], three had WBRT alone and survived 0.5, 7.25, and 8.25 months, one had craniotomy followed by WBRT and survived 10.5 months, one had SRS alone and survived 22.5 months, and one had WBRT + SRS and survived seven months. Although based on a small number of patients, the authors [28] concluded that extended survival may be achieved with more aggressive treatment such as surgery or SRS. Of 12 patients with brain metastases from cervical carcinoma reported by Chura et al. [40], four had WBRT alone and survived 0.3, 0.6, 1.1, and 1.5 months (median, 0.9 months), four had WBRT and chemotherapy and survived 3, 3.9, 4.4, and 7.9 months (median, 4.1 months), one had craniotomy followed by WBRT and chemotherapy and survived 6.2 months, and three had no treatment and survived 0.4, 0.5, and 3.3 months (median, 0.5 months). Since the median survival in patients who had WBRT followed by chemotherapy was significantly higher than in patients who had WBRT not followed by chemotherapy (4.4 months vs 0.9 months, p = 0.016), the authors [40] concluded that although the survival after diagnosis of brain metastasis is poor, the administration of chemotherapy after WBRT may improve the survival.

The overall survival after diagnosis of brain metastases according to mode of therapy of brain metastases in the 96 patients with brain metastases from cervical carcinoma documented in the literature was as follows [14-47] (Table 2): craniotomy followed by WBRT (± chemotherapy) – one to 72 months (median, 7.1 months), WBRT (± chemotherapy) alone – 0.1 to 22.6 months (median, three months), craniotomy alone – one to seven months (median, four months), SRS (either alone or combined with other treatment modality) – five to 22.5 months (median, 13.7 months), and no treatment – 0.25 to 3.3 months (median, 0.6 months). To sum up, the survival of patients having multimodal therapy for their brain metastases (craniotomy followed by WBRT) was considerably better than that of patients having WBRT alone or craniotomy alone. The worst survival was observed in patients having no treatment for their brain metastases. Although based on a very small number of patients, the best survival was noticed in patients having SRS either alone or in combination with other treatment modality.

### Conclusion

Brain metastases originating from cervical carcinoma are rare with approximately 100 cases documented in the literature and an incidence in living cervical carcinoma patients ranging from 0.31% to 1.18% (mean, 0.57%; median, 0.48%). The cervical carcinoma was most often Stage IB (42.2%) or Stage II (36.6%) at initial diagnosis, poorly differentiated (80.3%), and squamous cell carcinoma (65.1%). In most patients (93.2%), initial therapy for the cervical carcinoma was either pelvic radiotherapy or radical hysterectomy. Adjuvant therapy was given to 40.5% of the patients; mostly, pelvic radiotherapy. In the vast majority of the patients (96.3%), brain metastasis was detected after diagnosis of cervical carcinoma (“metachronous metastases”) with an interval between diagnosis of cervical carcinoma and brain metastases ranging from one week to 8.75 years (median, 18 months). Brain metastasis was diagnosed simultaneously with cervical carcinoma (“synchronous metastases”) in two (2.5%) patients and one week before diagnosis of cervical carcinoma in one (1.2%) patient. Thus, although brain metastasis originating from cervical carcinoma is usually considered a late event in the course of the primary disease, poorly differentiated cervical carcinomas with lymph-vascular space invasion may metastasize early in the course of the disease, even before clinical symptoms of the primary tumor become apparent. Brain metastasis originating from cervical carcinoma is either an isolated disease limited to the brain only (46.8%) or part of a disseminated disease (53.2%). Brain metastasis from cervical carcinoma is located most often in the cerebrum (73%) and is either single (50.6%) or multiple.
(49.4%) metastases in the brain. Treatment of brain metastases has evolved over the years from WBRT alone to multimodal therapy including surgical resection or SRS followed by WBRT ± chemotherapy. The median survival of all patients after diagnosis of brain metastases from cervical carcinoma was four months; nevertheless, a considerably better survival was observed in patients having multimodal therapy including craniotomy followed by WBRT ± chemotherapy (median survival, 7.1 months) compared to craniotomy alone (median survival, four months) or WBRT alone (median survival, three months). The worst survival was observed in patients having no treatment (median survival, 0.6 months). Although based on a very small number of patients, the best survival was noticed in patients having SRS either alone or in combination with other treatment modality (median survival, 13.7 months). Early detection of brain metastases is indispensable since the metastases at their early stage of development in the brain are still of small volume and thus much more feasible for surgical resection or SRS with less complications and longer survival than metastases at advanced stage of their development. Thus, the emergence of one of more neurological symptoms and signs in cervical carcinoma patients should provoke an immediate search for brain metastases with use of brain imaging studies.

References


Retromesenteric para-aortic lymphadenectomy in gynecologic malignancy

C. Altgassen¹, R. Bends², K. Kelling¹, D. Hornung¹, M. Friedrich², D. Salehin², K. Diedrich¹, A. Kavallaris¹

¹Department of Obstetrics and Gynecology, UK-SH, Campus Luebeck
²Department of Obstetrics and Gynecology, Baptist-Hospital, Koeln-Lindenthal (Germany)

Summary
In gynecologic oncology lymphadenectomy is of prognostic and therapeutic importance because recurrence-free time and survival depend on the metastatic involvement of lymph nodes. Lymphadenectomies are not performed to such an extent as they are indicated. This might be due to a laborious or problematic preparation. The authors therefore report their experience in a seldom taught preparation of the left para-aortic compartment in the form of a learning curve. Materials and Methods: To access the left para-aortic area, the descending colon is lifted to open the retroperitoneum along the line of Toldt. The mesentery of the descending colon was separated from the kidney along the fascia of Gerota by blunt preparation. Time was measured from the incision of the peritoneum until the renal vein was clearly visible. Results: The authors collected the data from the first 25 preparations. Mean duration for the left para-aortic preparation was 7.8 minutes compared to 5.9 minutes for the right side. Duration of preparation of the left area dropped from 11.0 minutes within the first patients (#1 to #5) to 3.8 minutes in the last patients (#20 to #25). No complications were observed in the study group linked to the retromesenteric approach described. Conclusion: Retromesenteric para-aortic lymphadenectomy is quick to learn. The authors needed 20 preparations to observe a significant drop in the time needed for preparation. Retromesenteric para-aortic lymphadenectomy offers an excellent overview that lightens lymphadenectomy and therefore reduces the risks for patients.

Key words: Para-aortic lymphadenectomy.

Introduction
In gynecologic oncology a lymphadenectomy is of prognostic and therapeutic importance because recurrence-free time and overall survival depends on the metastatic involvement of regional lymph nodes. Lymphadenectomies are not performed to such an extent as they are indicated. In patients with cervical cancer (FIGO-Stage IB and IIA) five-year survival drops from more than 90% to less than 75% if metastatic disease is demonstrated in regional lymph nodes [1]. In endometrial cancer, the removal of regional lymph nodes has a significant impact on survival [2]. Pelvic sidewall failures as well as para-aortic failures in endometrial cancer are strongly correlated to the initial lymph node status [3]. In patients with ovarian cancer, up to 75% of the patients show lymph node metastases [4]. If a complete macroscopic tumor resection was achieved, survival is independently influenced by the lymph node status [5].

Yet, there is still controversy about the circumstances in which a lymphadenectomy should be conducted, and to what extent. This is even more valid for para-aortic lymphadenectomies. In patients with cervical cancer, para-aortic lymphadenectomy up to the inferior mesenteric artery seems to be adequate. In patients with high-risk endometrial cancer, 67% have para-aortic metastases caudally and cranially of the inferior mesenteric artery [6]. As in ovarian cancer, para-aortic lymphadenectomy should be extended up to the renal vessels. Yet in the USA only 54% of the centers perform lymphadenectomy in patients with endometrial cancer [2, 7]. This might be due to the workload or expertise of the surgeons.

In a classic transperitoneal approach, the preparation and the presentation of the para-aortic area can be laborious or problematic. This might lead to an insufficient presentation that can place the patient at risk due to intraoperative complications such as laceration of the large vessels. Furthermore it can be a reason for not conducting an indicated para-aortic lymphadenectomy or for an incomplete lymphadenectomy. The authors therefore asked whether there might be an easier approach to the para-aortic area.

This study describes a retromesenteric preparation of the para-aortic area. The method is known to experts in gynecologic oncology. The reason why it has not spread within the community of gynecologic surgeons who also treat the diseases remains unanswered. One answer might be the fact that there is only one publication addressing this issue. Originally the authors intended to describe the development of a new technique and evaluated it in a prospective manner. They now describe the learning curve of a simple, safe, and easy access to the retromesenteric para-aortic areas.

Materials and Methods
This prospective study was approved by the institutional review committee (07-209). Between October 2007 and July 2008 all patients that were referred to this institution for para-aortic lymphadenectomy were considered for enrollment. They
were included after signing informed consent and if surgery was performed by one of the authors (C.A.). To take account of a possible learning curve, the authors measured the time needed for preparation of the para-aortic area in the first five patients (#1 to #5) and compared it with the last five patients (#21 to #25). Measurement commenced when the peritoneum was incised and ended when the renal vein was clearly visible and visibility had to be reconfirmed by independent members of staff.

Surgical technique

The day prior to surgery all patients received a bowel preparation. All patients were placed supine. The abdominal cavity was opened through a midline incision beginning at the pubic mound and leading around the umbilicus up to the middle between the navel and the xiphoid. In patients with cervical cancer, surgery began with para-aortic lymphadenectomy, as per the authors’ guidelines. In patients with ovarian cancer, para-aortic lymphadenectomy was the last surgical step if no residual disease remained. In patients with endometrial cancer, para-aortic lymphadenectomy was begun after a frozen section confirmed the indication for lymphadenectomy.

Preparation of the left para-aortic area

The descending colon was gently lifted to expose the paracolic gutter. Then the peritoneum was incised along the paracolic gutter from the pelvic rim, where the ureter crosses the common iliac artery, up to the splenic flexure close to the descending colon. The white line of Toldt marked the entrance precisely. Once a small space was developed, the mesentery was easily mobilized by gently wiping off the surrounding tissue which was in fact the left fatty-lymphatic tissue in front of the anterior leave of the fascia of Gerota. No sharp preparation was needed, but it was worth taking time at this step of the preparation. In the majority of preparations the ovarian vessels are attached to the mesentery. The authors realized that it was easier to develop the para-aortic area if these vessels were isolated from the mesentery right at the beginning. This method of preparation automatically leads to the correct space being developed. Before this observation, the authors easily developed the space behind the kidney and could not find the renal vessels, which led to a prolonged preparation. If the renal vessels cannot be identified, it is helpful to fully mobilize the splenic flexure, which is easily done. After presentation of the renal vein, the area of para-aortic lymphadenectomy is clearly delineated. The upper hypogastric plexus can be identified attached to the mesentery. All the fatty-lymphatic tissue between the aorta, the renal vein, and the ureter can be removed. The ovarian vessels can be easily dissected at their origin (Figures 1 and 2).

Preparation of the right para-aortic area

The preparation of the right para-aortic area was in accordance with the traditional approach. The peritoneum overlying the right common iliac artery was incised to identify the ureter and the ovarian vessels. The peritoneal incision was extended along the white line of Toldt along the right paracolic gutter up to the right colon flexure. Additionally, the peritoneum was incised towards the ligament of Treitz. The ascending colon, the coecum, the duodenum, and the small intestine can now be incised towards the ligament of Treitz. The ascending colon, the right colon, and the small intestine can now be mobilized by gently wiping off the surrounding tissue which was in fact the right fatty-lymphatic tissue between the vena cava, the renal vein, and the ureter can be exposed. By moving the descending colon to one side, as turning a page, one gains clear access to the contralateral side for lymphadenectomy.

Lymphadenectomy

The inferior mesenteric artery is neither under tension nor at risk of injury, because the preparation of the left para-aortic area is not usually done from the right side underneath the inferior mesenteric artery. At the end of lymphadenectomy, homeostasis was secured by bipolar coagulation. The bowel was then repositioned. One drain is placed in the right or left para-aortic area.

Statistics

Our primary objective was the description of the technique. The secondary objective was the prospective evaluation of a learning curve by comparing the time necessary for the preparation of the para-aortic area within the first five (#1 to #5) and the last five patients (#21 to #25). Time was measured from the incision of the peritoneal layer until clear visibility of the origin of the renal veins, and confirmed by two independent members of the stuff. Data was collected and managed within an Access database (Microsoft, Office 2007, Redmond, WA, USA). Explorative data description was calculated with Excess (Microsoft, Office 2007, Redmond, WA, USA). Comparison of the duration of the preparation was achieved by a one-sided Welch t-test. Data was analyzed with Software R (version 2.7.0; www.r-project.org).
Results

Data from 51 consecutive patients was collected between October 2007 and July 2008. Mean age was 57.8 ± 13.4 years (range 20 – 79). Mean body mass index (BMI) was 26.4 ± 6.7 kg/m² (range 17 – 46). Para-aortic lymphadenectomy was performed in 15 patients with cervical cancer (29%), in 19 patients with uterine cancer (37%), and in 14 patients with ovarian cancer (28%), in one patient with cancer of the coecum (2%), in one patient with primary cancer of the peritoneum (2%), and in one patient with ovarian and uterine cancer (2%).

Twenty-six patients were excluded from the evaluation of the learning curve (Figure 3). Two patients underwent laparoscopic para-aortic lymphadenectomy. In one patient with ovarian and uterine cancer, the uterus reached the navel. This patient was not considered an ideal candidate for this study because keeping the uterus out of the field of preparation was laborious and did not represent the average situs. Surgical strategy had to be changed in two patients: in one patient a suspected ovarian cancer turned out to be a primary colon cancer and in one patient, the authors had to deal with a primary cancer of the peritoneum. Twenty-one patients underwent classical transperitoneal para-aortic lymphadenectomy by other members of this department (Group R).

Patients undergoing retromesenteric para-aortic lymphadenectomy were consecutively grouped. Group A comprised the first five patients (#1 to #5) Group B comprised of patients #6 to #20. Group C comprises the last five patients (#21 to #25). Characteristics of these patients did not differ among these groups and from Group R with regard to age, BMI, and indication for para-aortic lymphadenectomy (Table 1).

Mean duration for the left para-aortic preparation was 11.0 ± 3.4 min (range 7 – 15) in the first five patients and dropped significantly to 3.8 ± 0.8 min (range 3 – 5) in the last five patients (p = 0.008). Mean duration for the right para-aortic preparation was 7.4 ± 1.7 min (range 6 – 10) in the first five patients and dropped significantly to 4.0 ± 0.7 min (range 3 – 5) in the last five patients (p = 0.007). (Figure 4 and Table 1).

Mean number of para-aortic lymph nodes resected was 12.0 ± 6.4 (range 2 – 29) for the overall population. Lymph node yield did not differ between group A and group C, but the lymph node yield in the study group (groups A, B, and C) was 13.8 ± 5.5 (range 5 – 27) compared to 9.9 ± 6.9 (range 2 – 29) in group R (p = 0.05) (Table 1).

One patient suffered from severe pulmonal embolism and cardiac arrest on the third day after surgery (group B, #6). Due to the severity of embolism, she underwent thrombolytic therapy after cardio-pulmonary reanimation and developed a retroperitoneal hematoma that had to be cleared twice. She left the hospital after 50 days of rehabilitation. Six months after this event, signs of cerebral hypoxia persisted (gait distribution, aphasia), but the patient was able to manage her daily life. No direct side-effects were related to the retromesenteric approach described.

Discussion

The present study demonstrates that the retromesenteric approach to the para-aortic area can easily be learned within the first 20 operations. It offers an easy and safe access to the left para-aortic area that facilitates para-aortic lymphadenectomy and resection of the renal vessels if indicated without placing structures such as the inferior mesenteric artery and the hypogastric nerves at risk.

Yet the authors’ preparation is not totally unknown. Initial mobilization of the descending colon and the left colic flexure is done routinely in general surgery after resection of the sigmoid to restore intestinal continuity. Only one report describes the type of retromesenteric preparation presented in this study for para-aortic lymphadenectomy in gynecological malignancies [8]. Authors emphasize that several steps in the para-aortic lymphadenectomy become easier and complications can be readily handled - experiences that the authors fully support. Mean duration of the left para-aortic node dis-
section was 35 minutes and 70 minutes for the entire para-aortic lymphadenectomy. The median number of aortic nodes removed was 29 lymph nodes. In a later report of the same group, the initial preparation seems to be identical but the present authors would have been delighted if other authors had more clearly emphasized whether they still use their approach to the para-aortic area that is not common in the field of gynecology [9].

Traditionally, para-aortic lymphadenectomy is done transabdominally either via an open approach [1, 10] or endoscopically [11, 12]. For a right para-aortic lymphadenectomy, the coecum and the caudal part of the ascending colon are mobilized via an oblique incision heading toward the ligament of Treitz [13, 14]. The ascending colon and the small bowel are lateralized to gain sufficient access to the right para-aortic area. Usually the left para-aortic area is cleared from the right side by traversing the aorta below the inferior mesenteric artery. Laparoscopically, the left para-aortic area is also cleared underneath the inferior mesenteric artery [15]. In experienced hands, the right-sided laparoscopic para-aortic lymphadenectomy lasts 36 min and the left sided para-aortic lymphadenectomy lasts 64 min [16]. The difference between the two sides might be due to the differing anatomies. Although the traditional approach to the left para-aortic area is safe, the preparation and presentation appear to be strenuous because traction and retractors are necessary to gain enough access without compromising the supply in the inferior mesenteric artery. To avoid post-operative adhesion formation, some groups perform a retroperitoneal approach that can be achieved via an open access [17] or endoscopically [18-21]. Again anatomy might be the reason for a limited para-aortic lymphadenectomy, especially for the contralateral side.

To assess an amelioration of technical skills, one has to define hallmarks to measure any effect. This can be the number of complications [16], the number of resected lymph nodes [22], the duration of the procedure [23], or an increasing radicality [24, 25].

When the authors designed this small series, they were confident that it would not take too many patients to make a difference. They also had the experience that the lymph node count depends on several aspects, including the pathologist’s motivation [23]. Additionally, the whole surgical procedure of a complete para-aortic lymphadenectomy depends on several co-factors. Therefore the authors decided to focus on the main step which was the preparation of the area of interest. From their point of view, the difference in the duration of the preparation of the two para-aortic areas will be due to the position of the

Figure 3. — Patients’ flow chart.
sigmoid with its blood supply and the easier mobilization of the ascending colon and the mesentery of the small bowel. The fact that the preparation of the right para-aortic side consists of known steps is the reason why the initial time for preparation was quicker, yet the authors could observe a significant improvement in the time needed for presenting the target area; the preparation of the left side, on the other hand, was completely new to them. Fortunately they observed no direct complication associated with this retromesenteric approach. Yet this series was large enough to draw attention to the increased wound surface that could lead to secondary hemorrhage. Larger populations would be necessary to detect possible differences like complication rate compared with the traditional transperitoneal approach.

The present findings and conclusions would no doubt be corroborated and enhanced by observing several different surgeons consecutively, but it is debatable whether a repeat of the same learning pattern in each surgeon would be witnessed, or (more likely) the handing over from one surgeon to the next of the experience gained in these first patients.

Though the present authors are not the first team to describe this technique, they can show that it is quick to learn. The essential steps are in entering the retromesenteric area along the line of Toldt to isolate the ovarian vessels from the posterior aspect of the mesentery, so that the retromesenteric space can be opened by blunt preparation by letting the kidney fall into its bed. The authors suggest naming this procedure a “retromesenteric” access to the left para-aortic area. The presentation of the left para-aortic area is easy and enables the surgical team to perform a suitable lymphadenectomy without causing strain or risk for the patient. Left and right para-aortic basins can be easily surveyed by maneuvering the mesentery - like turning over a page.

The authors hope readers are motivated to try this approach, so that they will become convinced of the ease of this preparation, just as they were overwhelmed when they started it.

References

Address reprint requests to:
C. ALTGASSEN, M.D.
Department of Obstetrics and Gynecology
University of Schleswig-Holstein
Campus Luebeck
Ratzeburger Allee 160
23538 Luebeck (Germany)
e-mail:christopher.altgassen@hohenlind.de

578
Prognosis of high-grade endometrial cancer: a comparison of serous-type and clear cell type to grade 3 endometrioid-type


Department of Obstetrics and Gynecology, National Defense Medical College, Tokorozawa, Saitama (Japan)

Summary

Objective: To evaluate prognosis of high-grade endometrial cancers, comparing serous (SC) and clear cell (CCC) types to grade 3 endometrioid carcinoma (ECG3). Methods: Among patients with endometrial cancer treated in two decades, medical records of patients with high-grade endometrial cancer were retrospectively investigated. Results: Of 447 endometrial cancers, 107 (24%) high-grade endometrial cancers were identified, with the increasing incidence in the last decade (28% vs 19%; p = 0.026). There were 24 SC, 14 CCC and 69 ECG3. Median age was 62, 68, and 61 years, respectively, with the CCC type showing an elder age than the ECG3 type (p = 0.012). The rates of patients with Stage IIIc-IV, lymph node assessment or complete resection at primary surgery, and post-operative chemotherapy were not significantly different; however, response rate to first-line chemotherapy in patients with measurable disease was lower in SC than ECG3 (3 / 11, 27% vs 14 / 19, 74%; p = 0.037), regardless of regimens. Five-year overall survival (OS) was 40%, 71%, and 71% respectively, and five-year progression-free survival (PFS) was 25%, 71%, and 61%, respectively, showing SC with worse prognosis than ECG3 on both OS (p = 0.026) and PFS (p = 0.0028). According to the multivariate analysis, age ≥ 70, Stage IIIc-IV and incomplete resection were independent prognostic factors on poor OS, whereas SC, Stage IIIc-IV and incomplete resection were on poor PFS. Conclusions: The increasing trend of high-grade endometrial cancer and different outcomes according to histological subtypes, especially poor PFS and chemotherapeutic response in SC, were suggested.

Key words: High-grade endometrial cancer; Endometrioid; Serous; Clear cell; Prognosis; Chemotherapy.

Introduction

Despite less frequencies among the endometrial carcinoma, International Federation of Gynecology and Obstetrics (FIGO) grade 3 endometrioid carcinomas (ECG3), along with serous carcinomas (SC) and clear cell carcinomas (CCC), relevant to type II endometrial cancers, are considered as high-risk endometrial carcinomas [1]. Most studies on endometrial carcinoma subtypes have compared SC, together with CCC [2-4], to endometrioid carcinoma including low-grade (grade 1 - 2) and grade 3 [5, 6] due to their rarity; thus there are relatively few data on the comparison of ECG3 with SC or CCC, respectively. Grade 1 - 2 endometrial carcinomas are associated with a favorable prognosis, but the effect of histological subtypes upon prognosis of high-grade endometrial carcinomas remains rather conflicting. Some studies reported no significant differences in outcomes between SC, CCC, and ECG3 [3, 7, 8]. In contrast, other studies have shown that SC or CCC are associated with an unfavorable prognosis compared with ECG3 [9-11]. A number of factors, such as a selection bias, criteria for pathologic diagnosis, and types of therapy including the extent of surgical procedure and the type of post-operative adjuvant therapy, could have differed between these studies, partly leading to the observed differences, together with their small number of cases. Moreover, although these type II endometrial carcinomas tend to have distant spread and recurrence, there is little information regarding responses to chemotherapy, as well as effective regimens in these subtypes. Controversy still remains whether these tumors should be treated differently, and there is no consensus regarding the most effective treatment for these subtypes without prospective data available to guide clinicians. The purpose of this study was to evaluate prognosis of high-grade endometrial carcinoma patients, comparing SC or CCC type to ECG3 carcinoma, respectively, and to explore the effective treatment strategy for these subtypes.

Materials and Methods

Among all patients who underwent primary surgery for endometrial carcinoma between 1990 and 2009 at the Institute, pathologic material and medical records of patients with high-grade endometrial carcinoma, including SC, CCC, and ECG3, were reviewed. Study protocol was approved by institutional ethics committee. Tumor cell type was assessed according to World Health Organization (WHO) criteria [12] and endometrial carcinomas were graded according to the FIGO grading system. Patients unsuitable for primary surgery due to advanced stage and poor medical conditions, precluding definitive pathologic diagnosis as primary endometrial carcinoma, were excluded from further analysis. Clinico-pathologic variables assessed included: patient age, histological subtype, FIGO stage, extent of primary surgery, and type of post-operative chemotherapy. Complete resection was defined as the surgical removal of all the visible tumors including primary and
metastatic lesions regardless of the extent of lymphadenectomy. Chemotherapy after primary surgery was recommended for all high-grade endometrial carcinoma patients, except for those not suitable for chemotherapy due to their medical conditions or advanced age; however, different post-operative chemotherapies have been introduced during the study period: cyclophosphamide plus doxorubicin plus cisplatin in earlier years and later changed to taxan plus platinum, reflecting the changes in standard chemotherapy for ovarian cancers.

Overall survival (OS) was measured from the date of primary therapy to the date of death or last follow-up and PFS was measured from the date of primary therapy to the date of disease progression or last follow-up. Associations between variables were analysed using Chi-square or Fisher’s exact test. Survival curves were estimated using Kaplan-Meier method and p values were generated using the log-rank test. Cox proportional hazards regression models were also used to examine OS and PFS for quantifying the relations between survival and covariates.

Results

Of 447 endometrial carcinomas during the study period, 340 (76%) low-grade and 107 (24%) high-grade endometrial carcinomas including SC, CCC, and ECG3 were identified. As shown in Table 1, there was a significant increase in the proportion of patients with high-grade endometrial carcinomas in the second decade (19% vs 28%; p = 0.026), despite the increasing number of patients both in low- and high-grade endometrial carcinomas. Median age of all endometrial carcinoma patients was also significantly higher in the second decade (59 vs 63 years; p = 0.026). The rate of patients with FIGO Stage IIIc-IV were not significantly different in the two periods of time.

There were 24 SC, 14 CCC, and 69 ECG3. Table 2 shows the comparative analysis of the characteristics of patients with SC type or those with CCC type to those with ECG3, respectively. Median age was 62, 68, and 61 years respectively, showing CCC with significant elder age compared to ECG3 (p = 0.012). The rates of patients with Stage IIIc-IV were not significant different, although there were only 3 Stage IIIc-IV cases among 14 patients with CCC. There were no significant differences in type of treatment and outcome, such as lymph node sampling or dissection at primary surgery, incomplete resection at primary surgery, and subsequent post-operative chemotherapy; however, response rate to first-line chemotherapy in patients with measurable disease was significantly lower in SC than that in ECG3 (27% vs 74%; p = 0.037). Responses to chemotherapy were poorer in SC than ECG3, either after taxan plus platinum (33% vs 80%), as well as after cyclophosphamide plus doxorubicin plus cisplatin (0% vs 67%). There were no data for CCC in regard to the chemotherapeutic response.

Kaplan-Meier test for OS (Figure 1) and PFS (Figure 2) demonstrated univariate and multivariate analysis of possible prognostic factors on OS and PFS. According to the multivariate analysis, age ≥ 70, Stage IIIc-IV, and incomplete resection were independent prognostic factors on poor OS, whereas SC type, Stage IIIc-IV, and incomplete resection were on poor PFS.

Discussion

The number of patients with endometrial carcinomas is rising, following the increasing life expectancy, as well as the prevalence of overweight and obesity especially in developed countries, such as North America and Europe [13]. Although the increase was observed in both low and high-grade endometrial carcinomas in the present study, the rate of high-grade endometrial carcinomas, which are typical of elderly women, significantly increased in the last decade. One of possible causes was the marked exten-
Prognosis of high-grade endometrial cancer: a comparison of serous-type and clear cell type to grade 3 endometrioid-type

Significant higher median age of endometrial carcinoma patients in the last decade from this study, together with other official statistics on trends in endometrial cancer patients across Japan [16, 17], could support these hypotheses indeed. Similar findings occurred in other countries, where an increase in the proportion of patients with grade 3 and serous-type endometrial cancers was reported [18].

As for prognosis in high-grade endometrial carcinomas, a study based on the largest cohort of patients including 2,316 ECG3, 1,473 SC, and 391 CCC by Hamilton et al. showed that patients with SC or CCC had a significantly poorer prognosis than those with ECG3, even after controlling for stages [10]. In this study, CCCs did not show worse prognosis compared with ECG3; however, statistical significance was not obtained due to the small number of cases with the higher rate of elderly patients, the low rate of advanced stage, and lack of cases with measureable disease unable to evaluate response to chemotherapy. Poorer outcomes were also reported by Boruta et al., with approximately 40% of five-year survival in SC compared to 75% in ECG3, showing close outcomes to the present study [9].

The impact of maximal cytoreductive surgery on the survival rate was explored, similarly to ovarian carcinoma and improved survival in advanced endometrial carcinomas was reported in several retrospective studies with the relatively small number of cases [19-21]. Bristow et al. reported 65 Stage IVb endometrial carcinomas treated with surgery as primary therapy and showed that patients with only microscopic residual disease had significantly better survival rate compared to those with optimal macroscopic (≤ 1 cm) residual disease [19]. In accordance with their study, the present study confirmed that complete resection with no gross residual tumors is an independent predictor for improved OS and PFS in

![Figure 1](image1.png)

**Figure 1.** — Comparison of overall survival rates of the patients according to histological subtypes. Patients with serous-type had significant worse overall survival rate than those with endometrioid grade 3 (p = 0.026).

![Figure 2](image2.png)

**Figure 2.** — Comparison of progression-free survivals of the patients according to histological subtypes. Patients with serous-type had significant worse progression free survival rates than those with endometrioid grade 3 (p = 0.0028).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td>PFS</td>
<td>OS</td>
<td>PFS</td>
<td>OS</td>
</tr>
<tr>
<td>HR</td>
<td>HR</td>
<td>p value</td>
<td>HR</td>
<td>p value</td>
</tr>
<tr>
<td>HR</td>
<td>HR</td>
<td>p value</td>
<td>HR</td>
<td>p value</td>
</tr>
<tr>
<td>HR</td>
<td>HR</td>
<td>p value</td>
<td>HR</td>
<td>p value</td>
</tr>
<tr>
<td>HR</td>
<td>HR</td>
<td>p value</td>
<td>HR</td>
<td>p value</td>
</tr>
<tr>
<td>Age &lt; 70</td>
<td>1.0</td>
<td>–</td>
<td>1.0</td>
<td>–</td>
</tr>
<tr>
<td>≥ 70</td>
<td>2.3</td>
<td>1.1-4.6</td>
<td>0.022</td>
<td>2.5</td>
</tr>
<tr>
<td>FIGO Stage I-IIIa</td>
<td>1.0</td>
<td>–</td>
<td>1.0</td>
<td>–</td>
</tr>
<tr>
<td>III-B</td>
<td>14.3</td>
<td>5.0-41.1</td>
<td>&lt; 0.001</td>
<td>4.8</td>
</tr>
<tr>
<td>Histology</td>
<td>Serous</td>
<td>2.3</td>
<td>1.1-4.9</td>
<td>0.033</td>
</tr>
<tr>
<td>Clear cell</td>
<td>1.0</td>
<td>0.3-3.3</td>
<td>0.959</td>
<td>–</td>
</tr>
<tr>
<td>Endometrioid G3</td>
<td>1.0</td>
<td>–</td>
<td>1.0</td>
<td>–</td>
</tr>
<tr>
<td>Primary surgery</td>
<td>Complete</td>
<td>12.1</td>
<td>5.1-28.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Incomplete</td>
<td>1.0</td>
<td>–</td>
<td>1.0</td>
<td>–</td>
</tr>
<tr>
<td>Post-operative</td>
<td>Yes</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>chemotherapy</td>
<td>No</td>
<td>1.2</td>
<td>0.6-2.6</td>
<td>0.574</td>
</tr>
</tbody>
</table>

**Table 3.** — Univariate and multivariate analysis of prognostic significance on overall survival (OS) and progression-free survival (PFS).

HR = hazard ratio; CI = confidence interval; G3 = grade 3.
patients with high-grade endometrial carcinoma, after tumor stage, which is the most important prognostic factor recognized overall. Age was also considered a significant factor affecting disease-related outcome, as well as the treatment strategy including aggressive operative procedures and adjuvant therapies. In this study, age was actually the independent prognostic factor not on PFS but on OS; on the contrary, SC type was an independent prognostic factor not on OS but on PFS. The majority of patients with SC received post-operative chemotherapy, but their chemotherapeutic responses for measurable disease were considerably low compared to ECG3. This poor response to treatment could explain low PFS in patients with SC in addition, to the knowledge of the authors, this is the first report with direct comparison of objective chemotherapeutic responses in ECG3 and SC according to regimens.

Despite frequent metastasis, as well as high risk for recurrence in high-grade endometrial carcinomas, there is little information regarding the responses of each histological subtypes to individual chemotherapeutic regimen [6, 22-26]. One of the largest analysis using pooled data of prospective studies, reported by McMeekin et al., showed 44% and 32% of response rates for doxorubicin, either alone, or in various combinations with doxorubicin and platinum and taxan in SC and CCC, respectively [6]. In that study, analyzing 1,203 patients with Stage III-IV or recurrent endometrial cancer, no significant difference occurred in the response to chemotherapy for SC or CCC when compared to all other histological types, including all grade endometrioid types and mixed types. In their separate analysis of 622 patients with endometrioid type, patients with ECG3 had an estimated odds of response 1.47 times that of those with grade 1 endometrioid carcinoma, showing rather better response rate in ECG3 like the present study. Goldberg et al. and Price et al. reported that response rate of 37% to first-line platinum-based chemotherapy in SC [22]. Levenback et al. and Price et al. reported that 2/11 and 3/11 of response rate to chemotherapy with cisplatin, doxorubicin, and cyclophosphamide in SC, showing poor response rates similar to the present study [23, 24]. These response rates in SC are considerably lower than those reported in ovarian serous adenocarcinoma. Although Zanotti et al. and Ramondetta et al. reported better response rates of 7/11 and 10/13 to chemotherapy using paclitaxel for SC, respectively [25, 26], the present results showed rather poor chemotherapeutic responses of 3/9 with taxan plus platinum as well in SC compared to 8/10 in ECG3 patients. Different chemotherapeutic response rates observed in all these studies including the present study are probably due to the small number of patients [22-26] or various chemotherapeutic regimens [6, 22, 25].

With relatively little evidence compared to ovarian carcinoma, patients with advanced endometrial carcinoma are treated with similar chemotherapeutic regimens following cytoreductive surgery, yet showing poorer outcomes. Landrum et al. reported that OS for endometrial carcinoma patients with intraperitoneal metastasis does not approach with that of patients with advanced ovarian cancer [27]. Although SCs of endometrium is considered to share similar tumor characteristics to ovarian cancer in terms of histological appearance and tendency of intra-abdominal dissemination, differences may exist, particularly in respect to the low chemotherapeutic responses. The reported gene profile analysis in the comparison of serous or endometrial tumors across endometrial and ovarian cancers by Zorn et al. showed unique gene expression patterns reflecting their organ of origin [28]. They suggested that these tumors have important gene expression differences, which makes it less likely that they can be clinically managed in an identical fashion. It is understandable that response rates in serous-type endometrial cancer are considerably lower than those reported in ovarian serous adenocarcinoma and that seems to mainly contribute to the poorer prognosis in serous-type endometrial cancer.

In addition to considering the introduction of highly intensive regimens, there is a need to identify novel agents to improve survival. Attention has turned to drugs that target molecular pathways. Over the past years, a considerable number of studies have been made in this field. In the recent report of phase II study of temsirolimus, response was seen in patients with all grades of disease, as well as in patients with serous histology [29]. In a phase II trial of bevacizumab, targeting VEGF, clinical responses were reported in serous-type and clear cell type as well [30]. Although patient numbers are still too small to formally evaluate the role of histologic subtype and response in these studies, it seems worthy of further studies. Targeting differential molecular characteristics of high-grade endometrial carcinomas could contribute to developing effective therapeutic strategies and multi-institutional prospective trials are needed to establish individual guidelines for these relatively infrequent types of tumors. In conclusion, the increasing trend of high-grade endometrial carcinoma and different outcomes among their histological subtypes suggested the necessity to explore differential approaches including surgery and chemotherapy for the management of these tumors.

References


Prognosis of high-grade endometrial cancer: a comparison of serous-type and clear cell type to grade 3 endometrioid-type


Octreotide is the favorable alternative for cisplatin resistance reversal of ovarian cancer in vitro and in nude mice in vivo

Y. Shen, M.L. Ren, Y.H. Shi, Y.X. Zhang, Y.L. Cai

Department of Obstetrics and Gynecology, Zhongda Hospital, Southeast University, Nanjing (China)

Summary
This study aimed to observe the effects of octreotide (OCT) on cisplatin resistance reversal of cancer cells in vitro and in nude mice in vivo. MTT method and flow cytometry were used to investigate the effect of cisplatin, OCT, or the combination of these two compounds on the proliferation and apoptosis of SKOV3-DDP cells. The size and weight of xenograft tumors from the nude mice model were measured. Real-time PCR was used to detect the mRNA expression of SSTR2, MDR1, MRP2, GST-π, and EGFR in SKOV3/DDP cells following different treatment. At the concentration of 2.5-20 g/ml, OCT significantly reduced IC50 (p < 0.05) and promoted apoptosis (p < 0.05) of SKOV3-DDP cells’ response to cisplatin. Unchanged expression was found in SSTR2 on the SKOV3/DDP cell in vitro after OCT treatment, but increased expression in vivo (p < 0.05). OCT increased GST-π expression (p < 0.05) and reduced MRP2 and EGFR expression (p < 0.05) in a dose-dependent manner. The similar results were obtained in mice in vivo experiment, except the reduced expression of GST-π. It is suggested that OCT could inhibit ovarian cancer proliferation and promote apoptosis, via the cell surface SSTR2, and reverse cisplatin resistance through inhibition of MRP2, EGFR, and even GST-π expressions.

Key words: Octreotide; Somatostatin; Epithelial ovarian cancer; Resistance reversal.

Introduction
Ovarian cancer is the most common cause of cancer death from gynecologic tumors in the world. Unfortunately, most cases are diagnosed in an advanced stage. Standard treatment involves aggressive debulking surgery followed by chemotherapy [1]. Platinum-based chemotherapy enhances the overall response rate, clinical remission rate, and median survival rate of ovarian cancer patients. However, it is also an obstacle to clinical treatment for primary and/or acquired multi-drug resistant (MDR) of tumor cells [2]. Therefore, there is a need to develop alternative new types of cytotoxic and non-cytotoxic drugs that can reverse chemotherapy resistance and enhance sensitivity to platinum-based chemotherapy drugs.

In a variety of non-cytotoxic agents, the somatostatin analogs (SSTA) have attached more attention in oncology community. It has been reported the somatostatin receptor (SSTR) is expressed in ovarian cancer cells [3,4], suggesting that SSTA could be involved in ovarian cancer. Recent studies have shown that SST and SSTA can enhance chemotherapeutic drug sensitivity in a variety of resistant tumor cells [5]. However, it is still unclear what the function of SST and SSTA are in the enhancement of cisplatin sensitivity to resistant ovarian cancer cells.

This study focuses on the effect of octreotide, one kind of octapeptide SSTA, in vivo and in nude mice in vitro, towards the cisplatin-resistant ovarian cancer cell SKOV3/DDP growth control and resistance reversal. The results provide a new understanding for the clinical treatment of ovarian cancer and drug resistance reversal.
The functions of cisplatin, OCT, and their combination in SKOV3/DDP cell apoptosis

According to the MTT results, the experiments were divided into four groups, including control, cisplatin (2.0 µg/ml), OCT (10.0 µg/ml), and two-drug combinations. Following the treatment for 36 h, the apoptosis test was done according to the Annexin V-FITC/PI staining kit’s instructions, and the results were read using flow cytometry.

In nude mice in vivo experiments:

SKOV3/DDP nude model preparation and group

Forty female BALB/c-nu/nu nude mice, six to eight weeks of age, weighing 18-22 g, specific-pathogen-free (SPF) breeding conditions, were purchased from the Experimental Animal Center of Chinese Academy of Sciences. Human ovarian cancer cells SKOV3/DDP in the logarithmic phase were made of the density of 5 × 10⁶/ml of single cell suspension to 5 × 10⁷ cells/ml inoculated into all nude mice subcutaneously near the right armpit, the daily observation of tumor growth and mice eating activity. Fifteen days after inoculation of tumor cells, the mice were randomly divided into four groups of 10 each: 1) octreotide group treated with octreotide 100 µg/kg, ip, qw, continuous four weeks, 2) cisplatin group treated with cisplatin of 4mg/kg, ip, qw, continuous four weeks, 3) combination group handled with the same dose of cisplatin of 4mg/kg, ip, qw, continuous four weeks, 4) control group only (0.1 ml/only) inoculated into all nude mice subcutaneously near the right armpit, the daily observation of tumor growth and mice eating activity. Fifteen days after inoculation of tumor cells, the mice were randomly divided into four groups of 10 each: 1) octreotide group treated with octreotide 100 µg/kg, ip, qw, continuous four weeks, 2) cisplatin group treated with cisplatin of 4mg/kg, ip, qw, continuous four weeks, 3) combination group handled with the same dose of octreotide and cisplatin above at the same time, continuous four weeks, 4) control group dealt with the saline of 50 ml/kg, sc, qd, continuous four weeks. Twenty-four hours after the last treatment, mice were sacrificed.

Evaluations of xenograft tumor status

Fifteen days after inoculation of tumor cells, the xenograft tumors were all at the similar volume in each group, of about 2 mm or so, which suggested the tumor formation of the same initial tumor volume, tumor growth, and good uniformity. After treatment of drugs in mice, the authors used a caliper to measure the size of tumor (a) as short-track and (b) long-track and the volume of tumor cells, the mice were randomly divided into four groups of 10 each: 1) octreotide group treated with octreotide 100 µg/kg, sc, qd, continuous four weeks, 2) cisplatin group treated with cisplatin of 4mg/kg, ip, qw, continuous four weeks, 3) combination group handled with the same dose of octreotide and cisplatin above at the same time, continuous four weeks, 4) control group dealt with the saline of 50 ml/kg, sc, qd, continuous four weeks. Twenty-four hours after the last treatment, mice were sacrificed.

Tumor cells extraction and preparation

Five of above fresh xenograft tumors in every group were randomly selected as part of polymerase chain reaction (PCR) experiments. The tumor was cut into small pieces, weighing about 80-100 mg, placed in liquid nitrogen, ground into powder, and every 100 mg of each tissue was added 1 ml of trizol reagent and homogenized with a homogenizer until it is particle-free homogenate and stainless steel. The cell lysates were transferred to a centrifuge tube at room temperature for 5 min, making the complete separation of nucleic acid protein complex. After centrifugation of 12,000 rpm at 4°C for 10 min, the supernatant was carefully draw into new tube.

Evaluation of SSTR2, epidermal growth factor receptor (EGFR), MDR1, and MRP2 mRNA expression in SKOV3/DDP cells in vitro and in nude mice

Total RNA was extracted from cells according to instructions of the RNeasy Mini Kit (Kaiji Company, KGA1203). The extracted RNA was dissolved in diethylpyrocarbonate (DEPC)-treated water. The absorption value at 260 nm and 280 nm were detected using a UV spectrophotometer. The RNA concentration was calculated using the following formula: RNA concentration = OD₂₆₀ × dilution fold × 0.04 µg/ul. The quality was considered good when the OD₂₆₀/OD₂₈₀ value was in the range of 1.8 to 2.1. The OD₂₆₀/OD₂₈₀ value was also investigated using ultraviolet spectroscopy. A 2 µl sample of cDNA was added to the reaction mixture, and the cDNA was synthesized according to instructions of the RT-PCR Kit (Kaiji company, KGA1303). Primers were designed and synthesized by the Kaiji Company, showed as follows: SSTR2 (96bp) 5’ TCAACCAACACCT-CAAACCAGAC 3’/5’ CCAAATGTGCAAGCCACCAAATA 3’, MDR1 (90bp) 5’ TGGACAGCTACAGCAGCGAAAG 3’/5’ GTCCGTTGGGATAGTGTGAAT 3’, EGFR (149bp) 5’ TAAACGGAAATGGTTGGTAAT 3’/5’ GAGGAGGAAGTATGTTGAAAGG 3’, MRP2 (156bp) 5’ CCATCATC -CATAGCTTCATTC 3’/5’ GTGCGTTCIIAACACTGCTC 3’, GST-π (128bp) 5’ GATGCGTTCCCTGCTCTC 3’/5’ CCAAACGCTCAGTTTCCC 3’, β-actin (136bp) 5’ GCAGAAAGGATCAGTCC 3’/5’ GCTGATCACA CTCTGGGAAAA 3’. Real-time PCR was performed in a Light Cycler (Roche Applied Science) with the following conditions: denaturation at 95°C for 5 min with the addition of 15 seconds at 94°C, 30 seconds annealing at 60°C. Comparing the threshold method and the mathematical method, the amount of target gene = 2⁻ⁿᶜᵗ. Ct is the number of cycles of fluorescence required for it to reach the threshold, ΔΔCt = (Ct objective gene-Ct reference gene ) experimental group - (Ct objective gene-Ct reference gene ) control group. Using this method, the authors could directly quantify the target gene relative to the reference gene ( -actin) and compare the common logarithm of the relative value of the target gene and control gene.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 software. The data was expressed as mean ± SD and was compared using Student’s t-test and ANOVA. A p value of < 0.05 was considered significant.

Results

In vitro experiments

The effect of OCT on SKOV3/DDP cells proliferation

As the treatment time progressed, cell growth became slow, and there were more falling floating cells, necrotic and debris cells. Figure 1 shows the cell morphology following 48 h of treatment. OCT showed an inhibition effect on SKOV3/DDP cell proliferation beginning at the concentration of 1.25 µg/ml. An increase in the treatment concentration enhanced the inhibition effect. When the concentration of OCT was higher than 5 µg/ml, the inhibition rate on SKOV3/DDP cell proliferation sharply increased. There were significant differences between the control group and each of the OCT treatment groups. There were also significant differences among the different concentrations of OCT treatment groups (p < 0.05).

Importantly, OCT (5, 10, 20 µg/ml) also inhibited proliferation in a time-dependent manner (p < 0.05, Table 1, Figure 2).
The functions of OCT and cisplatin combination on SKOV3/DDP growth inhibition rate and cisplatin IC50 value

Following treatment at the indicated concentrations of OCT and cisplatin, optical density (OD) was measured to calculate the cell growth inhibition rate. Compared to the control group, OCT decreased the IC50 value of cisplatin (p < 0.05). However, a higher dose of OCT (2.5-20 µg/ml) significantly inhibited IC50 (< 0.05) but not MDR1 and SSTR2 (p > 0.05).

The combination index (CDI) was calculated and is shown in Table 2. The CDI is equal to the survival rate(A drug+B drug)/(survival rate(A drug)/survival rate(B drug)). A CDI < 1 indicates synergistic effects of A and B drugs in the combined treatment. It was shown that the CDI < 1, when the concentration of OCT and cisplatin were higher than 5 µg/ml and 2 µg/ml, respectively, suggesting that there was a synergistic effect with OCT and cisplatin.

The effect of cisplatin, OCT and their combination on SKOV3/DDP cell apoptosis

In contrast to the control group, apoptosis was induced in both the OCT (10 µg/ml) and cisplatin (2 µg/ml) groups (p < 0.05). This effect was much more powerful in the combination treatment group than in the individual treatment groups (p < 0.05). However, there was no significant difference between the OCT and cisplatin treatment groups (p > 0.05). Table 3 shows that, compared with control group, treatment groups, the average tumor volume were significantly different (p < 0.05 vs control group; 2) p < 0.05 vs cisplatin group; 3) p < 0.05 vs octreotide group).

Table 1. — Inhibition rate of OCT on SKOV3/DDP proliferation at the indicated concentration and time.

<table>
<thead>
<tr>
<th>OCT (µg/ml)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>0.05 ± 0.05</td>
<td>0.03 ± 0.03</td>
<td>0.02 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Octreotide 0.12 ± 0.12</td>
<td>0.08 ± 0.08</td>
<td>0.06 ± 0.06</td>
<td>0.04 ± 0.04</td>
<td>0.02 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Combination 0.07 ± 0.07</td>
<td>0.05 ± 0.05</td>
<td>0.03 ± 0.03</td>
<td>0.02 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. — Combination drug index of OCT and cisplatin.

<table>
<thead>
<tr>
<th>Cisplatin (µg/ml)</th>
<th>OCT (µg/ml)</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00 ± 0.00</td>
<td>0.10</td>
<td>0.05</td>
<td>0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>0.05 ± 0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Octreotide</td>
<td>0.01 ± 0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Combination</td>
<td>0.00 ± 0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

In vivo in nude mice experiments

Observation of tumor tissue samples in each group

Seven days after inoculation, in the right armpit of nude mice. Also, two weeks after inoculation, visible pieces 1-2 mm of tumor growth can be seen, located subcutaneously near the right armpit and uplifted the skin surface with the characteristics of hard, good activity, skin color red. Luckily all mice were inoculated with tumor formation. Four weeks after, 40 mice were sacrificed for further observation and preparation. All the mice presented good growth of multi-section phyllodes solid tumor, coated with a layer of thin membrane, without local ulceration and other skin damage. It was clearly observed that the tumor surface had the clear boundary with the surrounding tissue, tumor gross specimen was pale yellow or white, and the cut surface was multiple cysts with thin yellow fluid and not smooth wall, shown in Figure 6. The tumor presented poorly-differentiated cancer, using hematoxylin and eosin (H&E) staining method, with the typical characteristics of density and flaky distribution cells, large stained and more mitotic nuclear, rare interstitial and large areas of necrosis, shown in Figure 7.

Comparison of tumor weight and tumor volume between each group

Table 3 shows that, compared with control group, treatment groups, the average tumor volume were significantly reduced (p < 0.05), respectively, the average size from biggest to smallest: control group (0.51 ± 0.03 cm³, Figure 6A) > cisplatin group (0.39 ± 0.02 cm³, Figure 6B) > octreotide group (0.25 ± 0.03 cm³, Figure 6C) > combination group (0.09 ± 0.01 cm³, Figure 6D). Among them, the combination therapy group is smaller compare to the octreotide and cisplatin groups (p < 0.05), while the octreotide group is smaller than cisplatin (p < 0.05).
Octreotide is the favorable alternative for cisplatin resistance reversal of ovarian cancer in vitro and in nude mice in vivo.

Table 3 also indicates that, compared with the control group, tumor weight of each treatment group was significantly decreased (p < 0.05), the average tumor weight from heavier to lighter are: the control group (0.55 ± 0.02 g, Figure 6A) > cisplatin group (0.37 ± 0.01 g, Figure 6B) > octreotide group (0.22 ± 0.004 g, Figure 6C) > combination group (0.07 ± 0.01 g, Figure 6D). Among them, the combination therapy group the tumor weight was significantly reduced more than the octreotide group, cisplatin (p < 0.05), while the octreotide group tumor weight was smaller than the cisplatin group (p < 0.05).

Tumor inhibition rate in different group sorted in descending order in descending was as follows: combination group (82.24%) > octreotide group (52.25%) > cisplatin group (24.62%), group differences were statistically significant (p < 0.05) (Table 3).

Table 4, Figure 8 shows the expression of SSTR2, MDR1, MRP2, EGFR, GST-π mRNA on xenograft tumors.

After drug intervention, the SSTR2 mRNA expression in the octreotide group and the combination group was significantly higher, compared with the control group, the differences were statistically significant (p < 0.05).
there were no significant differences between the cisplatin and the control group \((p > 0.05)\), even the octreotide group and the combination group \((p > 0.05)\).

After drug intervention, there was no significant difference of MDR1 mRNA expression between each other group \((p > 0.05)\). Compared with control group, the MRP2 mRNA expression was significantly reduced in every treated group \((p < 0.05)\), and even decreased more significantly in the octreotide and combination groups than the cisplatin group \((p < 0.05)\). Compared with the control or the cisplatin groups, the EGFR mRNA expression in the octreotide and combination groups significantly lower the cisplatin group \((p < 0.05)\), but the latter dropped more \((p > 0.05)\). Compared with the control group, the GST-\(\pi\) mRNA expression in each group was all significantly reduced \((p < 0.05)\), but the octreotide and the combination groups significantly lower the cisplatin group \((p < 0.05)\).

**Discussion**

Platinum-based combination chemotherapy is the most widely used method in the treatment of ovarian cancer. However, due to resistance, it often fails to cure patients [2]. Therefore, how to reverse platinum resistance for ovarian cancer and to increased sensitivity to platinum-based chemotherapy is the main focus for cancer researchers and clinicians.

SST is a cyclic polypeptide hormone, which is located in most human organs and tissues. It performs the functions of inhibition of hormone secretion, regulation of neural transmission, and cell proliferation [6, 7]. Natural SST is limited in clinical applications because of its low selectivity, short half-life, and re-increases in hormone levels after drug treatment termination. However, SSTA is clinically widely used and has shown to have much more powerful effects and a longer half-life. OCT is the most widely used somatostatin analogue in clinical applications. Recently, the inhibition effects of SST and SSTA on cancer cells caught the attention of more people [8]. A body of studies reported that SST and SSTA can inhibit the growth of several non-neuroendocrine tumors [6, 9-10]. It is accepted that the functions of SST and SSTA are mediated by SSTR [11]. SSTR2, followed by SSTR1, 3 and 4, are widely-expressed in tumor tissues. The expression and functions of SSTR in ovarian cancer are still not very clear.

Previous studies showed that SSTR is expressed in ovarian cancer [3, 12]. Halmos et al. used RT-PCR to investigate all the subtypes SSTR mRNA expression in 17 cases of primary ovarian tumor tissue and found that 76% of the cases had advanced primary malignant ovarian tumors with a high SSTR expression. Of the cases found to have malignant ovarian tumors with high expression of SSTR, 65% of the patients highly-expressed SSTR1 and SSTR2A, followed by the SSTR3 and then SSTR5. These data suggest that SSTR and SSTA could be potential targets for ovarian cancer therapy. In this study, the authors found that SKOV3/DDP cells expressed SSTR2 and that OCT effectively inhibited the cisplatin-resistant ovarian cancer cell SKOV3/DDP proliferation in a dose-dependent manner and suppressed apoptosis. The present data confirmed that SSTR and SSTA, as the
endogenous hormone, can regulate ovarian cancer proliferation. One or several SSTRs, especially SSTR2, were expressed on the surface of most of the tumor cells, which could be inhibited by SST and SSTA [13-14]. OCT presented the highest binding affinity to SSTR2 and subsequently inhibited the activity of tyrosine phosphatase and the proliferation of SSTR2 expressed cells [15]. However, OCT did not change the SSTR2 expression level in vitro and in vivo, even unexpectedly up-regulated in vivo, which is not consistent with a previous study done by Hua et al. [16]. Their study showed that the short-term application of OCT induced SSTR2 desensitization and internalization, which partially inhibited the effect of OCT on liver cancer cells [17]. These contrary results may be due to different cell characteristics, expression of the receptor and their subtypes, and high OCT treatment concentration. Furthermore, mRNA could not reliably be used as the index to reflect the status of receptor, and the authors should therefore focus on the cell membrane localized receptor protein [16]. Also, due to this experimental data, it is considered that the long-term chronic stimulation of octreotide might induce upregulation of SSTR2, and the change could further strengthen the anti-tumor effect of octreotide, which suggest the long-term use of octreotide may not increase its resistance.

It has been shown that the synergistic effects of SSTA with chemotherapy drugs increased their clinical efficacy. These effects could enhance the sensitivity of several gastrointestinal cancers to chemotherapeutic drugs [18]. Cisplatin plays a pivotal role in the treatment of ovarian cancer. It has rarely been reported that OCT increases its clinical efficacy when SSTR expressed ovarian cancer patients undergo chemotherapy. In the current study, the authors found that OCT suppressed the inhibition effects of cisplatin on SKOV3/DDP cell IC50 in a dose-dependent manner, inhibited SKOV3/DDP cell proliferation, increased chemotherapeutic agents’ sensitivity, and reversed chemotherapy resistance. In vivo results of the study also found the better inhibition effect of octreotide on the growth of SKOV3/DDP xenograft tumor in nude mice than cisplatin, and the combination of the two drugs enhanced anti-tumor effects, indicating that octreotide can inhibit the in vivo resistant ovarian cancer proliferation, and can play better than the inhibitory effect of cisplatin and a synergistic effect with cisplatin. These data suggest that OCT could reverse SKOV3/DDP cell resistance and increase the efficacy of chemotherapy of ovarian cancer, but the detailed mechanism is still not clear. The authors investigated the expression of resistance-related genes MDR1, MR2, GST-π, and EGFR using real-time PCR assays and compared the parameters before and after OCT treatment.

In this study, it was found that MR2 and EGFR are expressed on the SKOV3/DDP cell surface. OCT treatment increased the cisplatin sensitivity, induced the synergistic cellular cytotoxic effects with cisplatin, and decreased MR2 and EGFR expression in vitro and in vivo. These data demonstrate that OCT reverses ovarian cancer resistance and could be related to the down-regulated MR2 and EGFR expression. MR2 is an ATP-dependent membrane transport protein, with which MR2 participates in cisplatin transport in combination with glutathione. Both animal experiments and clinical studies have shown that MR2 could be associated with cisplatin resistance in ovarian cancer [19-20]. Based on these observations, the authors hypothesized that MR2 down-regulation could increase intracellular cisplatin concentration to efficiently reverse drug resistance. The mechanism of OCT-reducing EGFR expression in ovarian cancer is not clear. However, studies have shown that EGFR over-expression in ovarian cancer cells indicates an increase in drug resistance. Suppressing EGFR expression in ovarian cancer cells increases cisplatin sensitivity [21-22]. Following OCT binding with SSTR, the activation of the tyrosine phosphatase and reversal of EGFR-induced EGFR tyrosine kinase phosphorylation results in the reduction of EGFR, the termination of EGF signal transduction at the cell membrane and, eventually, the inhibition of cell proliferation [23]. EGFR is the producer of the oncogene ErbB1. It has the ability to connect the G-protein, cytokine receptors, integrins and other signals, and can affect many related gene-expressions [24]. Therefore, OCT may also, indirectly through EGFR, regulate the reversal of cisplatin resistance. These detailed mechanisms merit further investigations.

GST-π expression in ovarian cancer is not only related to primary cancer but also to acquired drug resistance [25]. In the current study, GST-π gene expression was increased but not decreased after treatment in vivo. Vanhoefer et al. reported that the drug has an outward flow caused by the non-specific binding of GST-π to the p-gp-induced drug pump at the early stage of resistance. Both GST-π and p-gp are involved in the modulation of the early-stage drug resistance development [26]. The synergistic effects of GST-π and p-gp may explain the phenomenon that GST-π and p-gp are not down-regulated in the OCT-induced reversal of cisplatin resistance. The resistance may be aggravated during the process of OCT-induced reversal of resistance for the increase of GST-π and p-gp. What is the meaning of this kind of change? Is it an accidental phenomenon or a necessity? Moreover, after octreotide intervention, in vivo, there has indeed been a reduction in GST-π. The result may be due to increased drug accumulation in the cells, and then depleted and lower-expressed GST-π after the long-term chronic effects of octreotide.

MDR1 can decrease the intracellular drug concentration by encoding cell surface transporter proteins p-gp, resulting in reduced or lost drug function, and induced resistance. It was shown that p-gp is expressed when ovarian cancer cells have high cisplatin resistance [27-28]. This study demonstrated that there is no MDR1 expression change in SKOV3/DDP cells following the combination treatment of OCT and cisplatin both in vitro and in vivo, suggesting that MDR1 is not involved in OCT inhibition of cell proliferation and that it reverses resistance.

However, the in vitro and in vivo results confirmed the
sensitization effect of OCT in ovarian cancer cisplatin resistance. This confirmation provided the new target for ovarian cancer therapy. Large-scale, randomized, double-blind, and controlled studies still need to be completed to confirm the OCT dose and timing issues of clinical treatment. In addition, it is suggested that the enhanced sensitivity to cisplatin maybe the mechanism through the changes in resistance gene expression following by action of the octreotide. However, the subsequent trial to explore the molecular mechanism of SSTR2 downstream signals transduction pathway in cisplatin resistance of ovarian cancer has been on-going. It will allow to acquire the theoretical basis for further studies.

Acknowledgments

This work was supported by the Technology Research Program of Nanjing City (No. 200901089) and Southeast University Technology Fund (KJ2010493).

References


HPV at the time of vaccine: has screening reached its goal?

E. Tartaglia¹, D. Iafusco², A. Cocca², S. Palomba⁴, M. Rotondi¹, P. Mastrantonio¹
¹University of Molise, Department of Health Sciences, Institute of Gynaecology & Obstetrics, Campobasso
²Second University of Naples “SUN”, Department of Paediatrics, Napoli
³University “Magna Graecia”, Department of Obstetrics & Gynaecology, Catanzaro (Italy)

Summary

Introduction: The human papillomavirus (HPV) prevalence recognized a geographic distribution of genotypes but, in the last years, the change of sexual behaviours, the increase number of sex partners, and the reduction of geographic distances have changed its prevalence and distribution. Objective: To determine the prevalence of HPV types among females in the Molise region and its evolution in 24 months. Materials and Methods: The authors, from February to August 2008, used a representative sample of a female population (n = 299) aged 17 to 64 years who were interviewed and submitted cervico-vaginal swab specimens. Swabs were analyzed for cytologic screening and HPV detection and typing. The patients with a positive cytology were submitted to colposcopy and eventually biopsy. Cytologic and colposcopic follow up was performed in 24 months. Results: The overall HPV prevalence was 30.1% and the prevalence of high- and low-risk HPV types was 22.41% and 18.06%, respectively. The prevalence of HPV vaccine types was relatively low for HPV-6-11-18. Only HPV-16 is well-represented in Molise, but recognizes a strictly geographic distribution. Conclusion: This study is one of the largest assessments of HPV genotypes to date in Italy. It is clear that several HPV-types are involved in cervical lesions, therefore the vaccine is profitable but limited by great number of types implicated in the pathogenesis of cancer and by their dishomogeneous distribution. Currently, a good campaign of screening is still necessary. In the future, second generation polyvalent HPV vaccines may be proposed for a wider and complete vaccine coverage.

Key words: HPV Prevalence; Risk factors; Vaccine; Screening.

Introduction

Human papilloma virus (HPV) is included in the family of Papillomaviridae, which now contains 29 genera formed by 189 papillomavirus types isolated from humans (120 types), in non-human mammals (64 types), in birds (3 types), and reptiles (2 types) [1]. HPV is actually estimated to be the most common sexually-transmitted infection in the world and its prevalence has been found to be the highest among young women within the first few years from sexual debut [2]. Genital HPV types are categorized according to their epidemiological association with cervical cancer in low-risk (LR-HPV) and high-risk (HR-HPV) HPV types. HR-HPV are detected in 99% of cervical cancers and approximately 70% of cervical cancers worldwide are due to HPV-16 (50%) and HPV-18 (20%) [2]. Although HPV infection is very common and has a high infective rate (66% of sexual partners), several studies suggest that approximately 90% of infections clear within two years [3, 4]. HPV prevalence recognized a geographic distribution of genotypes [5], but in the last years, changing of sexual behaviours, the increased number of sex partners, and the reduction of geographic distances have changed the prevalence and distribution of this virus [6]. Actually, there are two types of vaccines available to prevent HPV-infection and related diseases: both types contain hollow immunological virus-like particles (VLPs) assembled from recombinant HPV (16/18) coat proteins. One of these is tetravalent and also targeted for HPV-6 and HPV-11 which together currently cause about 90% of all cases of genital warts and 20%-30% of low-grade squamous intraepithelial lesions (L-SIL). Both types of vaccines require three doses (0.5 ml) given as intramuscular injections over six months (at times: 0; 2; 6). From 2008 also in Italy, the Health Office recommends the use of a prophylactic vaccine against HPV types 6, 11, 16, and 18 for routine use in females aged 11 to 12 years. The objective of vaccination is the progressive immunization of young adult female population exposed to the risk of infection reducing the incidence of precancerous lesions at brief-mid-term and of cervical cancer (almost 61%) at long-term. Such an immunization strategy involves a direct additional charge at the expense of the SSN National Healthcare System) to guarantee immunization to cover 90% of the target (in 2008 only, i.e., there was an outlay of Euro 60.000.000) [7]. Efficacy is based on completion of three doses of vaccine, which probably occurs in no more than 75% of females who initiate vaccination [8]. In Italy, i.e., only 53.1% of young adolescents born in 1997 had taken three doses of the vaccine and completed the immunization program by December 31, 2009 [9]. Different criticisms have led to a wide immunization program: available evidences are limited at this time and are small in comparison to the target of teenagers. The duration of protection and the requirement for booster doses are not known; the available information regarding a possible cruciform or widened protection to other genotypes induced by the vaccine are limited, neither it is possible to know if the
Specimen collection and processing

Cervico-vaginal swab specimens were performed in the structures of ASREM in Campobasso, Isernia, and Termoli. A collection device was utilized, which was a small cervical sampler on a plastic handle packaged in an individual re-closable, non-sterile plastic sleeve for single use only (Cervex-Brush). The swabs were stored in 20 ml of a PreservCyt LBC media (ThinPrep-liquid-PAPvial; Cytyc Corporation, USA); a methanol-based transport medium and preservative for cytologic samples at room temperature were also used and then sent to laboratories of the University of Molise. The swabs were analyzed for a thin-Prep Pap test and for the presence of HPV-DNA by Linear Array HPV genotyping test (Roche-Molecular-Systems, Inc. Branchburg, NJ, USA) – a qualitative in vitro test for the determination of 37 HPV-DNA genotypes. The authors considered as LR-HPV types: 6; 11; 40; 42; 54; 55; 61; 62; 64; 71; 72; 81; 83; 84; CP6108 and as HR-HPV types: 16; 18; 26; 31; 33; 35; 39; 45; 51; 52; 53; 56; 58; 59; 66; 67; 68; 69; 70; 73; 82; IS39.

Statistical analysis

All females who were submitted to an adequate swab for HPV evaluation were included in the final analysis (299/299). HPV prevalence was estimated within the 95% confidence interval (CI). CI were calculated by using the SE of log transformation with the SE of the log prevalence. Statistical analysis samples deemed positive for high- and low-risk HPV were categorized as single or multiple infections. To explore the association with age and overall HPV prevalence, age was categorized into four year intervals (17-25, 26-35, 36-45, and 46-64). Data were analyzed by an SPSS-12.0 Statistical Package for Windows. Pearson’s $\chi^2$ test was used to evaluate the significance of differences between designated groups. All tests were two-sided; a $p$ value of < 0.05 was considered statistically significant.

Results

The number of healthy women was 299, with a mean age of 34 years (range 17-64) entered in the study and 29/37 different HPV genotypes were identified. Overall, 206 viruses were detected in 90 women. Multiple HPV infections were observed in 55.64% (50/90) of HPV positive samples with a mean of three viruses per woman (range 2-8) (Table 1).

The overall HPV prevalence was 30.1% (95% CI, 25, 39-35, 28) among females of Molise aged 17 to 64 years.
HPV at the time of vaccine: has screening reached its goal?

(n = 299) and the overall prevalence of HR-HPV and LR-HPV types was 22.41% (95% CI, 18.14-27.35) and 18.06% (95% CI, 14.16-22.75), respectively. The prevalence of HR-HPV and LR-HPV is represented in Figure 1. The most common HR-HPV were: HPV-16: 6.69% (95% CI, 4.36-10.13), HPV-52: 6.02% (95% CI, 3.83-9.34), HPV-53: 4.35% (95% CI, 2.54-7.34), HPV-66: 4.35% (95% CI, 2.54-7.34), HPV-59: 3.68% (95% CI, 2.05-6.52), HPV-58: 3.34% (95% CI, 1.81-6.10), HPV-73: 3.01% (95% CI, 1.57-5.68), HPV-45: 2.34% (95% CI, 1.12-4.83), and HPV-39: 2.01% (95% CI, 0.90-4.39). Considering the LR-HPV, the most common types were HPV-CP6108: 5.35% (95% CI, 3.31-8.55), HPV-42: 4.68% (95% CI, 2.79-7.75), HPV-62: 4.35% (95% CI, 2.54-7.34), and HPV-84: 2.34% (95% CI, 1.12-4.83). The prevalence for other types of HPV was lower (≤2%).

There was a statistically significant difference of HPV prevalence among women across a broad age range representative of the population (Figure 2): from 36.45% (95% CI, 28.44-45.28) in the 26-35 years group to 14.63% (95% CI, 6.78-28.76) in those aged 46 years or more (p = 0.037) (Table 2). There was also a statistically significant difference of HPV prevalence related to marital status: 42.76% (95% CI, 35.68-50.14) in the single group that increased to 61.54% (95% CI, 39.87-79.43) in the widowed/separated/divorced group vs 14.18% (95% CI, 9.38-20.89) in the married/living with partner group (p < 0.001). No statistically significant difference was found in relation to geographic zone and education. There was a statistically significant difference of HPV prevalence related to low BMI (< 18.7: HPV prevalence 48.15% (95% CI, 32.39-64.28) (p < 0.05)), a high number of lifetime sex partners (≥3: HPV prevalence 84.21% (95% CI, 73.28-91.21) (p < 0.006), and to cigarette smoke (smokers: HPV prevalence 46.15% (95% CI, 37.29-55.27) (p < 0.001). Particularly, HR-HPV infections were associated with tobacco users. Indeed, compared with non-smokers, current smokers were at increased risk of HR-HPV infection: in fact the HR-HPV infection was 39.56% (95% CI, 30.79-49.05) in smokers vs 14.90% (95% CI, 10.72-20.35) in no smokers. Also a high prevalence of multiple infections were associated with tobacco users: 29.67% (95% CI, 21.54-39.33) in smokers compared to 11.06% (95% CI, 7.48-16.05) in non-smokers. No statistically significant difference was found in relation to menopausal state, pregnancy, cancer familiarity, utilization of oral contraceptives, history of previous HPV-related pathologies (previous LSIL/HSIL), and age of first sexual intercourse (Table 2). Among the 299 women screened, 271 (90.64% (95% CI, 89.14-91.94)) had normal cytology, ten (3.34% (95% CI, 1.81-6.10)) showed ASC (atypical squamous cells), thirteen (4.35% (95% CI, 2.54-7.34)) showed a LSIL, and five (1.67% (95% CI, 0.70-3.95)) a HSIL. Eight women with ASC resulted HPV-negative and only two ASC resulted HPV-positive; both HPV-positive cases developed LSIL during follow-up. Each case of LSIL and HSIL resulted HPV-positive and the cytological diagnoses were confirmed by colposcopic examination and cervical biopsy (Table 3). Six LSIL (46.15% (95% CI, 24.57-69.28) showed a CIN-1 at histology and seven LSIL (53.85% (95% CI, 31.76-74.52)) showed normal histology. Three HSIL resulted as CIN-2 (60.0% (95% CI, 26.39-86.26)) and two HSIL (40.0% (95% CI, 11.45-77.46)) showed as CIN-3.

### Table 1. — Multiple HPV infections.

<table>
<thead>
<tr>
<th>HPV Types Detected</th>
<th>Number of patients with two genotypes: 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>45/52</td>
<td>18/53/66</td>
</tr>
<tr>
<td>16/81</td>
<td>6/18/39</td>
</tr>
<tr>
<td>52/CP6108</td>
<td>42/52/58</td>
</tr>
<tr>
<td>72/CP6108</td>
<td>42/66/81</td>
</tr>
<tr>
<td>53/73</td>
<td>16/52/59</td>
</tr>
<tr>
<td>53/69</td>
<td>52/58/84</td>
</tr>
<tr>
<td>58/62</td>
<td>61/84/CP6108</td>
</tr>
<tr>
<td>51/59</td>
<td></td>
</tr>
<tr>
<td>52/58</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HPV Types Detected</th>
<th>Number of patients with three genotypes: 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>39/42/58/66</td>
<td>18/53/66</td>
</tr>
<tr>
<td>6/31/39/42/73/CP6108</td>
<td>52/58/62/73/CP6108</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HPV Types Detected</th>
<th>Number of patients with four genotypes: 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>16/33/45/53/73</td>
<td>16/33/45/53/73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HPV Types Detected</th>
<th>Number of patients with five genotypes: 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>39/42/45/53/54/CP6108</td>
<td>16/33/45/53/73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HPV Types Detected</th>
<th>Number of patients with six genotypes: 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>39/42/53/54/CP6108</td>
<td>16/33/45/53/73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HPV Types Detected</th>
<th>Number of patients with seven genotypes: 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HPV Types Detected</th>
<th>Number of patients with eight genotypes: 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/16/35/52/54/58/62/73</td>
<td></td>
</tr>
</tbody>
</table>

(HPV at the time of vaccine: has screening reached its goal?)
LSIL were treated with diathermoacogulation (DTC) or laser vaporization (5/13) or repeated cervical cytology and colposcopy at six and twelve months, allowing time for the abnormality to be resolved (8/13). HSIL were treated with a surgical conization of the cervix (5/5) (Table 3). No cancer was detected.

HPV-16 was detected in 4/5 cases (80.0% (95% CI, 51.79-93.71)) of HSIL and in 1/13 cases (7.69% (95% CI, 1.08-38.91)) of LSIL. HPV-6 was detected only in one case (20.0% (95% CI, 2.84-68.15)) of HSIL (associated to HPV-45 and-53) and no cases of LSIL or ASC. HPV-11 and HPV-18 were not detected in any patient with cytopathological abnormality. However, because there was a low number of samples, the difference did not reach statistical significance. The 84.44% (95% CI, 80.0-88.05) (76/90) of HPV-positive patients cleared the infection in 24 months.

Discussion

The authors have studied the prevalence of the HPV types in the Molise region, one of smaller Italian regions (321,000 inhabitants of which nearly 97,500 women aged between 17-64 years). HPV is a common infection among females in Molise [10,11]. The present data indicated that the burden of prevalent HPV infection among females was greater then previous estimates in Italy 30.1% vs 7%-16% (official data of Superior Health Institute) [12], however the prevalence of HPV vaccine types was relatively low for HPV-6 1.34% (95% CI, 0.50-3.51), HPV-11 1.00% (95% CI, 0.32-3.06), and HPV-18 1.00% (95% CI, 0.32-3.06). Only HPV-16 (6.69% (95% CI, 4.36-10.13)) was representative in Molise but it followed a strict geographic distribution: 65% (95% CI, 48.02-78.87) of them were diagnosed in high Molise (Isernia) and only 35% (95% CI, 18.55-56.01) in low Molise (Campobasso and Termoli). Perhaps, this is possibly due to the micro-economy in Molise which is primarily a rural and mountain economy, therefore the social and cultural exchanges are not favorable [13]. These data, also observed in other isolated regions [14], are in contrast to the study of Agarossi et al. [15] where no differences were observed in the prevalence rates of HPV infection among various Italian geographic areas; yet they are justified if we consider that different geographical areas have social, cultural, economic, and historical differences [16]. Other most common HR-HPV types detected in Molise were: HPV-52, HPV-53, HPV-66, HPV-59, HPV-58, HPV-73, HPV-45, and HPV-39. Some of them (HPV-16, 45, 52, and 58) are included in the eight most common HPV types involved frequently in pathogenesis of invasive cervical cancer (HPV-16, 18, 31, 33, 35, 45, 52, and 58) and cervical adenocarcinoma (HPV-16, 18, 45) [17]. Multiple HPV infections are common and were observed in 16.2% of the study samples (55.64% of all HPV positive samples) with a mean of three viruses per woman (range: 2-8). The prevalence reported in the literature ranged from 1% to 20%, particularly in relation to HPV detection methods used [18]. Currently, there is a lack of consensus within

Table 2. — Prevalence of HPV infection by demographic physical and behavioral characteristics.

<table>
<thead>
<tr>
<th>Demographics characteristics</th>
<th>Sample Size</th>
<th>Prevalence % (95% Confidence interval)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (Aged 17-64 years)</td>
<td>299</td>
<td>30.10 (25.39-35.28)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 – 25</td>
<td>68</td>
<td>35.29 (25.51-46.49)</td>
<td></td>
</tr>
<tr>
<td>26 – 35</td>
<td>107</td>
<td>36.45 (28.44-45.28)</td>
<td>0.037</td>
</tr>
<tr>
<td>36 – 45</td>
<td>83</td>
<td>25.30 (17.34-35.35)</td>
<td></td>
</tr>
<tr>
<td>46 – 64</td>
<td>41</td>
<td>14.63 (6.78-28.76)</td>
<td></td>
</tr>
<tr>
<td>Geographical zone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campobasso</td>
<td>123</td>
<td>27.64 (20.72-35.84)</td>
<td>0.74</td>
</tr>
<tr>
<td>Isernia</td>
<td>122</td>
<td>31.97 (24.68-40.26)</td>
<td></td>
</tr>
<tr>
<td>Termoli</td>
<td>54</td>
<td>31.48 (21.04-44.21)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single (unmarried)</td>
<td>145</td>
<td>42.76 (35.68-50.14)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Married/living</td>
<td>141</td>
<td>14.18 (9.38-20.89)</td>
<td></td>
</tr>
<tr>
<td>Widowed/separated/ divorced</td>
<td>13</td>
<td>61.54 (39.87-79.43)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graduated (degree)</td>
<td>45</td>
<td>26.67 (16.13-40.74)</td>
<td></td>
</tr>
<tr>
<td>Senior high school</td>
<td>136</td>
<td>36.03 (28.89-43.84)</td>
<td>0.2</td>
</tr>
<tr>
<td>Primary or junior high school</td>
<td>118</td>
<td>24.58 (17.83-32.85)</td>
<td></td>
</tr>
<tr>
<td>B.M.I.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 18.7</td>
<td>27</td>
<td>48.15 (32.39-64.28)</td>
<td>0.05</td>
</tr>
<tr>
<td>18.7 – 23.8</td>
<td>188</td>
<td>30.32 (24.44-36.92)</td>
<td></td>
</tr>
<tr>
<td>&gt; 23.8</td>
<td>84</td>
<td>23.81 (16.10-33.73)</td>
<td></td>
</tr>
<tr>
<td>Menopausal state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>11.76 (2.99-36.59)</td>
<td>0.09</td>
</tr>
<tr>
<td>No</td>
<td>282</td>
<td>48.35 (42.06-54.70)</td>
<td></td>
</tr>
<tr>
<td>Previous pregnancies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>117</td>
<td>22.22 (15.74-30.41)</td>
<td>0.2</td>
</tr>
<tr>
<td>No</td>
<td>182</td>
<td>35.16 (28.97-41.90)</td>
<td></td>
</tr>
<tr>
<td>Cancer familiarity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>70</td>
<td>0.00 (20.83-41.10)</td>
<td>0.98</td>
</tr>
<tr>
<td>No</td>
<td>229</td>
<td>30.13 (24.78-36.08)</td>
<td></td>
</tr>
<tr>
<td>Utilization of oral contraceptive</td>
<td>66</td>
<td>36.36 (26.37-47.69)</td>
<td>0.17</td>
</tr>
<tr>
<td>No</td>
<td>233</td>
<td>28.33 (23.12-34.19)</td>
<td></td>
</tr>
<tr>
<td>Previous pathologies HPV related</td>
<td>59</td>
<td>37.29 (26.71-49.24)</td>
<td>0.23</td>
</tr>
<tr>
<td>No</td>
<td>240</td>
<td>28.33 (23.20-34.10)</td>
<td></td>
</tr>
<tr>
<td>Age at first sexual intercourse y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 16</td>
<td>105</td>
<td>32.38 (24.54-41.35)</td>
<td>0.61</td>
</tr>
<tr>
<td>≥ 16</td>
<td>194</td>
<td>28.87 (23.16-35.33)</td>
<td></td>
</tr>
<tr>
<td>No. of lifetime sex partners *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>33.33 (15.38-57.91)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>38.46 (18.22-63.69)</td>
<td>0.006</td>
</tr>
<tr>
<td>3-5</td>
<td>19</td>
<td>84.21 (73.28-91.21)</td>
<td></td>
</tr>
<tr>
<td>&gt; 6</td>
<td>4</td>
<td>75.00 (40.17-93.06)</td>
<td></td>
</tr>
<tr>
<td>Smoke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>91</td>
<td>46.15 (37.29-55.27)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No</td>
<td>208</td>
<td>23.08 (17.98-29.11)</td>
<td></td>
</tr>
<tr>
<td>Smoke and HR-HPV infections</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>91</td>
<td>39.56 (30.79-49.05)</td>
<td>0.008</td>
</tr>
<tr>
<td>No</td>
<td>208</td>
<td>14.90 (10.72-20.35)</td>
<td></td>
</tr>
<tr>
<td>Smoke and multiple HPV infections</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>91</td>
<td>29.67 (21.54-39.33)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No</td>
<td>208</td>
<td>11.06 (7.48-16.05)</td>
<td></td>
</tr>
</tbody>
</table>

* Date available only for 51 of 299 patients.
the literature regarding the extent and implications of multiple HPV infections, but the present results are consistent with most recent international studies [19-22]. In the present research, multiple HPV infections were more frequently found in the youngest women; this observation is consistent with results of Mejilde et al. [19] and suggests that a greater sexual activity of younger women may be associated with sexual transmission of multiple HPV types. According to the most epidemiological data [15], this study shows a high prevalence in young women aged 17 to 35 years and a continuous decline of prevalence rates of infection with increasing age; nevertheless the peak of prevalence in Molise is observed among females aged 26-35 years (36.45%). The result of multivariate analysis shows that a never-married and separated/divorced status and total number of sexual partners in a lifetime are independent risk factors for HPV infections. These findings are in agreement with previous studies showing that having a never-married and separated/divorced status and number of sexual partners are all factors associated with high prevalence of HPV infection [23]. Also a low BMI (< 18.7) is related to a high prevalence of HPV infection (p < 0.05). No association was found between the age at first sexual intercourse and the HPV infections or in relationship to menopausal state, pregnancy, cancer familiarity, a history of previous pathologies HPV-related (previous LSIL/HSIL), and utilization of oral contraceptives. Particularly, these last findings are in contrast with the study of Cotton et al. [24] that found an association between oral contraceptive use and HR-HPV infections. Previous research on the effect of oral contraceptive use on HPV infection showed inconclusive results [25]. Nonetheless, the findings in the present study do not support the hypothesis that oral contraceptive users may acquire HPV more often. Unlike in this study, HPV detection, especially those related to HR-HPV and multiple infections, was associated with tobacco consumption. It is possible that smoking could increase the likelihood of HPV infection through a local decrease of immune response in the cervix and through an indirect effect related to metabolism of female hormones [26]. During a follow up of 24 months, in agreement with the literature [27], the authors have detected 3.34% of ASC, 4.35% of LSIL, 1.67% of HSIL, and cancer cases. Only two cases of ASC were HPV-positive and developed LSIL during follow up. Every case of LSIL and HSIL resulted HPV-positive and the cytological diagnosis were confirmed by colposcopical examination and cervical biopsy. LSIL were treated with DTC or laser vaporization (5/13) or repeated cervical cytology and colposcopy at six and 12 months, allowing time for the abnormality to be resolved (8/13). Every HSIL was treated with a surgical conization of the cervix (5/5). However, HPV-16 was involved in 80% of HSIL but only in 15% of LSIL.

<table>
<thead>
<tr>
<th>Table 3. — Management of patients with cytological abnormality.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9*</td>
</tr>
<tr>
<td>10*</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>17</td>
</tr>
<tr>
<td>18</td>
</tr>
<tr>
<td>19</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>21</td>
</tr>
<tr>
<td>9*</td>
</tr>
<tr>
<td>10*</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>23</td>
</tr>
<tr>
<td>24</td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>26</td>
</tr>
</tbody>
</table>

* Patients 9 and 10 with ASCUS developed LSIL during the follow up.
Conclusion

To the authors’ knowledge, this study is one of the largest to assess HPV genotypes to date in Italy. It is clear that several HPV-types are involved in cervical lesions; in the authors’ experience, the prevalence of HPV vaccine types (HPV-6, 11-, and 18) is relatively low in Molise and are not detected in any patient with cytological abnormality (excluded a coinfection with HPV-6 in a case of HSIL). Only HPV-16 is well-represented and is involved in 80% of HSIL, but follows a strict geographic distribution; therefore the vaccine in adolescents and younger women is profitable, but is limited by great number of genotypes implicated in the pathogenesis of cancer and by their dishonestogeneous distribution [13]. In accordance with de Sanjose et al. [17], HPV types (16, 18, 31, 33, 35, 45, 52, 58, and others) should be given priority when the cross-protective effects of current vaccines are assessed, and for formulation of recommendations for the use of second-generation polyvalent HPV vaccines and should focus particularly on these HPV types. Currently, a good campaign of accurate and efficient screening is still necessary.

Acknowledgments

The authors are indebted to many people who helped with performing specimens, genotyping HPV-DNA, and for the data shown in this manuscript. Particularly, the authors thank the MOLI.G.A.L. (Molise Group of Local Action) (Grant LEADER+), asse I, misura 1.4, azione 1.4.1, sub-azione 1.4.1.a3), the ambulatories and the Departments of Gynaecology and Obstetrics of ASREM (Health Authority of Molise Region), the Laboratories of the University of Molise for their help and collaboration, all medical doctors and midwives of “Consultorio Familiare ex ALS 3” (Campobasso), of “Veneziale Hospital” (Isernia), of “Saint Timoteo Hospital” (Termoli), and particularly doctors: Vitale F., Borgia G., and Carone V. for their availability and help. Last but not least, the authors thank doctors Giuliano B. and Senna T. (ASL NA/1C - Naples), Professor Lucariello A. (Department of Internal Medicine - University of Naples “Federico II”), and Professor Grasso G.M., Professor Ripabelli G.C., Professor Sammarco M.L., doctor Fanelli I., and doctor Leone A. of the University of Molise for their work.

References

HPV at the time of vaccine: has screening reached its goal?


Address reprint requests to:
M. ROTONDI, M.D.
Via Vesuvio, 14
80056 Ercolano (NA) Italy
e-mail: mariorotondi@fastwebnet.it

Eurogin 2013
International Multidisciplinary Congress
Florence (Italy) - November 3-6, 2013

www.eurogin.com/2013
Immunotherapy with Dendritic Cells for Gynecological Neoplasias: A New Therapeutic Approach?

M.A. Michelin, E.F.C. Murta

1Discipline of Immunology; 2Discipline of Gynecology and Obstetrics
Oncological Research Institute (IPON), Federal University of Triângulo Mineiro, Uberaba, MG (Brazil)

Summary
The immune system consists of a complex collection of mediators and cells that act in a coordinated way to eliminate neoplastic cells. One of immunotherapy’s promises is the development of cellular vaccines, or more specifically, vaccines with dendritic cells. However, we still have a lot left to study and learn, since we already know that patients with tumors of the same histological grade can have completely different behaviors when given the same immunological stimulus. We believe that antitumor immunotherapy will lead to a personalized vaccine, since the scheme of treatment, the stimuli and the dosages need to be tailored to each patient.

Key words: Immunotherapy; Dendritic cells; Neoplasia.

Introduction
Using the immune system as a tool to treat neoplasias is one of science’s oldest dreams. The origin of modern immunotherapy stems from studies performed in Europe and the United States at the start of the nineteenth century, which observed tumor regression in association with other infections, such as erysipelas [1].

The tumor microenvironment is, in fact, made up of a collection of neoplastic cells, but also of various components of the immune system. The particular constitution and character of this microenvironment determine the type of anti-tumor response, since at the same time as there are immune system cells that powerfully destroy neoplastic cells, such as natural killer and dendritic cells, the tumor cells themselves can create a suppressor environment in which myeloid suppressor cells and T regulatory lymphocytes predominate [2-6]. Understanding the complexity of this tumor microenvironment and the escape mechanisms used by tumors is of fundamental importance to the development of new immunotherapies.

During the last 100 years, there have been huge advances in immunology, which today have allowed us to take the first steps in developing an effective immunotherapy. Various kinds of immunotherapies have been and continue to be developed; from the point of view of applicability, some have not passed in vitro tests, while others are already in clinical use. Many components of the immune system have already been used as a tool in the effort to destroy tumors, such as immunostimulants, antibodies, cytokines, and cellular therapies.

Among the cytokines that are already in use, the one that has the most beneficial effects and the fewest side effects is IFN-alpha. This cytokine is used to treat various types of tumors, such as renal carcinoma, melanoma, head and shoulder tumors, non-Hodgkin’s lymphomas, chronic myeloid leukemia, and cervical tumors. Published studies show that IFN-alpha can act directly on tumor cells, interfering with growth and differentiation, affecting intracellular signaling and perhaps the tumor’s capacity for mitosis. Moreover, it is suspected that this cytokine can also act in an indirect way or by stimulating the immune system to exercise its anti-tumor action. Our research group has spent several years analyzing the use of IFN-alpha in patients with cervical intraepithelial neoplasia grade 3 (CIN 3). The data shows that this cytokine can act to stimulate patients’ immune systems, since a reversion to the Th1 pattern, both locally and peripherally, occurs [7-9].

In addition, we know that the immune system consists of a complex collection of mediators and cells that act in a coordinated way to eliminate neoplastic cells. One of promises of immunotherapy is the development of cellular vaccines, or more specifically, vaccines with dendritic cells.

Immunotherapy with Dendritic Cells
Since the 1960s, there have been great advances in cell immunology. In 1973, Steinman and Cohn published an article in which they affirmed: “A novel cell type has been identified in adherent cell populations prepared from mouse peripheral lymphoid organs (spleen, lymph node, Peyer’s patch). Though present in small numbers (0.1-1.6% of the total nucleated cells) the cells have distinct morphological features. The nucleus is large, retractile, contorted in shape, and contains small nucleoli (usually two). The abundant cytoplasm is arranged in processes of varying length and width and contains many large spherical mitochondria. In the living state, the cells undergo characteristic movements, and unlike macrophages, do not appear to engage in active endocytosis. The term, dendritic cell, is proposed for this novel cell type” [10]. This article heralded a great discovery, describing DCs for the first time.
This cell type originates in the bone marrow, via pluripotent CD34+ cells, and develops into two different types, plasmacytoid DCs and myeloid DCs. The first type originates in the lymphatic system, is present in a lower proportion than myeloids, and is found in lymphatic organs, such as the thymus, the spleen, the lymph nodes, and the bone marrow. Myeloid DCs are comprised of Langerhans cells, located in the stratified epithelium; dermal DCs, located in the dermis; and interstitial DCs, located in the interstitics of the tissues [11-13].

DCs leave the bone marrow in a premature form; during this phase, they have a large capacity for antigen processing and presentation, although they have a low ability to stimulate the lymphocytes. The migration process to the lymph node after antigen capture and the cytokines produced at the point of inflammation or of tumor response are crucial to the maturation and activation of the DCs, since this activation and migration implies the expression of new costimulatory molecules that are essentially made to activate the T lymphocytes [14, 15].

The first attempts to use DCs as anti-tumor immunotherapy took place in the 1990s. The clinical use of this type of cell in therapy to treat different types of tumors – such as renal tumors, melanoma, cervical cancer, and prostate cancer – has taught us a great deal. However, there are still gaps in various areas of our knowledge that need clarification, as, for instance, the best form of differentiation, the dosage, the mode of administration, and, above all, the mechanisms that these cells use in vivo to induce this anti-tumor response.

Our research group has worked on developing new protocols for the differentiation of these cells, as well as studying the mechanisms involved in the process of tumor regression after vaccination. After immunotherapy with autologous DCs in patients with different types of advanced-stage tumors, differentiated in vitro based on the patient’s own blood and pulsed with tumor antigens obtained by biopsies of the patients themselves, the systemic immunological profile changes. We have seen evidence that there is a stimulation of innate immune response, with an increase in T helper lymphocytes (CD4+) expressing IL-2, IFN-gamma, IL-12, TNF-alpha and IL-10 after the start of treatment. The same effect was observed for T cytotoxic lymphocytes (CD8+) expressing IL-2. The percentage of total T lymphocytes (CD3+) remains elevated during the whole of immunotherapy, while the levels of regulatory T cells (CD4+/CD25+/FOXP3+) go down after the start of treatment. The same effect (CD4+) expressing IL-2, IFN-gamma, IL-12, TNF-alpha response, with an increase in T helper lymphocytes, was observed for T cytotoxic lymphocytes (CD8+) expressing IL-2. The percentage of total T lymphocytes remains elevated during the whole of immunotherapy, while the levels of regulatory T cells (CD4+/CD25+/FOXP3+) go down after the start of treatment. The same effect was observed for T cytotoxic lymphocytes (CD8+) expressing IL-2. The percentage of total T lymphocytes remains elevated during the whole of immunotherapy, while the levels of regulatory T cells (CD4+/CD25+/FOXP3+) go down after the start of treatment.

However, our experience shows that despite the fact that the vaccine stimulates an initial immune response, in which there is regression and/or stabilization of the tumor, after some months, this response is reduced, and the tumor progresses. Therefore, we believe that a new tumor escape mechanism arises, and for this reason, we need to improve the protocol for the differentiation and stimulation of DCs.

Immunotherapy with DCs gives great hope for the treatment of tumors. Nevertheless, much more still needs to be done. We know that using autologous cells in immunotherapy is of fundamental importance, because when heterologous cells are used, these very cells must be irradiated to avoid rejection, which leads to a huge loss of function, since these cells must be able to migrate and to express new adhesion molecules and the mediators needed to adequately activate T lymphocytes. Another important point is the need to develop the cells on an industrial scale so that they will be more accessible to the population requiring treatment.

Conclusions
There are still a number of factors limiting the development of new protocols for treatment in humans, which range from ethical precepts to the understanding of the basic mechanisms involved in the interaction between the innumerable and complex components of the immune system.

Immunotherapy with DCs is immunology’s promise for the treatment of neoplasias. However, we still have a lot left to study and learn, since we already know that patients with tumors of the same histological grade can have completely different behaviors when given the same immunological stimulus. We believe that antitumor immunotherapy will lead to a personalized vaccine, since the scheme of treatment, the stimuli, and the dosages need to be tailored to each patient.

Acknowledgements
Fundação de Amparo À Pesquisa do Estado de Minas Gerais (Fapemig), Financiadora de Estudos e Projetos (FINEP), and Conselho Nacional de Pesquisa e Desenvolvimento (CNPq).

References


Address reprint requests to:
E.F.C. MURTA, M.D.
Oncological Research Institute (IPON)/
Discipline of Gynecology and Obstetrics
UFTM, Av. Getúlio Guaíra, s/n, Bairro Abadia
38025-440 Uberaba-MG (Brazil)
e-mail: eddiemurta@mednet.com.br.
The influence of interval between conization and laparoscopic radical hysterectomy on the morbidity of patients with cervical cancer

H. Li¹, ², J.Y. Jang², H. Li¹, K. Chen³, X.G. Shao²
¹The Second Affiliated Hospital of China Medical University, Shenyang
²The Affiliated Obstetrics and Gynecology Hospital of DaLian Medical University, DaLian
³The Maternal-Fetus Center of YingKou, LiaoNing (China)

Summary

Objective: To evaluate the correlations between postoperative sequelae and the intervals between conization and subsequent laparoscopic radical hysterectomy (LRH) and pelvic lymphadenectomy in patients with cervical cancer. Materials and Methods: A retrospective study was conducted in a tertiary care university hospital. The medical records of cervical cancer patients undergoing LRH and pelvic lymphadenectomy between April 2005 and August 2011 were reviewed. The subjects were divided into three groups according to time from conization to LRH: group 1 (within six weeks, n = 17), group 2 (> six weeks, n = 38), and group 3 (no previous conization, n = 40). Results: The three groups showed no significant differences with respect to patient and tumor characteristics, intraoperative variables such as surgical time, blood loss, conversion to laparotomy, and perioperative morbidity, while the complications in group 1 showed a significant difference compared to groups 2 and 3. Conclusions: LRH is feasible for the treatment of cervical cancer patients with previous conization and the appropriate time interval is after six weeks. A careful separation of the bladder and ureters from the cervix is recommended to minimize morbidity associated with this surgery.

Key words: Cervical cancer; Laparoscopic radical hysterectomy; Conization; Learning curve.

Introduction

Since the first laparoscopic radical hysterectomy (LRH) and lymphadenectomy for cervical carcinoma was performed in 1992, laparoscopy for uterine malignancies has evolved rapidly [1]. The advantages of laparoscopy vs open uterine surgery are clear, including decreased postoperative pain, shorter length of hospital stay, more rapid return to normal activities, and a substantial equivalent of lymph nodes removed [1, 2]. In the management of early invasive cancer of the cervix, LRH with proper patient selection and performance of the surgery by an experienced gynecologist that is well-trained in laparoscopic surgery is encouraged in the practice of gynecologic oncology. However, it is associated with unique challenges and complications [3]. To reduce perioperative complications, the determination of an appropriate time for LRH in patients who have undergone cervical conization is required and this study was undertaken to verify a possible correlation between the clinical aspects and the time interval.

Materials and Methods

Patient groups
Data of patients who underwent total LRH and pelvic lymphadenectomy for cervical cancer between April 2005 and August 2011 were retrospectively collected and recorded. During this period, 1,167 cervical cancer patients received radical hysterectomy and systematic lymph node dissection, and 167 patients were eligible to undergo LRH; of these, 55 (32.9%) had recent conization of the cervix and were the basis of this report. Surgery was performed by the team including six surgeons under the direction of Dr. JiYoung Jiang, who had previous and extensive experience in gynecologic oncology and laparoscopic procedures. Inclusion criteria were: good general condition, no evidence of lymph node metastasis according to imaging study, before Stage IIA. All patients were staged based on clinical evaluation according to the most recent FIGO clinical staging system, conversion to laparotomy was mandatory if safety of the patient and the tumor incision margin could not be assured during the laparoscopic procedure. Patients were informed of risks and possible complications of the laparoscopic procedure and signed a written informed consent for conversion to laparotomy. All patients received a preoperative bowel preparation, antibiotic prophylaxis, and antithrombotic treatment with subcutaneous low molecular weight heparin for five to seven days.

Total LRH technique
Many authors have described the techniques used to perform LRH [4, 5]. Briefly, with the patient in the Trendelenburg position with the foot raised at 1-15° after the placement of a uterine manipulator, a 10-mm trocar for the 0° laparoscope was placed at the level of the umbilicus. Once the trocar had been safely introduced into the abdominal cavity, insufflation was performed and the intra-abdominal pressure was maintained at 11-12 mmHg. Two additional 5 mm trocars in the right and left quadrants and one suprapubic trocar were placed. The round ligaments were transected bilaterally, and the paravesical and pararectal spaces were developed with ultrasonic energy instruments to achieve a blunt dissection. Complete pelvic lymphadenectomy up to the common iliac nodes and para-aortic...
nodes was performed and the lymph nodes were placed into a bag and removed vaginally at the end of the operation. Total LHRH was performed identifying and cutting uterine vessels at their origin from hypogastric vessels using a bipolar knife. With the transected uterine vessels in tension, the ureter was then unroofed and dissected out of the tunnel, and the uretersocardial and cardinal ligaments were isolated and resected as close as possible to the pelvic sidewalls. The bladder was mobilized inferiorly to ensure adequate vaginal margins. Finally, the vaginal wall was transected by monopolar coagulation and all the specimens were then retrieved from the vagina. In patients less than 40 years and with squamous cervical cancer, the ovaries were preserved, and the vaginal cuff was sutured vaginally. In order to facilitate laparoscopic uterine manipulation and minimize risk of ureteral damage, ureteral bilateral stents were placed preoperatively and removed at 7-14 days postoperatively.

Postoperative treatment

The vital signs of the patients, the characteristics, and quantity of liquid from the drainage tube were observed. Febrile morbidity was defined as a temperature of 38°C on two separate occasions during at least four hours, excluding the initial 24 hours post-surgery. Adjuvant therapy was proposed to patients according to risk factors, such as parametrical involvement, positive resection margins, and positive lymph nodes. Follow-up consisted in a pelvic examination every three months during the first two years, three times annually from the third to the fifth year, then annually from then onwards.

Statistical analysis

The SPSS software (version 13.0) was used for statistical analysis, differences between continuous variables were evaluated through one-way ANOVA for normally distributed variables and by the Bonferroni test for variables that were not normally distributed, differences of proportions were compared with the Fisher exact test, and p < 0.05 was considered statistically significant.

Results

Patients’ characteristics

A total of 95 patients were subdivided into 17 patients in group 1 (previous conization within six weeks), 38 patients in group 2 (previous conization > six weeks), and 40 patients in group 3 (no previous conization) were enrolled. The mean age was 44.2, 43.5, and 46.2 years, the mean delivery histories were 1.4, 1.2, and 1.3 times, and mean body mass index (BMI) was 22.5, 21.4, and 22.7 kg/m², and previous cervix LEEP was 5 (29.4%), 13 (34.2%), previous cold knife was 12 (70.6%), 25 (65.8%), respectively. The aforementioned data showed no significant differences between the three groups (Table 1).

Comparison of intraoperative variables

The uterine dimension was 10.5 ± 2.1, 11.8 ± 1.8, 10.4 ± 2.3, surgical time (min) 263 ± 65, 217 ± 74, 210 ± 65, estimated blood loss (ml) 367 ± 115, 229 ± 123, 240 ± 119, pelvic node 24.1 ± 4.2, 25.3 ± 5.0, 23.7 ± 7.4, conversion to laparotomy 2 (11.6%), 1 (2.6%), 1 (2.5%), respectively. The aforementioned data showed no significant differences between the three groups (Table 1).
The influence of interval between conization and laparoscopic radical hysterectomy on the morbidity of patients with cervical cancer

(11.6%), 1 (2.6%), and 1 (2.5%) respectively. Group 1 and 2 patients experienced uncontrolled parametrial bleeding during dissection of the vesicocervical ligament to unroof the ureter. In group 2, one patient experienced right external iliac vein laceration during debulking lymphadenectomy and thrombosis occurred on the fifth day. In group 3, one patient experienced left internal iliac vein injury when the uterosacral ligaments were transected. The patients in group 1 therefore had more blood loss, longer surgical time, and more risks of conversion to laparotomy, compared to the patients in groups 2 and 3, and there was no difference between these latter two groups (Table 3).

Comparison of postoperative morbidity and complications

In group 1, one patient with previous transverse incision Cesarean section at the lower uterine segment experienced bladder injury during opening of the cervicovesical space and there was extensive intraoperative bleeding during the course of hemostasis, which was repaired laparoscopically. One patient experienced inadvertent ureteric injury during retroperitoneal ureteric dissection and was then repaired lapasocopically. One patient complained of water vaginal discharge from the tenth postoperative day, the ureteric injury (ureterovaginal fistula) was discovered 18 days postoperatively and required surgical repair. One patient experienced pyrexia ranging from 38.0°C to 39.8°C and more vaginal discharge for seven days postoperatively, and vaginal cuff cellulitis and cuff separation were found, but then successfully repaired. Two women experienced fever of unknown origin, but disappeared ten days later. In group 2, one patient had acute renal failure and retroperitoneal hematoma was found; she then received a blood transfusion of 800 ml, and healed by conservative treatment after three days. In group 3, one patient experienced bowel obstruction on the fourth day postoperatively and recovered by means of gastrointestinal decompression. In all cases, the aforementioned data showed perioperative morbidity and complications in group 1 was significantly higher compared to groups 2 and 3, and there was no significant difference between the same groups (Table 4).

Discussion

This study showed that LRH can be successfully performed in women with cervical carcinoma undergoing prior excision of the cervix. Although the study sample was small, in most (95.8%) of these patients, the procedure was completed laparoscopically.

The topic of interval between conization and subsequent hysterectomy seems surpassed. Concerns of hysterectomy in women with previous electrical loop or cold knife excision of the cervix include: the risk of urinary injury, infectious morbidity, and severe hemorrhage due to inflammatory reaction of the paracervical tissue. It has been proposed that subsequent hysterectomy after conization should be conducted within 48 hours or after six weeks [6]. Many patients had conization performed elsewhere, and these patients were frequently referred at varying intervals after conization and often had a radical surgical procedure at that time. Hysterectomy cannot be easily conducted within 48 hours, especially for cervical cancer patients, because the histotype cannot be determined early. Furthermore, a hysterectomy after six weeks after conization can lead to several problems, such as fear and anxiety about cancer metastasis during the waiting period. Attribution to the efficacies of antibiotics, to rapid and sufficient transfusion, and to advances in surgical techniques, the appropriate time is of current debate and each hospital has different guidelines. Korean doctors report that abdominal extended hysterectomies could be conducted at any time when the patient is in a good condition, not precisely within 48 hours or > six weeks after LEEP [7]. Thailand doctors reported that total laparoscopic hysterectomy could be feasible and safe in patients with prior diagnostic excision of the cervix [8], but in a brief review of articles of LRH, no information regarding the significance of conization -LRH interval was found [9-11] thus this study was undertaken to verify a possible correlation between the clinical aspects and the time interval.

The paracervix has two parts: the medial part is a condensation of connective tissue, and the lateral part is made of fatty tissue that contains lymph nodes and surrounds vessels and nerves; the stable anatomical landmark that marks the limit between these two parts is the terminal ureter. It has been recognized that prior operation of the cervix may lead to inflammatory reaction and hypervascularity of the paracervical tissue [12], and these changes continue until six weeks postoperatively. If hysterectomy is performed after 48 hours or before the cervix has healed, the risk of severe intraoperative bleeding and serious postoperative infectious morbidity is increased. In this surgical procedure in group 1, during the course of ligation of uterine artery, dissection of the ureteral tunnel and resection of the cardinal ligament of the vagina, the authors found that the parametrical blood vessel was dilated, the fatty tissue was in edema, and the authors experienced more hemorrhage, more time with ultrasonic energy, and bipolar device was required to arrest hemorrhage. Increased surgical time often associated with increased technical difficulty, predisposes the patient to increased perioperative morbidity [13]. One patient suffered from febrile, watery diarrhea, peritonitis, and leakage of urine through the vagina nine days postoperatively, ureteronecystostomy was performed abdominally, but in groups 2 and 3, these complications did not exist.

Urologic structures are at risk for injuries at the time of LRH and pelvic lymphadenectomy procedures, in particular this injury is mainly related to injury of the bladder or ureter because the authors dissected part of the vesicocervical ligament. In this series, the incidence of bladder injury was 5.8% and the incidence of uretal injury was 5.8% in group 1, 2.6% in group 2, and 2.5% in group 3. Steed [13] and Milad [14] reported similar high numbers of urinary tract complications of 8% and 6%. Although it...
has been argued that the restriction of the technique can be overcome with suitable training, the authors’ experience still indicated a reduction in such complications within the proper interval between conization and subsequent LRH.

The authors also found that in group 1, the febrile morbidity was higher than the other two groups and this was in accordance to the previous work of Osoba [15], but Cavanagh [16] reported that while febrile morbidity may be increased when hysterectomy was performed within six weeks of cone biopsy, no conclusive evidence could be found for this; however even if it did increase, it was apparently not a serious problem. If hysterectomy was not postponed for six weeks, it was unimportant if it was performed within that period.

Compared to abdominal and vaginal hysterectomies, laparoscopic hysterectomy requires greater surgical expertise, a longer time to master, longer surgical time, and increased incidence of urinary tract injury. To prevent and reduce these complications, gynecologists must acquire skilled laparoscopic techniques through repeated training based on a slow learning curve with the aim to improve these laparoscopic skills. In a recent review of laparoscopic gynecologic complications, Possover [17] suggested that a minimum of 50 LRH and lymphadenectomy cases are needed for a surgeon to acquire adequate laparoscopic skills. Furthermore, complications decreased with increased experience, therefore it is important to respect the learning curve. In this department, the initial learning experience of the primary complications was from laparoscopic hysterectomy as well as LRH. In this study, four patients (23.5%) in group 1, ten patients (26.3%) in group 2, and 11 patients (27.5%) in group 3 were included in the first 50 cases; the proportion had no significant difference, therefore the authors could exclude the impact of the learning curve.

In conclusion, the data revealed differences in surgical morbidity at distinct time intervals between conization and subsequent LRH. LRH within six weeks of conization of the cervix is fraught with the danger of high morbidity and it is reasonable to perform LRH six weeks after conization; moreover, careful dissection of the bladder from the cervix and identification of both ureters are recommended to minimize morbidity associated with this procedure.

References


Address reprint requests to:
J.Y. JANG, M.D.
The 10 floor of Dalian Maternity Hospital
1 Dunhuang Road
Shuhekou District DaLian City
116033 Liaoning Province (P.R. China)
e-mail: jiangjijyong@dl.cn.
Management of ASCUS findings in Papanicolaou smears. A retrospective study

C. Iavazzo, I. Boutas, C. Grigoriadis, N. Vrachnis, N. Salakos
Second Department of Obstetrics and Gynecology, University of Athens Aretaieion Hospital, Athens (Greece)

Summary
Aim: Atypical squamous cells of undetermined significance (ASCUS) are a cervical cytologic finding category suggestive but not definitive of squamous intraepithelial lesions. ASCUS remains an incompletely described entity and accounts for even 5%-10% of reported Papanicolaou (Pap) smears. The management of women with such cytologic findings remains controversial. The aim of this study was to evaluate the cytology laboratory findings with regards to ASCUS diagnosis, using cervical Pap smears, and colposcopic biopsies, as well as their management. Materials and Methods: This is a retrospective study of patients with ASCUS Pap smears taken during the period January 2010 – December 2010 in the Second Department of Obstetrics and Gynecology, Aretaieion Hospital. Results: During the study period, 657 Pap smears were examined at the Aretaieion Hospital; moreover, seven patients, whose Pap smears were cytologically diagnosed with ASCUS, were referred from other clinics, providing a total of 42 cases with a descriptive diagnosis of ASCUS for review. Of the 42 cases, eight were not studied because they were either lost in follow-up or they did not have available data. The remaining 34/42 patients were evaluated by colposcopic examination and directed biopsies where necessary. The ratio of ASCUS to low-grade squamous intraepithelial lesion (LGSIL), high-grade squamous cell intraepithelial lesion (HGSIL) or squamous cell carcinoma (SCC) was 5/34, 1/34, and 0/34, respectively. In the 34 ASCUS cases evaluated by colposcopy, the age distribution varied from 22 to 54 years. Eight of 34 cases did not have a child, 7/34 were primigravida, 18/34 were secondigravida, and 1/34 had four children. Four out of 34 cases were postmenopausal, 3/34 referred no history of abnormal bleeding, 21/34 were smokers, 6/34 used oral contraceptives, 2/34 used intrauterine devices, 1/34 took replacement of hormones, 4/34 had prior abnormal Pap smears human papillomavirus (HPV), or 1/34 had previous cancer (breast cancer). Colposcopy was inconclusive in 4/34 patients, while 8/34 cases were negative for Schiller and acetic acid tests and also had normal colposcopy. Infectious organisms were found in 8/34 patients with ASCUS, including actinomyces (1/8), trichomonas (5/8), and candida albicans (2/8). Histologic tests revealed 16/34 koiloyctosis cases, 5/34 LGSIL, 1/34 HGSIL, and 0/34 SCC. Conclusion: The dilemma in the management of patients with an ASCUS diagnosis still exists as a significant problem for clinicians.

Key words: ASCUS; Papanicolaou smear; Colposcopy; Management.

Introduction
Human papillomavirus (HPV) is considered the primary etiologic agent for cervical cancer in women worldwide [1-3]. Over 100 subtypes of HPV are known with approximately 50 infecting the urogenital tract. Fifteen to 20 of these are associated with cervical cancer and types 16, 18, 31, 45, and 58 cause the majority of all cervical dysplasias and carcinomas [2-5]. Although HPV infection is considered a necessary precursor for the development of the majority of cervical carcinoma, only a small percentage of women with persistent infection progress from low- to high-grade lesions [6]. A significant percentage of cervical cytologic examinations do not yield a definitive diagnosis of dysplasia or a diagnosis within normal limits. These are designated as atypical squamous cells of undetermined significance (ASCUS).

More specifically, the ASCUS category is a cervical cytologic finding that is suggestive, but not definitive, of squamous intraepithelial lesions (SILs). ASCUS remains an incompletely described entity and accounts for even 5%-10% of reported Papanicolaou (Pap) tests [7-8, 4]. ASCUS diagnosis may indicate the presence of histological lesions ranging from cervical intraepithelial neoplasia (CIN) I to cancer in 5% - 17% and 0.2% of cases, respectively [9]. Aside from being commonly found in Pap smears, ASCUS should be considered because it can mask a higher-grade lesion, and the management of these patients remains controversial [10].

The management of women with such cytologic modifications also remains debatable [10-12]. According to the American Society for Colposcopy and Cervical Pathology, women with an initial cervical cytologic diagnosis of ASCUS should be managed by using a program of two repeated cervical cytology tests at six month intervals, immediate colposcopy, or DNA testing for high-risk types of HPV [4, 13]. These three proposed management strategies for patients with ASCUS diagnoses have been compared by using results from the randomized ALTS group trial. This comparison determined that Hybrid Capture II (HCII) testing for cancer-associated HPV DNA has a greater sensitivity to detect high-grade lesions than does cytologic testing and has a comparable specificity to a single additional cervical cytology [14]. In an earlier study, Wright et al. [4] compared the cost per case of detecting high-grade squamous intraepithelial lesions (HGSILs) in women with ASCUS or low-grade squamous intraepithelial lesions (LGSILs) in initial cervical...
cytology. In this study, the patients were managed by repeat cervical cytology or HCII and then followed up by colposcopic examination.

The option of reflex HPV DNA testing in triaging ASCUS for colposcopy seems sensible, particularly in settings where compliance to follow up Pap schedules is a challenge. It may also assure women negative for high-risk HPV, that they do not have a significant lesion, and offer an opportunity for prompt management of those with high-risk results [15].

The practice of regular Pap smear screening to detect premalignant changes in the cervix has been proven to be an effective means of preventing cervical carcinoma. These abnormalities are of concern because approximately 10%-15% of patients with LGSIL [16, 17] and an uncertain proportion of women with ASCUS [18-22] will be found to have a higher-grade lesion at colposcopy. Although immediate referral to colposcopy is advocated by some, it remains to be proven that this is necessary and cost-effective [10, 23, 24]. There are also valid concerns that many women with minor cervical abnormalities are being tested repeatedly or treated unnecessarily, resulting in costs to the healthcare system, estimated to be more than six billion annually in the US alone [10].

The aim of this study was to evaluate the cytology laboratory performance with regard to ASCUS diagnosis, using cervical Pap smears, and colposcopic biopsies obtained from a referral colposcopy clinic over a one-year period. The objectives of this study were: 1) to determine the ASCUS rate and ASCUS/SIL ratio in the laboratory; 2) to compare the colposcopy clinic and referring Pap smear results.

Materials and Methods

A retrospective study of patients with ASCUS Pap smears, taken during the period January 2010 – December 2010, was performed. Most patients enrolled in the present research had an ASCUS report following a Pap smear in the Second Department of Obstetrics and Gynecology, Aretaieion Hospital, and the remainder was referred to this Hospital from other clinics. The study protocol was approved by the Ethical Committee of the Hospital. Women who had been previously diagnosed with SIL or cervical carcinoma, and pregnant women were excluded. Cytological criteria for diagnosis of ASCUS focused on nuclear enlargement, variation in size and shape of the nucleus, mild nuclear hyperchromasia with evenly distributed chromatin, and regularly smooth nuclear outlines. More specifically, ASCUS was established as a diagnostic category to allow the reporting of cervico-vaginal smears that could not be definitively assigned to the normal and benign cellular changes or to SIL categories. ASCUS refers to cellular abnormalities that are more marked to the normal and benign cellular changes or to SIL categories. The diagnostic criteria for ASCUS included: bland nuclear enlargement (two- to three-fold the area of squamous cells nuclei), smooth nuclear membranes, nuclear hyperchromasia, cellular shape, and dimensional changes. These cytological abnormalities are characterized by epithelial changes associated with reactive/inflammatory processes, sampling artifacts, or SIL [10].

All women underwent a colposcopic examination by an experienced colposcopist. Colposcopic examination was performed with a binocular colposcopic device after applying 5% acetic acid solution and painting of the cervix with Lugol’s solution. The examination was considered satisfactory when the entire squamo-columnar junction (SCJ) and the margin of any visible lesion could be visualized with the colposcope. A colposcopy was considered positive when a flat or slightly elevated, mostly well-demarcated, aceto-reactive lesion, a punctuate capillary pattern, or a mosaic pattern could be found after acetic acid application. Colposcopies were defined non-satisfactory if SCJ was not visible during the examination. If the examination suggested any cervical abnormality, a directed biopsy was conducted.

The medical records of all ASCUS patients were reviewed and the retrieved data consisted of the woman’s age, gravidity, parity, menopausal status, as well as any history of abnormal bleeding, smoking, use of oral contraceptives, intrauterine devices, hormonal replacement, prior abnormal Pap smear, or previous cancer. The surgical-pathological files were searched, and clinic records and subsequent cervical vaginal cytologies were checked to determine follow-up diagnosis. Clinicians following and treating these patients were contacted when records were incomplete or not available in colposcopy or in the gynecology clinic. The pathologic material was reported by three pathologists using relatively uniform criteria and terminology. In cases without tissue biopsies, diagnoses were based on the colposcopic examination. Then final diagnoses were correlated to the ASCUS smears. For the patients followed only by cytology, the outcome diagnosis was determined by the most recent Pap smear. For the patients who underwent colposcopic evaluation, the outcome diagnosis was the more significant of either the biopsy or Pap smear taken at the time of colposcopy.

Data was analyzed by SPSS software. When a probability was less than 0.05, the comparison was documented to be significant.

Results

During the study period, 657 Pap smears were examined at the Aretaieion Hospital. Moreover, seven patients, whose Pap smears were cytologically diagnosed with ASCUS, were referred from other clinics, providing a total of 42 cases with a descriptive diagnosis of ASCUS for review. Of the 42 cases, eight were not studied because were either lost in follow-up or did not have available data. The remaining 34/42 patients were evaluated with colposcopic examination and with directed biopsies if necessary. The ratio of ASCUS to LGSIL, HGSIL, or SCC was 5/34, 1/34, and 0/34, respectively.

In the 34 ASCUS cases evaluated by colposcopy, the age distribution varied from 22 to 54 years. Eight of 34 cases did not have a child, 7/34 were primigravida, 18/34 were secondigravida, and 1/34 had four children. Four out of 34 cases were postmenopausal, 3/34 referred no history of abnormal bleeding, 21/34 smoked, 6/34 used oral contraceptives, 2/34 used intrauterine devices, 1/34 took hormonal replacement, 4/34 had prior abnormal Pap smears (HPV), or 1/34 previous cancer (breast cancer).

Colposcopy was inconclusive in 4/34 patients, while 8/34 cases were negative for Schiller and acetic acid tests and also had normal colposcopy. Infectious organisms were found in 8/34 patients with ASCUS, including actin-
or HGSIL in any group (p > 0.05).

No correlation was demonstrated between age, smoking, parity, contraceptive method, or menopausal status at detection of ASCUS, and subsequent detection of LGSIL or HGSIL after ASCUS was diagnosed, the final cytologic diagnosis was made three months after the first ASCUS diagnosis in 5/6 of patients; for 1/6 of patients, the diagnosis was made six months after the initial diagnosis.

No correlation was demonstrated between age, smoking, parity, contraceptive method, or menopausal status at detection of ASCUS, and subsequent detection of LGSIL or HGSIL in any group (p > 0.05).

**Discussion**

In the current study, the authors attempted to evaluate outcomes in the largest group of women diagnosed with ASCUS according to cervical smear reports.

The prevalence of ASCUS varied from 1.8% to 10% in different studies [25-27]. In the present study, ASCUS was found in 42/664 of the Pap smears performed for screening. Such a wide variation may be due to differences in population characteristics and differences in the characteristics of the samples studied. In the current study, the specimens for cytopathologic evaluation were collected at clinics for the purpose of cervical carcinoma screening. Most patients were asymptomatic and were representative of a population at low-risk for cervical carcinoma. It has been claimed that the Thin Prep method lowers the percentage of borderline Pap smear diagnoses such as ASCUS, and increases the percentage of more accurate Pap smears diagnoses such as SILs [28].

In general, the Bethesda System (TBS) recommends that the frequency of ASCUS diagnoses should not exceed two or three times the rate of SIL in any given laboratory [10]. In the present study, the authors found those values to be 42/664 for ASCUS and 12/664 for SIL (4/12 LGSIL, 8/12 HGSIL), which are in accordance with TBS guidelines.

The optimal method for managing a patient diagnosed with ASCUS has not yet being established. Conservative follow-up with repeated cytologic evaluations at shortened intervals has been used as standard practice. The interim guidelines published by the National Cancer Institute suggest that a patient should be referred for colposcopy after the second ASCUS diagnosis within two years [10]. The American College of Obstetrician and Gynecologists however, suggests that patients with at least two consecutive ASCUS diagnoses, or one ASCUS diagnosis with the presence of high-risk factor (such as HPV infection, cigarette smoking, or multiple sexual partners) should be referred for further evaluation [29].

Most reports agree that patients whose cervical smears fall within the ASCUS diagnostic category have a significant incidence of SIL on follow-up biopsies, with rates varying from 13.5% to 58% and the majority being LGSIL [25, 30, 36]. This was also confirmed in the present study. Manos et al. [37] have proposed that the rate of progression from ASCUS to HGSIL is significant, but substantially less than the rate of progression from LGSIL to HGSIL. These same authors suggested that the risk of progression (within two years) from ASCUS to HGSIL is greater than the risk of progression from normal or reactive findings to HGSIL, but less than the risk of progression from LGSIL to HGSIL [37]. ASCUS, therefore, truly is an intermediate cytologic category.

In the present study, among patients with ASCUS who were followed for two years, approximately 5/34 developed LGSIL and 1/34 developed HGSIL. All the diagnoses were confirmed histologically. The incidence of carcinoma in situ or invasive cervical cancer among patients with ASCUS varied between 0.14 and 0.46% [28]. In the present study, the authors did not identify such a patient.

No correlation was found in the present study regarding LGSIL or higher-grade findings with age, parity, menopausal status, smoking, contraceptive methods, or hormonal replacement treatment. This interpretation, however, is limited by the small number of women detected with LGSIL or HGSIL in this segment of the study population.

It should be mentioned that in the postmenopausal period, most common diagnostic tools (Pap smear and colposcopy) used for the diagnosis of CIN have several limitations. Postmenopausal hypoestrogenism could be followed by atrophic changes in the urogenital female tract, such as the involution of cervical tissue. A common finding in postmenopausal women is an increase in the number of diagnosis of ASCUS with a higher ASCUS/LGSIL ratio. However, in postmenopausal women ASCUS has a low-positive predictive value. Mature squamous cells with enlarged nuclei have been classified as ASCUS. However, in postmenopausal women, as opposed to younger women, this bland nuclear enlargement is relatively common and rarely associated with a significant histologic abnormality [38]. This bland nuclear enlargement due to atrophic changes might be the cause of the increased number of ASCUS diagnosis in menopause. Therefore, with a single course of local estrogen replacement therapy, it may be possible to distinguish between benign chorionic villus sampling mimicking atrophy and true preneoplastic changes. Estrogen therapy will often cause enough ectropion of the endocervical cells so that the entire SCJ can be visualized. Moreover, it may reduce

---

Table 1. — Final histological diagnoses of atypical squamous cells of undetermined significance (ASCUS).

<table>
<thead>
<tr>
<th>Final diagnosis</th>
<th>ASCUS n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervicitis</td>
<td>8/34</td>
</tr>
<tr>
<td>Koilocytosis</td>
<td>16/34</td>
</tr>
<tr>
<td>LSIL</td>
<td>5/34</td>
</tr>
<tr>
<td>HSIL</td>
<td>1/34</td>
</tr>
<tr>
<td>SCC</td>
<td>0/34</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
</tr>
</tbody>
</table>

---

Management of ASCUS findings in Papanicolaou smears. A retrospective study
the number of endocervical curettage or loop excision or cone procedure for women with inadequate colposcopic examination [38].

From the study of Rader et al. [26], the association of a diagnosis of ASCUS and the presence of HPV infection also seems clear. Although, the authors did not perform HPV-DNA testing due to the high cost, a number of studies have shown the advantages of using HPV DNA testing to determine which patients with a cytologic diagnosis of ASCUS should be referred to colposcopy and biopsy [4, 13, 14, 38]. These studies have demonstrated that HPV testing is the preferred triage technique for ASCUS specimens. Prior studies have generally investigated ASCUS triage by HPV DNA testing, using the HCH method. Kim et al. [13] pointed out that HPV testing in patients with ASCUS smears may provide the best prognostic information and thus may be the most cost-effective management strategy for these women. For women with ASCUS cytology interpretations, the ALTS data demonstrate that HPV triage is at least as sensitive as immediate colposcopy in the detection of underlying CIN III, while nearly halving the number of women referred for colposcopy. Repeat cytology with colposcopic referral at an ASCUS threshold is also sensitive in detecting CIN III but requires repeated visits and leads to significantly more colposcopic examinations than does a single HPV test [14].

HPV testing was highly-sensitive for detecting cancer in women ASCUS, especially in older women [29]. Thus, HPV testing should be used as a triage test if the cost is not considered. In a situation where follow-up care is uncertain and the patient might be at increased risk, immediate colposcopy could be more effective [10].

Conclusion

The management of patients with an ASCUS diagnosis remains controversial and a significant problem for clinicians. The dilemma in the management of ASCUS patients also stems from the uncertain clinical significance of this diagnosis and the variable prevalence of SIL in these patients.

References


Management of ASCUS findings in Papanicolaou smears. A retrospective study


Address reprint requests to:
C. IAVAZZO, M.D.
Second Department of Obstetrics and Gynecology
University of Athens
Areteiaion Hospital
38, Seizani Str., Nea Ionia
14231 Athens (Greece)
e-mail: christosiavazzo@hotmail.com
Prognostic factors determining recurrence in early-stage endometrial cancer

S. Misirlioglu¹, A.B. Guzel¹, U.K. Gulec¹, D. Gumurdulu², M.A. Vardar¹
¹ Department of Obstetrics and Gynecology, Faculty of Medicine, Cukurova University, Adana
²Department of Pathology, Faculty of Medicine, Cukurova University, Adana (Turkey)

Summary

Objective: This study aimed to determine the clinically important prognostic factors for loco-regional or distant recurrence in early-stage endometrial cancer. Materials and Methods: This study complied with the Declaration of Helsinki, and the local ethics committee approved the study. Cases who underwent primary surgery of early-stage endometrial cancer at the Institution from 2000 to 2012 were reviewed retrospectively. Patients who did not detect recurrence were classified as group 1 (n = 200); those who detected recurrence were classified as group 2 (n = 23). Clinically prognostic factors were evaluated by univariate analyses. Results: The average age for group 2 (LUSI) was 63.8 years (p = 0.0001). Patients with grade 3 histology were all detected within group 2 (p = 0.0001). Endometrioid adenocarcinoma displaying squamous differentiation was found with a rate of 58.3% in group 2 (p = 0.0001). Lower uterine segment involvement (LUSI) and lymphovascular space invasion (LVSI) rates were 86.9% in group 2 (p = 0.0001). The rate of tumor size > 2 cm was 56.6% in group 2 (p = 0.0001). The median depth of myometrial invasion (DMI) was 5.1 mm (p = 0.034) and the average in myometrial thickness was 14.5 mm in group II (p = 0.0001). The percentage of myometrial invasion was 35.8% in Group II (p = 0.0001). Tumor free-distance was 9.4 mm in group 2 (p = 0.0001). Conclusion: Age and clinicopathological parameters of the tumours are significant predictors for recurrence in early-stage endometrial cancer.

Key words: Early-stage endometrial cancer; Prognostic factors; Recurrence.

Introduction

Endometrial cancer is the most common gynecologic malignancy accounting for approximately 43,470 new cancer diagnoses in the United States in 2010 and around the world there are over 198,000 new cases of endometrial cancer per year, and over 50,000 deaths [1, 2]. Although one in 40 women will be diagnosed with uterine cancer in their lifetime, endometrial cancer is one of the most treatable gynecologic malignancies as it often presents early in natural course [3]. Once a diagnosis is made, the cornerstone of management is surgery, consisting of total hysterectomy, bilateral salpingo-oophorectomy, with or without pelvic and para-aortic lymphadenectomy, to determine the Stage of the disease and guide adjuvant treatment [4]. An estimated 90% of patients with endometrial cancer will present symptoms of abnormal or post-menopausal bleeding that allows for early detection. Thus, over 75% of cases are confined to the uterus at the time of diagnosis, resulting in high rates of overall survival. As the majority of patients are diagnosed with Stage I disease, the risk of recurrence within this group is relatively low, ranging from 2-15% [5]. Furthermore, a subset of patients within this group, those with low-grade histology and disease confined to the endometrium, have an even lower rate of recurrence. However, recurrence develops in 40-60% of patients who had metastases to the adnexa or lymph nodes [6]. Therefore, after initial treatment, outpatient follow-up is necessary to detect subclinical recurrence, which may be curable by salvage therapy. For these reasons, endometrial cancer patients make up a large proportion of the patient population routinely followed by gynecologic oncologists.

There is a group of factors which are effective in the determination of the prognosis to consider during post-operative patients’ evaluation. These prognostic factors can be summarized as age, histological grade, histopathological type, LUSI, LVSI, tumor size, tumor free-distance (TFD), depth of myometrial invasion (DMI), myometrial thickness (MT), and percentage of myometrial invasion (MIP) [4-7]. To further improve treatment and follow-up for uterine corpus cancers, a number of molecular markers have been extensively studied. DNA ploidy, hormone receptors, p53, bcl-2, and proliferation markers have already been shown with consistent results to be prognostic factors through retrospective studies [4]. Post-operative evaluation of early-stage endometrial cancers in terms of clinicopathological prognostic factors is to determine the patients who carry the risk of recurrent disease and likely to benefit from adjuvant treatment, consisting in modalities such as radiation, chemotherapy, and hormonal therapy.

The goal of this study was to evaluate clinicopathologic parameters associated with development of recurrence in early-stage endometrial carcinoma by drawing on 12 years of experience at a single institution.

Materials and Methods

All patients who underwent primary surgical staging for endometrial cancer at the Gynecologic Oncology Unit at the Cukurova University Medical Faculty from January 2000 to December 2011 were identified retrospectively. Inclusion criteria were then based on surgical pathological staging system.
which had been accepted by International Federation of Gynecology and Obstetric (FIGO) for endometrial cancer in 1988 and revised in 2009. Patients categorized in the former classification as Stage IA, IB, and IA in the current classification were included to this study. Of those patients, 223 underwent a primary surgery with a total hysterectomy and bilateral salpingo-oophorectomy and peritoneal cytology with or without pelvic and/or para-aortic lymphadenectomy at the Institution. Patients previously provided curative treatment through surgery was not given adjuvant treatment. With reference to the complaints of the patients developing recurrence, they were mainly vaginal bleeding, abdominal pain, and difficulty in breathing. Disease recurrence was diagnosed when physical examination was performed. Cytological or pathological examination, and systemic enhanced computed tomography (CT) indicated recurrent or metastatic tumors during the follow-up period. In 2005, positron emission tomography (PET), with or without CT scan, was performed instead of a routine CT scan in some patients. The histopathologic types of lesions were defined through the tissue biopsies obtained in accordance to the location of the re-laparotomy and lesion under office conditions. These recurrences were classified as isolated vaginal, pelvic, and extra-pelvic. Pelvic relapse was assumed as all of the tumors throughout the actual pelvis not isolated to vagina and pelvic node involvement. Extra-pelvic involvement, on the contrary, was defined as positive para-aortic nodes, abdominal masses, and distant metastasis. Distant recurrence was defined as any metastasis outside the abdominal or pelvic compartment. None of the patients developing recurrence received adjuvant treatment after primary surgery. Seventy-nine percent (n = 176) of these patients were subject to total abdominal hysterectomy and bilateral salpingo-oophorectomy, and 21% (n = 47) were subject to laparoscopic procedure. Pelvic and para-aortic lymphadenectomy was applied in cases in which endometrial sampling and preoperative diagnosis was considered as high-grade and there was both endometrioid histology. Similar to ovarian cancers, omentectomy was performed in cases with clear cell and serous papillary differentiation within the tumor. Among these, those whose lymph node was negative and tumors limited to the uterus were included to this study. Microscopic histopathologic characteristics of surgical specimens were evaluated by an experienced gynecopathologists at our medical faculty. Patients with clear cell and serous papillary within the tumor were all considered to have grade 3 tumors. DMI was measured between the endomyometrial junction and the maximal MI. TFD was calculated by subtracting the DMI from MT. MI was derived by dividing DMI by MT and expressed as a percentage of MT. LUSI was defined as the transition area between corpus and cervix. The size of the tumor was defined as < 2 cm and > 2 cm to be measured on the vertical axes. The clinico-pathological factors analyzed include the age at diagnosis, DMI, MT, TFD, histological grade and type, lymphovascular space invasion, LUSI and tumor size. The study was carried out in accordance with the Helsinki Declaration, and the Ethical Committee of the Çukurova University Faculty of Medicine. Informed consent was obtained from all of the participants. Statistical methodology included analysis of data SPSS 15.0 Evaluation Version (Statistical Package for Social Sciences Chicago, IL, USA) software was used. Categorical measurement was summarized as numbers and percentages; permanent measurement was summarized as mean and standard deviation (where necessary median and minimum-maximum). Chi-square test statistics method was used for the comparison of categorical variables. Mann Whitney U test statistics was used for the comparison of continuous measurement between groups. Odds ratio was calculated for risk measurement. A p value of < 0.05 was considered statistically significant.

### Results

The clinicopathological variables of 223 women with early-stage endometrial cancer (FIGO 1988: Stage 1A and 1B; FIGO 2009: Stage 1A) were analyzed. Histopathological subtypes of the studied cases are shown in the Table 1. Patients who did not detect recurrence were classified as group 1 (n = 200); those who detected recurrence were classified as group 2 (n = 23). The median age at initial treatment and recurrence was 56.0 years (range, 32-79 years) and 63.8 years (range 55-80 years), respectively. Women aged over 60 years were considered an unfavourable factor for the development of recurrence (p = 0.0001) (Table 2).

The mean follow-up time determined was 44.6 months (range 24-60 months). Eighteen (78.2%) recurrences were asymptomatic: the most frequent symptom was vaginal bleeding, which was noted in fifteen patients, followed by abdominal pain in two and cough in one. Five (21.8%) recurrences were asymptomatic within this period. In fifteen (65.3%) women recurrence was vaginally-isolated, in seven (30.4%) women it was pelvic relapse, and one woman (4.3%) developed extrapelvic metastasis (lung). Isolated vaginal recurrence was the most commonly detected in group II (p = 0.0001). Table 2 shows the number of women by site of recurrence (Table 2).

Endometrioid adenocarcinoma with squamous differentiation was found in 14 cases (58.3%) within the recurrence group. In endometrioid adenocarcinomas, it was found that the existence of squamous differentiation increased the risk of recurrence development by 9.3 times in univariate analysis (OR = 9.3, 95% CI 3.77, 23.28; p = 0.0001). In this study, mucinous adenocarcinomas within the group displaying recurrence were not found. Although the p value = 0.023, it was not significantly important because there were only four cases within the group not displaying recurrence. Within the group not displaying recurrence, it was found that four cases consisted in clear cell component (16.7%) and two cases consisted in uterine papillary serous component (8.3%).

#### Table 1. — Histopathological subtypes distribution of 223 early-stage endometrial carcinomas at initial diagnosis.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Group 1 n (%)</th>
<th>Group 2 n (%)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid adenocarcinoma, with squamous differentiation</td>
<td>26</td>
<td>14</td>
<td>9.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>196</td>
<td>23</td>
<td>0.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>31</td>
<td>0</td>
<td>0.8</td>
<td>0.023</td>
</tr>
<tr>
<td>Uterine serous papillary carcinoma</td>
<td>0</td>
<td>2</td>
<td>0.1</td>
<td>0.011</td>
</tr>
<tr>
<td>Clear cell adenocarcinoma</td>
<td>0</td>
<td>4</td>
<td>0.1</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

OR: odds ratio, CI: confidence interval. Group 1: non-recurrent, Group 2: recurrent. A p value < 0.05 was considered statistically significant.
Most uterine corpus cancers are diagnosed at an early stage and have a favorable prognosis. However, a substantial number of patients undergo disease recurrence after primary treatment [4]. As endometrial cancer is the most common gynecologic malignancy and boasts high survival rates due to early detection, it is not surprising that over ten percent of all female cancer survivors are affected by this condition alone [5]. In a meta-analysis by Fung-Kee-Fung et al. [5], recurrence rates for patients with early-stage, low-risk endometrial cancer have been detected in less than 5% [8]. This investigation found a recurrence rate of approximately 10.31%, which is consistent with that found in the literature for all endometrial cancers [range, 6%-25%] [9-11].

In the present study, the authors analyzed factors predictive of pelvic or distant recurrence in a set of 223 patients with FIGO-1988 Stage 1A and 1B, FIGO-2009 Stage 1A, early-stage endometrial carcinoma. The strengths of this study are the sample size and the fact that all patients were staged by histopathology. More than 80% of the recurrences in this study appeared within two years of diagnosis, which concurs with other studies that report a 70%-80% rate within three years [12-14]. Although these values show similarities with the literature, cases in this study were not subjected to risk group-
Prognostic factors determining recurrence in early-stage endometrial cancer

613

recurrence risk and prognosis in uterine carcinoma. These studies have identified prognostic variables such as age, stage at initial diagnosis, histologic grade, DMI, and lymph node status as being associated with recurrence risk [10, 11, 15-18]. Numerous studies have highlighted grade according to this setting. Morrow et al. demonstrated that in patients with Stage I or II endometrial carcinoma, the greatest determinant of recurrence was grade 3 histology with a relative risk of 15 [18]. Creutzberg et al. also described high-risk patients in early-stage endometrial cancer as those with two out of the three following risk factors: older than 60 years, greater than 50% MI, and grade 3 histology [16]. Mariani et al. showed that MI greater than 66% was a significant predictor of distant failure and death in Stage I endometrial cancer patients [11]. Mundt et al. performed a large study including 455 patients with endometrial cancer. They showed that age was significant in a univariate analysis, but was not proven a significant prognostic factor in a multivariate analysis. They suggested that higher rates of recurrence and poorer survival rates reported in the elderly are more likely the result of imbalances in pathological factors and less aggressive therapy [19]. Several studies have also found age to be an independent prognostic factor [14, 20, 21]. Although in this present study cases were not evaluated in terms of prognosis and survival, a significantly important relationship was found between high-grades and recurrences. Furthermore, the average age for recurrent group found was 63.8; this value is statistically significant and compatible to the literature. The very often observation of LUSI and the differences in the definition of histology prevents to come up with a definite conclusion. Lavie et al. have reported that LUSI was significantly associated with grade 3 tumor, deep MI, and the presence of capillary space-like involvement [22]. Gemer et al. showed in patients with apparent Stage I endometroid endometrial cancer, the presence of LUSI was a poor prognostic factor, associated with a significantly higher risk of distal recurrence and death [23]. It is not possible to comment exactly on whether LUSI is an independent prognostic factors or not. However, data on hand shows that there is a significantly important correlation between this parameter and recurrence. LVTI was a predictor of nodal disease and an independent prognostic factor for relapse of disease in all stages of endometrial cancer [24]. In this study, however, the grading of LVTI was not classified as severe or mild. The presence of LVTI was significantly related to poor histological grade and deep MI. The data in the present study demonstrated a strong recurrent rate in patients with LVTI-positive tumors. These results are similar to the previous studies of Briet et al. and Cheewakriangkrai et al. in a general population of women with endometrial cancer [25, 26]. In the present study, it is not possible to state that tumor size was an independent factor; but when the tumor size was > 2 cm, a significant correlation appeared between the disease and recurrence. Shah et al. have reported tumor size correlates with extraterine disease, but it was not an independent prognostic variable [27]. In a multivariate model, TFD was shown to correlate with surgicopathologic variables, recurrence risk, and survival by Lindauer et al. [28]. TFD, like DMI, is predictive of many surgicopathological variables and patient outcome in surgically-staged endometrial cancer. Although the performance characteristics may not be as powerful as DMI, the ease and reproducibility of this measurement may justify its inclusion in synoptic reporting of endometrial cancer. As is mentioned in the literature [28-30], TFD in the recurrence developing cases an almost one cm increased depth of MI. A TFD of one cm maximized the balance of sensitivity and specificity in predicting recurrence. In the present study, there was a statistically significant correlation between the development of recurrence and following clinicopathologic prognostic factors: age, parity, histological grade, histopathological type, LUSI, lymphovascular space invasion, tumor size, tumor free-distance, DMI, MT, and MIP which were in the early-stage endometrial cancer. The data on hand is univariate analysis and as is emphasized in the literature, these variables are effective prognostic factors for predicting recurrence development. Most of the data are characterized by retrospective design, large sample size, multi-prognostic variables, single institute experience, and sufficient follow-up. These data suggest that, in those patients who do recur, it is the intrinsic biology of the tumor that has the greatest prognostic importance.

Conclusion

Today, however, despite several studies that are being conducted in subjects of proto-oncogenes, proliferation markers, endometrial proteins, enzymes and angiogenesis, the clinical use of them is debatable. Although some of them are clinically significant, the additional knowledge they will contribute to the routine evaluation is uncertain. The increase in accumulated knowledge about the prognostic factors predicting recurrence in the early-stage endometrial cancer and an effective analysis of them will help the gynecologic oncologists in choosing an appropriate treatment modality for the prevention of recurrences. The determination of risk groups and avoidance from unnecessary adjuvant treatment will have a positive impact on both medication of the prognosis and cost analysis. Since this study was based on univariate analysis, it could not be concluded whether factors having predictive values for recurrences are independent variables or not. However, data on hand show that there is a strong correlation between clinically important prognostic factors and recurrence development. In order to develop a consensus on the prognostic factors determining recurrences in early-stage endometrial cancers, comprehensive, randomized, and prospective multivariate analyses which also cover molecular mechanisms are needed. Prospective multi-center trials should be performed to make more progress in the treatment of gynecologic cancer patients, including uterine corpus cancer.
References


Address reprint requests to:
A.B. GUZEL, M.D.
Department of Obstetrics and Gynecology
Faculty of Medicine
University of Cukurova
01330 Adana (Turkey)
e-mail: abguzel@gmail.com
Ten years survival of FIGO Stage IIIC epithelial ovarian cancer cases due to lymph node metastases only

E. Grossi¹, S. Noli¹, G. Scarfone¹, A. Villa¹, F. Parazzini¹, S. Cipriani², G. Bolis¹,²

¹First Obstetric and Gynecologic Clinic, University of Milan and IRCCS Foundation, Ospedale Maggiore Policlinico, Mangiagalli, Regina Elena
²Department of Maternity, University of Milan and IRCCS Foundation, Policlinico Mangiagalli Regina Elena, Milan (Italy)

Summary

Purpose of investigation: In this paper the authors have analyzed the long-term survival of women with Stage III ovarian cancer due to lymph node metastasis. Materials and Methods: This retrospective study included 27 patients with FIGO Stage IIIC epithelial ovarian carcinoma due to lymph node metastases observed consecutively at the Mangiagalli Clinic of Milan from 1982 to 2008. Results: Two cases had Fallopian tube carcinoma. A total of ten recurrences were observed. Median time to recurrence was 158 months. The five-year disease-free survival (DFS) was 57.7%. The ten-year corresponding value was 53.2%. Median survival time was 158 months, with median follow-up time of 169 months. The five-year (overall survival) OS rate was 77.1%; the ten-year rate was 55.4%. Conclusion: Women with ovarian cancer Stage IIIC due to nodal involvement have a five-year OS of about 80% and a ten-year OS of about 50%.

Key words: Ovarian cancer; Survival; Node metastases.

Introduction

According to the FIGO staging system, ovarian cancer patients with lymph node metastases, even if the primary tumor is confined to the ovary, are still classified in Stage III (FIGO). These patients represent a low percentage of Stage III ovarian cancers, thus few studies have analyzed their survival [1-6]. Most of them have shown that Stage III epithelial ovarian cancers classified solely by lymph node metastases have a more favorable prognosis than other types of Stage III epithelial ovarian cancers. In particular, few data are available regarding long-term survival, which is the object of the present study.

Materials and Methods

This retrospective study included all patients at FIGO Stage IIIC epithelial ovarian carcinoma due to lymph node metastases observed consecutively at the Mangiagalli Clinic of Milan from 1982 to 2008. They were identified among a total of 1,120 ovarian cancer cases diagnosed and/or treated during the study period.

The inclusion criteria involved positive retroperitoneal lymph nodes in patients with disease apparently confined to the ovary. Patients with omentum, Douglas, and diaphragm metastases, also if microscopic, were not included. A total of 27 cases were identified.

Surgical staging included total hysterectomy, bilateral salpingo-oophorectomy, complete removal of the omentum, peritoneal washing, plus lymph node biopsies (seven cases), or total lymphadenectomy (20 cases).

A total of 25 cases were treated with first-line platinum-based chemotherapy, either alone or in combination with taxol or cyclophosphamide, for six cycles, according to the ongoing studies at the time of diagnosis. Second-line chemotherapy was also administered according to the ongoing studies at the time of diagnosis.

Median follow-up was updated on April 2010 and was 169 months. Overall survival (OS) and disease-free survival (DFS) were calculated from the date of surgery. Survival curves and rates were computed using the Kaplan-Meier method [7].

Results

Table 1 shows the characteristics of the study patients. Mean age at diagnosis was 54 years (range 30 to 73).

Twelve women had serous carcinoma (44.4%), two mucinous (7.4%), three endometrioid (11.1%), two clear cell (7.4%), and six mixed types (22.2%). A total of three cases had grade 1 tumor, one had grade 2, and 23 had grade 3 (85.2%). Two cases had Fallopian tube carcinoma.

During lymphadenectomy, the average number of lymph nodes removed was 13. Aortic lymph nodes were affected in 16 cases (59.3%), pelvic nodes in 19 cases (70.4%) and both in all cases. Two patients (7.4%) had metastases in the hypogastric nodes.

Cytoreductive surgery was optimal (residual tumor measured < 1 cm or absent) in all cases, except in one case. Cytology of peritoneal washing was positive in five patients. Twenty-two patients had complete response to chemotherapy, three patients had partial response, and two had no response. A total of ten recurrences were observed. The sites of recurrence included: iliac, aortic, inguinal, and cervical nodes (three cases); pelvis (three cases); liver, lung, spleen (two cases); peritoneum (one case); vagina (one case).

The five-year DFS was 57.7%. The ten-year corresponding value was 53.2%. A total of 13 deaths were observed; of these, three patients died for causes not related to neoplasm and had a brief survival after diagnosis. Median survival time was 158 months. The five-year OS rate was 77.1%, and the ten-year rate was 55.4% (Figure 1).
Discussion

The results of this study show that women with ovarian cancer Stage IIIC due to nodal involvement have a five-year OS of about 80% and ten-year OS of about 50%. This high survival rate is consistent with previous published studies showing a better survival in patients with Stage IIIC epithelial ovarian cancer based on nodal involvement only, than patients with the same Stage with abdominal disease and/or carcinomatosis [1-3].

Onda et al. [1] compared patients with ovarian cancer limited to the pelvis, but upstaged to Stage IIIC based on lymph node positivity vs patients with tumor limited to the pelvis and negative lymph node and patients with intraperitoneal tumor spread beyond the pelvis irrespective of lymph node status. That study showed that patients with intraperitoneal tumor limited to the pelvis and positive lymph nodes had fairly good five-year survival (84%). The survival difference between this group and the group with a tumor limited to the pelvis without nodal involvement was not statistically significant (84% vs 96%, p = 0.107). Additionally, the five-year survival of patients with nodal involvement and disease limited to the pelvis was significantly better than patients with intraperitoneal tumors spread beyond the pelvis at the same lymph nodal status (84% vs 26%, p = 0.042), although these two groups belonged to the same Stage IIIC according to FIGO staging.

Kanazawa et al. analyzed retrospectively 125 patients according to clinical, histological criterions, especially according to nodal status. Patients lymph node only upstaged to Stage IIIC had a better survival than patients classified as Stage IIIC because of abdominal disease larger than two cm at diagnosis (< 0.0001) [2].

Cliby et al. [3] analyzed 115 patients with Stage IIIC epithelial ovarian cancer to describe the clinical behavior of occult Stage IIIC. In their series, 36 patients were upstaged to Stage IIIC by virtue of positive nodes and 69 patients were classified as Stage IIIC because of abdominal disease larger than two cm. The five-year survival of this study group was 76%.

In conclusion, this study shows a high long-term survival in women with Stage IIIC due to nodal involvement ovarian cancer.

Table 1. — Characteristics of the study subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean, range)</td>
<td>54 (30-73)</td>
</tr>
<tr>
<td>Histologic type</td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>12 (44.4)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Endometroid</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Mixed</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>Fallopian tube adenocarcinoma</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td>2</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>3</td>
<td>23 (85.1)</td>
</tr>
</tbody>
</table>

References


Address reprint requests to:
F. PARAZZINI, M.D.
First Obstetric and Gynecologic Clinic
University of Milan and IRCCS Foundation
Policlinico Mangiagalli Regina Elena
Via Commenda, 12
20122 Milan (Italy)
E-mail: fabio.parazzini@marionegri.it
Epidermoid or dermoid cysts of the ovary?
Clinicopathological characteristics of 28 cases and a new pathologic classification of an old entity

A. Kondi-Pafiti, A. Filippidou-Giannopoulou, E. Papakonstantinou, C. Kleanthis
C. Iavazzo, C. Grigoriadis
Pathology Laboratory, Aretaieion Hospital, University of Athens, Medical School, Athens (Greece)

Summary

Objective: The aim of this study was to present the clinical and pathological findings that aid in the differential diagnosis between epidermoid and dermoid ovarian tumors. Materials and Methods: This was a 15-year retrospective clinicopathological study. A total of 28 cases of epidermoid ovarian cysts histologically confirmed after pathological examination at the Pathology Laboratory of Aretaieion University Hospital between January 1996 and December 2010, were analyzed and a literature review was performed. Results: Patients with epidermoid cysts presented with a main complaint of either abdominal pain or a palpable abdominal mass. In the 28 cases studied, 18 patients underwent cystectomy and four cases underwent oophorectomy. In six cases of post-menopausal women, abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. No recurrent disease in the pelvis was reported during the available follow-up period which was from 12 to 30 months. Discussion: Epithelial epidermoid ovarian tumors represent less than one percent of ovarian surface epithelial tumors. The differential diagnosis of epidermoid cysts includes dermoid (mature cystic teratomas) tumors of the ovary. However, it should be mentioned that up to 17% of teratomas may include epidermoid tumors. In comparison to dermoid cysts which present at an earlier age but with a greater size, ovarian epidermoid tumors present as small- to medium-sized cystic lesions occurring at a significantly older age. The treatment of choice is conservative surgical therapy.

Key words: Ovarian tumors; Mature cystic teratomas, Dermoid cysts.

Introduction

Ovarian tumors with epithelial elements constitute common epithelial tumors, which are mainly composed of various glandular cell types, with the exception of Brenner or transitional cell-type tumors. Ovarian tumors with squamous cell elements are rare and until recently were represented only by mature cystic teratomas, characterized by the presence of stratified squamous cell epithelia in the form of skin, found in the interior of the cysts. Ovarian tumors with morphology of squamous cell carcinomas which are the only squamous cell malignant neoplasms of the ovary, develop from the malignant transformation of epithemoid elements of teratomas.

Mature cystic teratomas or dermoid cysts, are one of the most common tumors (up to 20% of all ovarian neoplasms) that occur in women during their reproductive life [1]. The current classification of teratomas comprises a number of histological types of tumors, such as mature cystic teratomas and monodermal teratomas. Mature cystic teratomas are characterized by the development of various mature tissues, of ectodermal (skin, brain), mesodermal (muscle, fat), and endodermal (mucinous or ciliated epithelium) origin. Monodermal teratomas present basically one tissue type (thyroid tissue in struma ovarian and neuroectodermal tissue in carcinoid tumor). The basic characteristic of all teratomas is the presence of mature or immature tissues of germ cell (pluripotential) origin.

Materials and Methods

This was a 15-year retrospective study. Between January 1996 and December 2010, 2,500 cases of ovarian tumors were examined in the Pathology Laboratory of Areteiaion University Hospital. The cohort included 225 cases of mature cystic teratomas that were identified and re-evaluated, and 24 cases originally diagnosed as dermoid cysts that were re-classified as epidermoid cysts. During the last five years, four cases of epidermoid cysts were classified as epithelial tumors according to the last WHO classification criteria. Pathologic examination of the epidermoid cysts was performed according to standard histological examination procedures, including histological sectioning of paraffin-embedded formalin-fixed tissues, stained by Hematoxylin &
Discussion

Epithelial epidermoid ovarian tumors represent less than one percent of ovarian surface epithelial tumors. The literature review revealed reports with a limited number of patients ranging from one up to 14 patients presenting a heterogeneous group of tumors with histogenesis not yet clarified [1]. Peters et al. [5] suggested a possible common origin from pluripotential coelomic epithelium, based on the similar immunohistochemical profile between epidermoid and endometrioid ovarian neoplasms. Young et al. [6], based on a comparative study of epidermoid cysts, Walthard cell nests, and the epithelial components of a Brenner tumor, support the hypothesis that epidermoid ovarian tumors originate from epithelial cell nests by the same mechanism as Brenner tumors do. However, according to Nogales et al. [7], some cases of epidermoid cysts may be of germ cell origin, and with the same mechanism can be expressed in the respective lesions of the testis. In five cases in the present study, the squamous epithelium lining of the cysts resembled transitional-type cells by standard H&E staining but immunohistochemical investigation of the cells for the expression of uroplakin urothelial marker was negative. There are reports in the literature, that small epidermoid cysts may be incidental findings in hysterectomy specimens [2, 8, 9].

The differential diagnosis of epidermoid cysts includes dermoid (mature cystic teratomas) tumors of the ovary [2] (Figure 2). However, it should be mentioned that up to 17% of teratomas may include epidermoid tumors [4]. Khedmati et al. [2] studied sixteen ovarian epidermoid cysts, representing 1.5% of ovarian surface epithelial tumors, and reported that all tumors were unilateral and small in size. The mean age of the patients was 55 years and is the same as in the present study. In comparison to dermoid cysts which present in an earlier age but with a greater size, ovarian epidermoid tumors present as small-to-medium-sized cystic lesions occurring at a significantly older age. The treatment of choice is conservative surgical therapy.
A re-classification of ovarian epidermoid tumors as of epithelial origin rather than including them in the large group of teratomas, was suggested by WHO in 2003. This is based on the fact that they are characterized by different pathological findings and benign behavior. Because of the rarity of this ovarian tumor, there is yet little evidence in order to understand its origin and the possibility of malignant behavior; therefore, an international database is needed to collect all necessary information in order to recommend the best treatment options.

References


Address reprint requests to:
A. KONDI-PAFITI, M.D.
Areteion Hospital
Pathology Laboratory
Vas. Sofigas 76 Av.
Athens 11528 (Greece)
e-mail: akondi@med.uoa.gr
A retrospective analysis of endometrial carcinoma cases surgically treated with or without para-aortic lymph node dissection followed by adjuvant chemotherapy

M. Okazawa¹, Y. Ueda², T. Enomoto², K. Yoshino², K. Kono², S. Mabuchi², T. Kimura², M. Nagamatsu¹

¹Department of Obstetrics and Gynecology, Kaizuka City Hospital, Hori, Kaiduka, Osaka
²Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine, Yamadaoka, Suita, Osaka (Japan)

Introduction

The incidence of endometrial cancer is already the most common gynecological cancer in the United States and has increased significantly over the last three decades. Surgical endometrial cancer therapy consists in a hysterectomy, bilateral salpingo-oophorectomy, and retroperitoneal lymph node dissection [1, 2]. Aggressive cytoreductive surgery for advanced cases with extra-pelvic and distant metastatic diseases has been demonstrated to possibly improve the prognosis [3, 4]. However, the prognostic significance of routine dissection of the retroperitoneal para-aortic lymph nodes (PAN) has been unclear. Several recent randomized studies have indicated that systematic dissection of the pelvic lymph nodes (PLN) plus para-aortic lymph nodes (PAN) or PLN-only dissections were performed for endometrial carcinomas. An adjuvant chemotherapy using paclitaxel, epirubicin, and carboplatin was conducted for all such patients. A retrospective comparison of the efficacy of PAN dissection was conducted. Results: Disease-free and overall survivals and frequency of PAN involvement at the first recurrence did not exhibit a statistically significant difference between the PLN-only group and the PLN + PAN group. Operation time was significantly longer in the PLN + PAN group than the PLN-only group, and the total blood loss was also significantly greater in the PLN+PAN group. Conclusion: PAN dissection may be omitted, without adverse effect on prognosis, for endometrial carcinoma patients with recurrence risks who undergo adjuvant chemotherapy using platinum, anthracycline and taxane derivatives.

Key words: Endometrial carcinoma; Para-aortic lymph node dissection; Adjuvant chemotherapy; Prognosis.

Summary

Purpose: To analyze the efficacies of para-aortic node (PAN) dissection for patients undergoing surgery and adjuvant chemotherapy for endometrial carcinomas. Methods: At the Osaka University Hospital and the Kaizuka City Hospital in Osaka, Japan, either pelvic lymph nodes (PLN) plus para-aortic lymph nodes (PAN) or PLN-only dissections were performed for endometrial carcinomas. An adjuvant chemotherapy using paclitaxel, epirubicin, and carboplatin was conducted for all such patients. A retrospective comparison of the efficacy of PAN dissection was conducted. Results: Disease-free and overall survivals and frequency of PAN involvement at the first recurrence did not exhibit a statistically significant difference between the PLN-only group and the PLN + PAN group. Operation time was significantly longer in the PLN + PAN group than the PLN-only group, and the total blood loss was also significantly greater in the PLN+PAN group. Conclusion: PAN dissection may be omitted, without adverse effect on prognosis, for endometrial carcinoma patients with recurrence risks who undergo adjuvant chemotherapy using platinum, anthracycline and taxane derivatives.

Key words: Endometrial carcinoma; Para-aortic lymph node dissection; Adjuvant chemotherapy; Prognosis.
In the present study, the authors also performed a retrospective comparison of PLN-only versus PLN + PAN dissection in endometrial carcinoma cases with intermediate or high risk factors for recurrence, all of whom received TEC chemotherapy as an adjuvant therapy.

Materials and Methods

A retrospective comparison of the efficacies of PLN-only versus PLN + PAN dissection was conducted in the endometrial carcinoma cases treated from 2002-2009 at the Osaka University and Kaizuka City hospitals. Both types of dissections were performed under the same indications, including a myometrium invasion depth of > one-half of total thickness and / or an atypical histology (such as endometrioid adenocarcinoma grade 3, clear cell carcinoma, or serous papillary carcinoma). In these cases, a regimen of TEC (150 mg / m² paclitaxel, 50 mg / m² epirubicin, and AUC 4 carboplatin) was administered every three to four weeks for three courses. For more advanced Stage III and IV cases, six courses of TEC therapy were given.

The approved protocol for TEC administration was that it was only to be given to patients who were 70 years of age or less; the present comparative analysis was thus somewhat artificially limited to those under 70 years of age. In addition, chemotherapy was performed only in those patients who were expected to have an estimated remaining survival rate greater than three months.

The cases in which a PAN swelling occurred that was already easily detectable by computed tomography (CT) or magnetic resonance imaging (MRI), and that were therefore strongly suspicious for metastasis, were excluded from the present study. Advanced cases with a tumor that could not be completely excised were also removed from the study. Eligibility for TEC chemotherapy required that the patients had adequate findings in the following: hematology (WBC ≥ 3,000 / µl, platelets ≥ 100,000 / µl, granulocytes ≥ 1,500 / µl and hemoglobin > 10 g / dl), renal (creatinine ≥ 2 mg / dl) and hepatic (bilirubin ≥ 3 mg / dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≥ 2 times the international normal value). A relative performance status of zero to two was needed. The tumors needed to be histopathologically diagnosed as an endometrial carcinoma. The histological diagnoses were made by authorized pathologists from the Departments of Pathology of the Osaka University and Kaizuka Hospitals, who were all trained at the Osaka University Hospital. The gynecologic surgeons who performed the surgical treatments were also all trained at the Osaka University Hospital, and the surgical procedures and indications for retroperitoneal lymph node dissection were identical in the two hospitals. Moreover, adjuvant chemotherapy was performed using similar indicators.

In the current study, the clinicopathological features of the cases, including age of the patient, histology, stage of the disease, metastatic status of PLN and PAN, frequency of PAN involvement at the first recurrence, were analyzed by the Fisher’s exact test. DFS and OS curves were calculated. DFS was measured from the administration of chemotherapy to the date of the radiologic or pathologic diagnosis of recurrence, or to the date of the last follow-up. OS was defined as the period from the beginning of chemotherapy to the patient’s disease-related death, or to the date of the last follow-up.

Statistical analysis

MedCalc (MedCalc Software, Mariakerke, Belgium) was used for the statistical analyses. The distribution of patients’ age, operation time, and blood loss during surgery was analyzed by the Mann-Whitney U-test. Tumor histology and Stage and the frequency of PAN involvement at the first recurrence, were analyzed by the Fisher’s exact test. DFS and OS curves were calculated.
Table 1. — Characteristics of patients treated with or without PAN dissection.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PLN</th>
<th>PLN + PAN</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (cases)</td>
<td>35</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Median age (years)</td>
<td>58 (40 - 69)</td>
<td>58 (44 - 70)</td>
<td>0.83</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td>0.24</td>
</tr>
<tr>
<td>endometrioid</td>
<td>22</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>non-endometrioid</td>
<td>13</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td>0.062</td>
</tr>
<tr>
<td>I/II</td>
<td>22</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>III/IV</td>
<td>13</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

Background characteristics of the two groups, PLN-only and PLN + PAN, were not significantly different.

PLN: patients in which only PLN dissection was performed (Kazuka City Hospital cases).
PLN + PAN: patients in which both PLN and PAN dissection were performed (Osaka University Hospital cases).

Table 2. — Frequency of PLN and PAN metastases in the patients who underwent PLN and PAN dissection.

<table>
<thead>
<tr>
<th>Metastasis</th>
<th>PLN</th>
<th>PAN</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>46 (67%)</td>
<td>6 (9%)</td>
<td>52 (75%)</td>
</tr>
<tr>
<td>positive</td>
<td>11 (16%)</td>
<td>17 (25%)</td>
<td>32 (42%)</td>
</tr>
<tr>
<td>Total</td>
<td>57 (83%)</td>
<td>37 (52%)</td>
<td>94 (100%)</td>
</tr>
</tbody>
</table>

Number of the cases with or without metastasis to PLN and PAN in the patients who underwent PLN + PAN dissection are shown.

Results

Clinical characteristics of the cases in which PLN dissection and those in which both PLN and PAN dissections were performed

Under the same indications of retroperitoneal lymph node dissection and adjuvant TEC chemotherapy, 35 patients underwent PLN dissection followed by TEC therapy at the Kazuka City Hospital, and 69 patients underwent PLN and PAN dissection followed by TEC therapy at the Osaka University Hospital during the study period. Clinical characteristics, including the age distribution of the patients and the histology and Stage of the disease, did not demonstrate a significant difference (Table 1); however, the cases in which dissection of both PLN and PAN was performed tended to be in more advanced stages (p = 0.062 by Fisher’s exact test).

Frequency of metastasis at PLN and PAN among the patients with an indication of retroperitoneal lymph node dissection

The status of metastasis to the PLN and PAN was analyzed (Table 2). Among the 69 women with risk factor indications who received both PLN and PAN dissections followed by TEC therapy, 46 patients (67%) had no metastasis at either their PLN or PAN, and 11 patients (16%) had metastasis in both PLN and PAN. There were six cases (9%) without PLN metastasis but with PAN metastasis. Because these cases did not have any other metastasis, it was the PAN dissection that detected the PAN metastasis, which led to an upgrade of the cases to a higher Stage. If these patients had received only a PLN dissection without a PAN dissection, they would have been incorrectly classified as Stage I / II. This is the primary reason why the cases in which dissection of both PLN + PAN performed numerically tended to be in more advanced stages (Table 1).

Survival effect of PAN dissection in patients with an indication of retroperitoneal lymph node dissection

The DFS and OS curves of the PLN-only and PLN + PAN dissection groups are shown in Figure 1. The median follow up period was 29 months (3 - 63 months) and 36 months (3 - 91 months), respectively. DFS and OS did not exhibit a statistically significant difference between the PLN group and the PLN + PAN group (p = 0.039 by the log-rank test, Hazard Ratio: 1.4316; 95% CI: 0.5916 - 3.4643; and p = 0.66 by the log-rank test, Hazard Ratio: 1.2473; 95% CI: 0.4488 - 3.4665, respectively).

Frequency of the first recurrence to the PAN

The frequency of PAN involvement during the first recurrence of the tumor was compared between the two study groups (Table 3). In the PLN-only group, the first recurrence at PAN was detected in three (33%) of nine cases, and on the other hand, it was observed in four (29%) of 14 cases in the PLN + PAN group. A statistically significant difference was not detected (p = 0.81 by Fisher’s exact test).

Table 3. — Comparison of the first recurrent site between the cases in which only PLN was dissected and those in which both PLN and PAN were dissected.

<table>
<thead>
<tr>
<th>First recurrent site</th>
<th>PLN</th>
<th>PLN + PAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAN</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Others</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

The frequency of PAN involvement at the first recurrence between the two groups was not different significantly (p = 0.81).

PLN: patients in which only PLN dissection was performed. PLN + PAN: patients in which both PLN and PAN dissection were performed.

Table 4. — Comparison of operation time and blood loss during surgery between the cases in which only PLN was dissected and those in which both PLN and PAN were dissected.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PLN</th>
<th>PLN + PAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation time (min)</td>
<td>214 (167 - 389)</td>
<td>385 (184 - 670)</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>590 (150 - 2590)</td>
<td>770 (200 - 3300)</td>
</tr>
</tbody>
</table>

Operation time was significantly longer in the PLN + PAN group than the PLN-only group (p < 0.001 by Mann-Whitney U test), and total blood loss was also significantly more in the PLN + PAN group than the PLN group (p = 0.015 by Mann-Whitney U test).

PLN: patients in which only PLN dissection was performed. PLN + PAN: patients in which both PLN and PAN dissection were performed.
A retrospective analysis of endometrial carcinoma cases surgically treated with or without para-aortic lymph node dissection etc.

Adverse effects of PAN dissection

The total operation time and total blood loss during surgery were compared between the PLN group and the PLN + PAN group (Table 4). As would be expected for the more extensive surgery required, the operation time was significantly longer in the PLN + PAN group than the PLN-only group ($p < 0.001$ by Mann-Whitney U test), and the total blood loss was also significantly more ($p = 0.015$ by Mann-Whitney U test).

Discussion

Chemotherapy following surgery has superseded radiotherapy in the treatment of endometrial carcinoma. However, both the significance of PAN dissection and the optimal regimen of chemotherapy have been controversial issues. Although improved survival rate has been shown following TAP therapy, a number of severe toxicities were observed [13]. In the authors’ own recent phase I / II prospective studies in the Japanese population, it was shown that TEC therapy was a safer and more effective regimen.

Although a few randomized studies have demonstrated that systematic dissection of the PLN has little therapeutic value for early-stage endometrial carcinoma [5, 6], a retrospective SEPAL cohort study showed that there was a possible therapeutic role for a combined dissection of PLN and PAN in those endometrial carcinoma cases with intermediate or high risk factors of recurrence [7]. However, even after the Todo et al. study, the significance has remained unclear for PAN dissection in women who had received adjuvant chemotherapy, especially those using platinum, anthracycline, and taxane derivatives (which are regarded as gold standard drugs for treatment of advanced or recurrent endometrial carcinomas) [9, 10, 12].

In the present study, the authors undertook a retrospective comparison of PLN versus PLN + PAN dissection in endometrial carcinoma cases with intermediate or high risk factors for recurrence, all of whom received TEC chemotherapy as an adjuvant therapy. Among the 69 women with adverse indications who received PLN + PAN dissections followed by TEC therapy, there were six cases (9%) without PLN metastasis, but with PAN metastasis (Table 2). This result suggests that PAN dissection is important for accurate staging of the disease. This upgraded staging led to a higher frequency of Stage III / IV cases in the PLN + PAN group than in the PLN group (Table 1). However, even including these cases, it was demonstrated that PAN dissection did not improve the prognosis of the endometrial carcinoma patients with intermediate or high risk factors for recurrence who underwent adjuvant TEC chemotherapy (Figure 1). The present results might imply that the role of PAN dissection is currently limited to staging of the disease. PAN dissection may not be required for patients with sufficient known adverse risk factors for recurrence, as they would receive adjuvant chemotherapy regardless, and especially when the regimen is going to be one of the more advanced combinations of platinum, anthracycline, and taxane derivatives.

The current study also includes additional evidence that PAN dissection was unnecessary. The frequency of PAN recurrence after surgery followed by adjuvant TEC chemotherapy was 33% (three of nine cases) in the PLN-only group and 29% (four of 14 cases) in the PLN + PAN group, suggesting that initial PAN dissection did not guarantee later reduced PAN recurrence (Table 3). Moreover, PAN dissection led to more adverse effects. The operation time was significantly longer (with its associated risks) and total blood loss was significantly more in the PLN + PAN group than the PLN group (Table 4).

In this retrospective study, the authors showed that PAN dissection did not reduce PAN recurrence and did not improve the overall prognosis of patients with recurrence risks in cases where they received adjuvant chemotherapy using platinum, anthracycline, and taxane derivatives. It was also shown that PAN dissection was accompanied with a surgical burden. Further prospective studies that analyze the necessity of PAN dissection, followed by current modalities of chemotherapy, are still required.

Conclusion

PAN dissection may be omitted without having an adverse effect on prognosis, in endometrial carcinoma patients with recurrence risks also receiving an adjuvant chemotherapy using platinum, anthracycline, and taxane derivatives.

Acknowledgements

The authors would like to thank Dr. G. S. Buzard, U.S. CDCP, for his constructive editing of this manuscript. The authors are also grateful to Ms. S. Sugiyama and Ms. K. Nakano for their dedicated and excellent bioinformatics work extracting patient data from medical records.

References


Address reprint requests to:
Y. UEDA, M.D., Ph.D.
Department of Obstetrics and Gynecology
Osaka University Graduate School of Medicine
2-2, Yamadaoka, Suita
Osaka 565-0871 (Japan)
e-mail: zvf03563@nifty.ne.jp
Overexpression of c-Met in cervical intraepithelial neoplasia

C. Çomunoğlu1, K. Boynukalın2, M.G. Uğur3, R. Al4, G. Mocan Kuzey1, C. Baykal5

1Near East University, Faculty of Medicine, Department of Pathology, Nicosia, Turkish Republic of Northern Cyprus, 2Anatolia Women’s Health Center, Ankara 3Gaziantep University School of Medicine, Department of Obstetrics & Gynecology, Gaziantep 4Atatürk University School of Medicine, Department of Obstetrics & Gynecology, Erzurum 5Florence Nightingale Hospital, Department of Obstetrics & Gynecology, Istanbul (Turkey)

Summary

Purpose of investigation: The purpose of this study is to evaluate the significance of the c-Met / Hepatocyte Growth Factor Receptor (HGFR) expression in cervical intraepithelial neoplasia (CIN). Materials and Methods: Twenty-one patients from two types of cervical intraepithelial neoplasias (LSIL and HSIL), diagnosed in our clinic were studied with c-Met immunohistochemistry. Of the 21 cases, five were diagnosed as LSIL and 16 as HSIL. Normal cervical mucosas from five patients were studied with c-Met as control cases. Results: Overexpression of c-Met was found in all five of LSIL specimens. C-Met overexpression was observed in 11 cases of HSIL. No c-Met overexpression was seen in any of the five control cases. Conclusion: These results revealed that c-Met oncogene overexpression is an important parameter in cervical early oncogenesis and may have a role in malignant transformation of cervical epithelial cells.

Key words: c-Met / HGFR overexpression; Cervical intraepithelial neoplasia; Immunohistochemistry.

Introduction

A variety of growth factors, such as epidermal growth factor (EGF), transforming growth factor-alfa (TGF-α), and transforming growth factor-beta (TGF-β), appear to play a crucial role in human carcinogenesis. The hepatocyte growth factor HGF / receptor system has multifunctional properties, such as: cell proliferation [1], cell movement [2] and morphogenesis [3, 4]. The receptor for HGF is a protein product of a protooncogene c-Met [5, 6] which encodes a transmembrane tyrosine kinase (P190 c-Met) with structural and functional features of a growth factor receptor [7-9]. Autophosphorylation of this receptor by ligand binding stimulates its intrinsic tyrosine kinase activity with resultant changes in cellular morphology, motility, and growth.

Overexpression of this oncogene was shown in different human solid tumors such as hepatomas, carcinomas of colon, rectum, stomach, pancreas, thyroid, kidney, ovary, endometrium, bladder, breast, and prostate [10-22]. As cervical preinvasive pathologies seem as a perfect model to analyze carcinogenesis step by step from the disturbance of cell proliferation and/or differentiation in squamous epithelium, via low and high grade CIN, also called squamous intraepithelial neoplasia (SIL), to invasive carcinoma [23]. The certain role of human papilloma virus (HPV) in the development of genital intraepithelial neoplasia has been shown with enough evidence [24]. All genital condylomas, and most intraepithelial neoplasia and cervical invasive cancers contain HPV DNAs [23, 25]. HPV types 16, 18, 31, and 33 are most commonly found in cervical, vaginal or vulvar neoplasia, whereas types 6 and 11 are linked to condyloma acuminata or regressive dysplasia [26].

This study included 21 patients diagnosed with CIN in Near East University Hospitals, TRNC during year 2010 and five patients with normal mucosa in 2011. All patients were evaluated and diagnosed by gynecologists according to the American Society of Colposcopy and Cervical Pathologies (ASCCP) guidelines. Five of the specimens were CIN 1, and 16 were CIN 2-3.

Materials and Methods

Immunohistochemical Analysis

Immunohistochemical evaluation was performed according to the authors’ previous studies [31-33]. Formalin-fixed and paraffin-embedded specimens of primary lesions were studied simultaneously. The observers were unaware of the clinical data. Four micrometer sections were deparaffinized in xylene and rehydrated. Antigen retrieval procedure was performed in 50x Tris ethylenediaminetetraacetic acid (EDTA) buffer (pH: 9) in pressure cooker and incubation was done in 20x TBS (Tris buffered Saline Solution) for 15 minutes. Nonspecific protein blockage was performed with peroxidase blocking reagent. All of the immunohistochemical procedures were performed at room temperature. Slides were incubated with polyclonal anti-c-Met antibody (Novocastra, Leica Microsystems; dilution: 1/25). Subsequent procedures were performed using Dako

XXXIII, n. 6, 2012

Revised manuscript accepted for publication April 16, 2012
EnVision Flex (Dako K8000 En Vision Flex Kit, Denmark) immunoperoxidase staining kit. The antibody was visualized by freshly prepared solutions of 0.04% 3',3'- diaminobenzidine tetrahydrochloride and 0.03% hydrogen peroxide and the sections were counterstained with haematoxylin, cleaned and mounted. Positive immunostaining was localized to cytoplasmic membrane. Immunoreactivity was evaluated according to the number of the stained cells and the intensity of staining. An overexpression criterion was defined as discrimination of the dysplastic epithelium and neighboring normal epithelium by staining difference. Extensiveness of staining was scored as 0 = 0%, 1 = 1 - 30%, 2 = over 30%. Intensity was scored as 1 (mild), 2 (moderate), and 3 (intense). A numerical value is gained by product of these two scores. A final score between 0 - 3 was accepted as negative; a score greater than 3 was accepted as overexpression.

Statistical analysis

Chi-square was used where appropriate. Differences between groups were tested using the log-rank test; p values of < 0.05 were considered statistically significant. Calculations were done using the SPSS for Windows version 14.0 statistical software.

Results

Results of immunohistochemical analysis are summarized in Table 1. Overexpression of c-Met was found in all 5 of LSIL specimens (100%). C-Met overexpression was observed in 11 of 16 of HSIL cases (69%). Overall c-Met overexpression was found in 16 out of 21 (76%) cervical dysplasia cases (Figures 1 and 2). Staining was membranous and cytoplasmic, distinct in dysplastic tissue. C-Met overexpression was not seen in any of the five control cases.

Discussion

Cervical carcinogenesis and early development of cervical preinvasive lesions are currently being extensively evaluated from several perspectives. Early carcinogenesis of the cervix is directly related to the oncogenic changes caused by HPV [34, 35]. Many genetic changes and growth factors are found to play role in these changes; Epithelial Growth Factor Receptor (EGFR), c-erbB2, Insulin Like Growth Factor (IGF-1 and IGF-2) and Vascular Endothelial Growth Factor (VEGF) are some well-known ones [36-44]. Apart from their oncogenic role, some of them are found to be related to patients' survival and prognosis and are being used for therapeutic aims [45-47].
Hepatocyte Growth Factor Receptor (HGFR) is a protein product of a proto-oncogene c-met encoding a trans-membrane tyrosine kinase, has structural and functional features of other growth factor receptors [8, 9, 48]. Its auto phosphorylation by ligand binding stimulates its intrinsic tyrosine kinase activity with resultant changes in cellular morphology, motility, and growth. Ligand of HGFR and HGF/SF is implicated in the mode of stromal invasion or aggressiveness of human cervical squamous carcinoma cell lines [49]. It is well known that transfection of cells that are c-Met negative with the HGF/SF receptor gene results in an increased motile and invasive nature [48-51], demonstrating the potential of this oncogene in enhancing cellular properties that are central to the initiation and development of metastasis. It is therefore possible that preinvasive status and malignant cells with aberrant expression of the c-Met protein are prone to have more aggressive and severe cell behaviors.

The step-by-step progression model of cervical preinvasive diseases to invasive cancer that takes place in CIN continuum, remains and states an important histopathological concept to evaluate a perfect carcinogenesis model. Years of epidemiologic and preventive research focused on the HPV cervical cancer model have revolutionized the knowledge and understanding of cervical precancer [52]. HPV infection serves as a broad transition and discrimination state between normal tissue and cancer. This malignant transformation zone gives a big opportunity to evaluate and understand all histologic, hormonal, genetic, and growth factor changes that takes place. CIN 3, particularly full thickness carcinoma in situ, shares the same HPV-type spectrum and cofactor profile as invasive cancer with the same aneuploid DNA content and genetic instability. CIN 2 demonstrates greater heterogeneity in biology and definition [53]. CIN 1 is no longer considered as not representing precancer because it usually reflects HPV infection only. Persistent HPV infection with oncogenic HPV types is strongly linked to precancer which then may progress to high grade SIL (CIN 2-3) and invasive cancer in some proportion of the cases. HGF and its receptor c-Met expression level under the effect of HPV persistent infection may be one of the parameters that regulates the CIN prognosis and progress to more severe degree. As found in this study, c-Met seems to have a dominant role in this transition. Tissue studies of hysterectomy or colisation patients with CIN diagnosis may evaluate the HGF ligand and receptor expression levels in neighboring normal and pathologic cervical tissue and possible difference will play an important role in future diagnostic and therapeutic plans for these patients.

Conclusion

CIN cases display immunohistochemical c-met overexpression. The authors believe that c-Met may play a role in transformation of cervical squamous cell dysplasia.

References

16 2212-32 - Overexpression of c-Met:P-2208-32  27/11/12  09:32  Pagina 628


Address reprint requests to:
C. COMUNOĞLU, M.D.
Near East University, Faculty of Medicine
Department of Pathology
Near East Blvd.
Nicosia, Mersin-10 (Turkey)
e-mail: cemcomunoglu@gmail.com
Analysis of epidermal growth factor receptor (EGFR) status in endometrial stromal sarcoma

G. Capobianco¹, F. Pili², M. Contini², M.R. De Miglio², V. Marras², D.A. Santeufemia¹, C. Cherchi¹, M. Dessole¹, P.L. Cherchi¹, P. Cossu-Rocca²

¹Gynecologic and Obstetric Clinic, University of Sassari
²Department of Clinical and Experimental Medicine, University of Sassari (Italy)

Summary

Purpose: Endometrial stromal sarcomas (ESSs) are rare neoplasms, which are currently treated by surgery, whereas effective adjuvant therapies have not yet been established. Recently, epidermal growth factor receptor (EGFR) expression has been described in ESS, and a potential role of EGFR-targeted adjuvant therapies has been proposed. The aim of this study was to analyze EGFR status in an ESS series and to evaluate their potential role as molecular targets. Materials and Methods: EGFR status was investigated in a total of ten cases of ESS, which included seven low-grade ESS and three undifferentiated ESS cases. EGFR expression levels were assessed by immunohistochemistry, and gene amplification analysis was performed with dual-color fluorescence in situ hybridization (FISH). Results: Nine out of ten ESS cases showed positive immunostaining, whereas FISH analysis demonstrated constantly negative results. Conclusions: This study confirmed that EGFR is frequently overexpressed in ESS. FISH analysis did not show EGFR amplification in any of the tumors, therefore EGFR expression in ESS should be related to different genetic mechanisms.

Key words: EGFR; Endometrial Stromal Sarcoma (ESS); Immunohistochemistry; FISH.

Introduction

Endometrial stromal sarcomas (ESS) are rare neoplasms, which are currently classified in low-grade (LG-ESS), with indolent growth, tendency to local recurrences and, rarely, to metastasize, and undifferentiated endometrial sarcomas (UES), with ominous prognosis [1, 2].

Histologically, LG-ESS is a well-differentiated neoplasm composed of oval to spindle-shaped cells resembling stromal cells of proliferative endometrium, admixed with numerous small arterioles, similar to the endometrial spiral arterioles. Conversely, UES is defined as a high-grade neoplasm that lacks specific differentiation and shows no histological resemblance to endometrial stroma. Furthermore, UES displays marked nuclear pleomorphism with high mitotic rate and shows destructive myometrial invasion [2, 3].

In LG-ESS, the tumor cells are usually immunoreactive for estrogen and progesterone receptors, CD10, vimentin, and sometimes focally with actin, while they are generally negative for desmin, and h-caldesmon. On the other hand, UES are often estrogen and progesterone receptor-negative [1, 4].

Surgery is still the treatment of choice for ESS. While hormonal therapy has been claimed as a successful therapy to decrease recurrences in LG-ESS, nonetheless, effective adjuvant therapy to prolong survival, either radiation therapy or combination of chemotherapeutic agents, has not yet been established [5-9]. Thus, alternative approaches, such as molecularly targeted therapies, as tyrosine kinases inhibitors, need to be investigated.

The aim of our study was to analyze epidermal growth factor receptor (EGFR) expression and gene amplification in a series of ESS, to evaluate their potential role as molecular targets.

Materials and Methods

Selection of patients

A series of ten cases of ESS, including seven LG-ESS and three UES cases, was selected from the archives of the Department of Histopathology of the University of Sassari. All the cases were critically reviewed by two experienced pathologists, and categorized according to the current classification.

From formalin-fixed, paraffin-embedded (FFPE) specimens, three micron sections were obtained for haematoxylin and eosin (H&E) stains and immunohistochemical analyses. Consecutive sections were also obtained for fluorescence in situ hybridization (FISH) analysis.

Immunohistochemistry

Immunohistochemistry was performed in serial four µm sections, with the EGFR pharmDx Kit (DakoCytomation, Glostrup, Denmark) according to manufacturer’s instructions, as previously described [10]. Antigen retrieval was performed in a proteinase K solution for five minutes. Inactivation of endogenous peroxidase activity was obtained by incubating monoclonal mouse anti-human EGFR primary antibody (100 µl) for 30 minutes, and then incubated with labeled polymer HRP (100 µl) for 30 minutes. Immunohistochemical results were evaluated in a semi-quantitative manner and scored according to the intensity of immunostaining (1+, 2+, 3+) and the percentages of positively staining cells. Only cases with more than 1% of immunoreactive cells were considered positive. Membrane and/or cytoplasmic immunoreactivity was also assessed for each positive case.
Fluorescence in situ hybridization

FISH was performed as previously described [11]. Dual-color FISH was performed by using a mixture of a chromosome 7 centromeric region (CEP7 Spectrum Green) DNA probe, and EGFR gene (LSI Spectrum Orange) DNA probe. Probes (5 µl) were added to the slide in a reduced light condition. The slides were covered with a 22 x 22 mm cover slip and sealed with rubber cement. Denaturation was achieved by incubating the slides at 75°C for ten minutes in a humidified box and then hybridized at 37°C overnight. The cover slips were removed and the slides were washed extensively and further air-dried and counterstained with ten ml DAPI (Insitus, Albuquerque, NM, USA). The slides were examined using an Olympus fluorescence microscope with appropriate filters for DAPI, Spectrum Green (CEP7) and Spectrum Orange (EGFR gene). From each tumor section, at least 50 neoplastic nuclei were scored for both orange and green signals, under the fluorescence microscope with x1,000 magnification, and the ratio between orange and green signals was subsequently calculated. Only cases with ratios of two or higher were considered amplified.

Results

Patients’ age ranged from 42 to 71 years (mean 52). Eight out of ten cases (five LG-ESS and three UES) occurred in the uterus, whereas the remaining two cases were extra-uterine, arising from foci of pelvic endometriosis, with involvement of large intestine in a single case. Tumor sizes ranged from two to 17 cm (mean seven). All the cases showed immunoreactivity for CD10 at the time of the diagnosis, with positively-stained tumor cells ranging from 10% to 90%. Hormonal receptors expression was reported to be variable, with estrogen receptors (Er) positivity in 60% of the cases, and progesterone receptors (PGr) positivity in 70% of the cases, respectively.

Nine out of ten cases (90%) showed positive immunostaining. Six out of seven LG-ESS cases were positive, showing both membranous and cytoplasmic (five cases), or only membranous staining (one case). The staining intensity was interpreted as 3+ (three cases), or 2+ (two
cases), with percentages of positive cells ranging from 60% to 80%. All three cases of UES were positive for EGFR, with membranous and cytoplasmic (two cases) or only membranous (one case) staining. The staining intensity was evaluated as 1+, 2+ and 3+, with percentages of positive cells ranging from 60% to 80%. No immunoreactivity was recognizable in normal, peritumoral tissues.

FISH analysis showed EGFR/CEP7 ratios constantly below the cut-off value, ranging from 0.9 to 1.3. The results are summarized in Table 1. Figure 1 shows morphologic, immunohistochemical, and FISH features.

Discussion

This study confirmed that EGFR expression is frequently observed in ESS. EGFR immunohistochemical expression in ESS was firstly assessed by Moinfar et al. [11] in a series of 23 cases, specifically 20 LG-ESS and three UES cases. EGFR immunoreactivity was appreciable in 17 out of 23 cases (74%), namely 14 out of 20 LG-ESS (70%) and three UES (100%). No genetic analyses were performed in this study. An additional ESS clinical case report by Mitsuhashi et al. described focal EGFR immunohistochemical expression in a UES variant, with temporary response to imatinib-mesylate [13]. Recently, Cheng et al. performed a comprehensive analysis of targeted tyrosine kinases receptors on 13 LG-ESS by means of immunohistochemistry, describing EGFR negative results in their series [14].

The results in this study confirm and strengthen Moinfar’s data, since an immunoreactivity was found in 90% of ESS, specifically 86% of LG-ESS, and 100% of UES.

Genetic analyses on EGFR gene amplification are very scarce. Until now, only Mitsuhashi et al. reported EGFR immunoreactivity associated with gene amplification, as detected by FISH [13].

In this study, EGFR gene amplification was not identified in all the investigated cases by FISH, hence suggesting that EGFR overexpression in ESS does not involve gene amplification. Therefore, EGFR overexpression should be related to different genetic alterations, or post-translational regulation machinery, with anomalous protein stabilization or defective receptor downregulation increasing its ligand-mediated activation [15, 16].

Clinical significance of EGFR gene abnormalities has been previously stated in non-small-cell lung cancer (NSCLC), and the presence of specific mutations on EGFR gene exons 18-21 has been claimed to determine sensitivity to biologic targeted treatments, such as tyrosine kinase inhibitors gefitinib and erlotinib. Furthermore, concurrent EGFR gene amplification and somatic mutations have been described as increasing neoplastic sensitivity to targeted therapies [17].

In conclusion, in the authors’ experience, ESS failed to demonstrate EGFR gene amplification, suggesting that these tumors are likely to be less sensitive to specific tyrosine kinase inhibitors; nevertheless, EGFR protein overexpression, determined by immunohistochemistry, could as well be taken into account as a potential target for anti-EGFR monoclonal-antibody-based therapies.

Acknowledgments

This work was supported in part by a grant from Fondazione Banco di Sardegna, Sassari, Italy.

References


Address reprint requests to:
P.L. CHERCHI, M.D.
Gynecologic and Obstetric Clinic
Sassari University
Viale San Pietro 12
07100 Sassari (Italy)
e-mail: capobia@uniss.it
Correlated expression of Fas, NF-κB, and VEGF-C in infiltrating ductal carcinoma of the breast

X.L. Dai¹, S.L. Zhou¹, J. Qiu², Y.F. Liu³, H. Hua³

¹Department of Medical Technology, Yancheng Health Vocational and Technical College, Yancheng City, Jiangsu Province
²The Third People’s Hospital of Yancheng City, Yancheng City, Jiangsu Province
³Affiliated Hospital of Nantong University, Yancheng City, Jiangsu Province (P. R. China)

Summary

Objective: To investigate the expressions of Fas, NF-κB, and vascular endothelial growth factor-C (VEGF-C) in infiltrating ductal carcinoma of the breast and provide scientific basis for early diagnosis and prognosis of breast cancer. Materials and Methods: The immunohistochemical technique (SP method) was used to detect expression of Fas, NF-κB, and VEGF-C in 137 cases of breast-infiltrating ductal carcinoma, 17 cases of intraductal carcinoma of the breast, and 20 cases of normal breast tissues, and analyze its relationship with clinicopathologic factors of breast cancer and patients’ survival rate, as well as the correlation among their expression, clinicopathologic factors, and survival rate. Results: Fas expression was less commonly detected in infiltrating ductal carcinoma than in intraductal carcinoma and normal tissue. In contrast, both NF-κB and VEGF-C were more commonly detected in infiltrating ductal carcinoma than in intraductal carcinoma and normal tissue. Fas expression was correlated with tumor size, histological grade, and clinicopathological stage; NF-κB expression was correlated with tumor size, histological grade, lymph node metastasis; VEGF-C expression was correlated with lymph node metastasis and clinical and pathological stages of breast cancer (p ≤ 0.05). Spearman rank correlation analysis revealed a negative correlation between Fas expression and both NF-κB and VEGF-C expression in infiltrating breast cancer (p < 0.05) Additionally, Kaplan-Meier survival analysis demonstrated that five-year survival was higher for patients with Fas-positive samples but lower for those with VEGF-C-positive samples. Conclusions: The present results demonstrate that Fas and NF-κB play a role in the initiation and development of breast cancer, while VEGF-C appears to promote lymph node metastasis. Thus, these proteins may serve as useful diagnostic and prognostic markers of invasive breast cancer.

Key words: Breast-infiltrating ductal carcinoma; Fas; NF-κB; VEGF-C; Immunohistochemistry.

Introduction

Breast cancer is the most common malignant tumor among women. Approximately 1.3 million new cases of breast cancer arise worldwide each year, and 460,000 women die annually from this disease [1]. Indeed, breast cancer is predicted to account for 29% of malignant tumors in women in 2012 [2]. Both the occurrence and progression of breast cancer are caused by interactions of genetics, hormones, and immune function, as well as various environmental factors. Additionally, cancer cell invasion and early metastasis are closely related to disease prognosis.

Given the high incidence of breast cancer and its prognosis, much research has been devoted to identifying and employing biological indicators of breast cancer for aiding in diagnosis and treatment of the disease. Several different molecules have received some attention as potential markers of breast cancer development. First, apoptosis inhibitor (Fas antigen/Apo-1/CD95), one of the members of the death receptor superfamily [3], regulates apoptosis by combining with specific ligand FasL and initiating signaling through the death-inducing signal cascade (DISC) [4]. Absence of Fas is related to tumorigenesis, and indeed, Fas expression can reflect the biological behavior of tumors, including breast cancer [5-7]. Another potential marker is nuclear factor-kappa B (NF-κB), a transcription factor that binds DNA response elements and regulates expression of many genes. This protein specifically binds with the immunoglobulin κ light chain gene enhancer κB sequence and is involved in immune response, inflammation, cell proliferation, and apoptosis [8, 9]. NF-κB is dysregulated in many tumor types, with inappropriate activation stimulating tumor cell growth and inhibiting tumor cell apoptosis [10-12]. In fact, NF-κB is constitutively active in some breast tumors, making it potentially useful as a breast cancer biomarker [13, 14]. Finally, human vascular endothelial growth factor-C (VEGF-C), a platelet-derived growth factor, promotes angiogenesis and lymphangiogenesis [15]. Specifically, VEGF-C can induce formation and expansion of lymph vessels in and around solid tumors and increase the contact area of tumor cells and lymphatic vessels, contributing to lymphatic metastasis of tumors [16]. In fact, VEGF-C is overexpressed in some breast and many other tumors and as such, is used as an indicator of lymph node metastasis and long-term prognosis [17, 18].

While many studies have investigated expression of Fas, NF-κB, and VEGF-C in various tumors, little correlation has been observed between their expression. The authors assessed in the present study the expression of Fas, NF-κB, and VEGF-C in infiltrating ductal breast carcinoma. Statistical correlations were used to establish a link between expression of these proteins and lay a foundation for understanding their contribution to breast cancer metastasis.
Materials and Methods

Specimens

Archived paraffin blocks were collected from January to December 2004 in the Pathology Division, The Third People’s Hospital of Yancheng. Blocks included 137 specimens of infiltrating ductal breast carcinoma, 17 specimens of intraductal carcinoma, and 20 specimens of normal breast tissues removed due to benign lesions. All infiltrating ductal carcinoma specimens were obtained from females who ranged in age from 34 to 75 years (mean age 50.95 ± 4.7 years). Prior to surgery, patients had not received any radiotherapy, chemotherapy, or biological therapy. Paraffin blocks were serially sectioned at 4 µm, and sections were placed onto slides and stained with hematoxylin and eosin (H&E) using conventional methods. Cases were classified according to criteria for Histopathological Diagnosis of Tumors [19]: 21 specimens were grade I, 63 were grade II, and 53 were grade III; and 75 cases had lymph node metastasis, while 62 cases had no metastasis. Clinical tumor, node, metastases (TNM) staging was performed according to the criteria established by both Union for International Cancer Control (UICC) and American Joint Committee on Cancer (AJCC) [20]: 19 specimens had a carcinoid tumor diameter ≤2 cm, 95 cases had a diameter of two cm to five cm, and 23 cases had a diameter greater than five cm; additionally, 33 cases were TNM grade I, 51 were grade II, 57 were grade III, and 16 cases were grade IV. Follow-up was completed on December 31, 2009, and the survival rate was calculated from surgery date to follow-up cutoff date or death date due to recurrence, metastasis, or other reasons.

Immunohistochemistry

Sections were dewaxed and rehydrated. Antigens were retrieved at room temperature in citric acid (pH 6.0). Endogenous peroxidase was blocked with 0.3% H2O2, then sections were washed in distilled water followed by 0.1 M phosphate-buffered saline (PBS). Sections were sealed with ten percent goat serum and incubated at room temperature. Primary antibodies against Fas, NF-κB, or VEGF-C (Santa Cruz Biotechnology, CA, USA) were applied and slides were placed at 4°C overnight. Sections were washed with PBS, then secondary antibodies (Zhongshan-Golden Bridge Biotechnology Co., Ltd, Beijing, China) were applied for incubation at 37°C. DAB chromogen (Zhongshan-Golden Bridge Biotechnology Co., Ltd, Beijing, China) was used according to manufacturer’s instructions to develop staining color. Sections were washed with distilled water, lightly re-stained with hematoxylin, dehydrated in an alcohol gradient, treated with dimethylbenzene, and sealed with neutral resins. PBS was used as a negative control in place of primary antibody, and known positive sections were used as a positive control.

Determination of staining results

Staining criteria for Fas, NF-κB, and VEGF-C were based on criteria modified from the Shimizu method [21]: for each protein, brownish-yellow particles observed in the cytoplasm under a light microscope indicated positive staining. Semi-quantitative assessment was performed by two blinded pathologists using a combination of staining proportion and intensity. Five fields were randomly selected under 400× magnification. Staining proportion was scored as the percentage of positively-stained cells from all cells in the field, and was scored as follows: ≤1% staining as 0, 1%-2% as weak positive (+), 2%-5% as positive (+), 5%-10% as moderate positive (++), 10%-20% as strong positive (+++), 20% as very strong positive (++++)

Correlation of Fas, NF-κB, and VEGF-C in breast tissues

Fas, NF-κB, and VEGF-C proteins were each detected using immunohistochemistry in infiltrating ductal breast cancer specimens (Figures 1, 2, and 3). For Fas, expression was detected in 50.4% (n = 137) of infiltrating ductal carcinoma samples, 64.7% (n = 17) of intraductal carcinoma samples, and 85.0% (n = 20) of normal breast tissues. In contrast, NF-κB expression was detected in 62.0% of infiltrating ductal carcinoma samples, 41.2% of intraductal samples, and just 10.0% of normal breast samples; similarly, VEGF-C was expressed in 68.6%, 47.1%, and 0%, of infiltrating ductal carcinoma, intraductal carcinoma, and normal breast samples, respectively. Expression of Fas, NF-κB, and VEGF-C were significantly different among infiltrating ductal carcinoma, intraductal carcinoma and normal breast (p < 0.05, Table 1).

Statistical analysis

Statistical analysis was performed with SPSS15.0 statistical software, and 2 test was performed to detect the correlation between expression of Fas, NF-κB, VEGF-C, and clinicopathological parameters of infiltrating ductal carcinoma. Correlation of Fas, NF-κB, and VEGF-C expression in tissues was analyzed by Spearman method. Kaplan-Meier survival analysis was performed on the relationship of each variable and five-year survival, and survival curves were generated. Meanwhile, log-rank test was performed for inter-group survival test, and p < 0.05 was considered as statistically significant.

Correlation of Fas, NF-κB, and VEGF-C expression with clinicopathological characteristics of infiltrating ductal carcinoma

Fas expression was correlated with tumor diameter, histological grade, and TNM stage (Table 2). NF-κB expression was related to tumor size, histological differentiation degree, and lymph node metastasis (Table 3). VEGF-C expression was related to lymph node metastasis, TNM stage, and degree of malignancy; furthermore, its expression rate increased with increasing tumor diameter (Table 4).

Correlation of Fas, NF-κB, and VEGF-C expression in infiltrating ductal carcinoma tissues

To determine whether expression of these proteins is related to one another, the authors assessed correlation of expression of one protein within a sample with expression of the other proteins. Twenty (14.6%) of 137 infiltrating ductal carcinoma samples were negative for both Fas and NF-κB, and 37 samples (27.0%) were positive for both Fas and NF-κB. Of the 68 samples lacking Fas expres-
Correlated expression of Fas, NF-κB, and VEGF-C in infiltrating ductal carcinoma of the breast

Table 1. — Positive expression rates of Fas, NF-κB, and VEGF-C in infiltrating ductal breast carcinoma, intraductal carcinoma, and normal breast tissues (%).

<table>
<thead>
<tr>
<th>Tissues</th>
<th>n</th>
<th>Fas Positive (%)</th>
<th>NF-κB Positive (%)</th>
<th>VEGF-C Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltrating ductal</td>
<td>137</td>
<td>50.4</td>
<td>62.0</td>
<td>68.6</td>
</tr>
<tr>
<td>carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraductal carcinoma</td>
<td>17</td>
<td>64.7</td>
<td>41.2</td>
<td>47.1</td>
</tr>
<tr>
<td>Normal breast tissue</td>
<td>20</td>
<td>85.0</td>
<td>10.0</td>
<td>0</td>
</tr>
</tbody>
</table>

*p = 0.002, χ² = 9.099; *p = 0.034, χ² = 6.301; *p = 0.031, χ² = 11.162.

Table 2. — Correlation between Fas expression and clinicopathological characteristics of infiltrating ductal breast carcinoma.

<table>
<thead>
<tr>
<th>Clinicopathological characteristics</th>
<th>n</th>
<th>Fas Pos</th>
<th>Fas Neg</th>
<th>Positive expression rate (%)</th>
<th>χ² value</th>
<th>p value</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) ≤ 45</td>
<td>46</td>
<td>21</td>
<td>25</td>
<td>45.6</td>
<td>0.019</td>
<td>0.892</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>91</td>
<td>48</td>
<td>43</td>
<td>52.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma diameter (cm) ≤ 2</td>
<td>19</td>
<td>17</td>
<td>2</td>
<td>89.5</td>
<td>5.094</td>
<td>0.03</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>95</td>
<td>40</td>
<td>45</td>
<td>42.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 5</td>
<td>23</td>
<td>2</td>
<td>21</td>
<td>8.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histologic grade Grade I</td>
<td>21</td>
<td>14</td>
<td>7</td>
<td>66.7</td>
<td></td>
<td></td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Grade II</td>
<td>63</td>
<td>38</td>
<td>25</td>
<td>60.3</td>
<td>5.992</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Grade III</td>
<td>53</td>
<td>17</td>
<td>36</td>
<td>32.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis Yes</td>
<td>75</td>
<td>53</td>
<td>22</td>
<td>52.0</td>
<td>0.177</td>
<td>0.073</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>No</td>
<td>62</td>
<td>30</td>
<td>32</td>
<td>48.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNM Staging Stage I-II</td>
<td>84</td>
<td>48</td>
<td>36</td>
<td>57.1</td>
<td>3.990</td>
<td>0.046</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Stage III-IV</td>
<td>53</td>
<td>21</td>
<td>32</td>
<td>39.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. — Correlation between NF-κB expression and clinicopathological characteristics of infiltrating ductal breast carcinoma.

<table>
<thead>
<tr>
<th>Clinicopathological characteristics</th>
<th>n</th>
<th>NF-κB Pos</th>
<th>NF-κB Neg</th>
<th>Positive expression rate (%)</th>
<th>χ² value</th>
<th>p value</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) ≤ 45</td>
<td>46</td>
<td>25</td>
<td>21</td>
<td>54.3</td>
<td>1.742</td>
<td>0.190</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>91</td>
<td>60</td>
<td>31</td>
<td>65.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma diameter (cm) ≤ 2</td>
<td>19</td>
<td>9</td>
<td>10</td>
<td>47.4</td>
<td>4.346</td>
<td>0.038</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>95</td>
<td>58</td>
<td>37</td>
<td>61.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 5</td>
<td>23</td>
<td>18</td>
<td>5</td>
<td>78.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histologic grade Grade I</td>
<td>21</td>
<td>13</td>
<td>8</td>
<td>38.1</td>
<td></td>
<td></td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Grade II</td>
<td>63</td>
<td>41</td>
<td>22</td>
<td>65.1</td>
<td>6.139</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Grade III</td>
<td>53</td>
<td>36</td>
<td>17</td>
<td>67.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis Yes</td>
<td>75</td>
<td>53</td>
<td>22</td>
<td>70.7</td>
<td>5.233</td>
<td>0.022</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>No</td>
<td>62</td>
<td>32</td>
<td>30</td>
<td>51.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNM Staging Stage I-II</td>
<td>84</td>
<td>49</td>
<td>35</td>
<td>58.3</td>
<td>1.269</td>
<td>0.263</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Stage III-IV</td>
<td>53</td>
<td>36</td>
<td>17</td>
<td>73.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. — Correlation between VEGF-C expression and clinicopathological characteristics of infiltrating ductal breast carcinoma.

<table>
<thead>
<tr>
<th>Clinicopathological characteristics</th>
<th>n</th>
<th>VEGF-C Pos</th>
<th>VEGF-C Neg</th>
<th>Positive expression rate (%)</th>
<th>χ² values</th>
<th>p values</th>
<th>r values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) ≤ 45</td>
<td>46</td>
<td>29</td>
<td>17</td>
<td>63.0</td>
<td>0.530</td>
<td>0.470</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>91</td>
<td>63</td>
<td>28</td>
<td>69.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma diameter (cm) ≤ 2</td>
<td>19</td>
<td>10</td>
<td>9</td>
<td>52.6</td>
<td>3.955</td>
<td>0.08</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>&gt; 2-5</td>
<td>95</td>
<td>66</td>
<td>29</td>
<td>69.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 5</td>
<td>23</td>
<td>18</td>
<td>5</td>
<td>78.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histologic grade Grade I</td>
<td>21</td>
<td>12</td>
<td>9</td>
<td>57.1</td>
<td></td>
<td></td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Grade II</td>
<td>63</td>
<td>43</td>
<td>20</td>
<td>68.3</td>
<td>1.895</td>
<td>0.185</td>
<td></td>
</tr>
<tr>
<td>Grade III</td>
<td>53</td>
<td>39</td>
<td>14</td>
<td>73.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis Yes</td>
<td>75</td>
<td>58</td>
<td>17</td>
<td>77.3</td>
<td>5.852</td>
<td>0.015</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>No</td>
<td>62</td>
<td>38</td>
<td>24</td>
<td>58.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNM Staging Stage I-II</td>
<td>84</td>
<td>51</td>
<td>33</td>
<td>60.7</td>
<td>6.291</td>
<td>0.012</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Stage III-IV</td>
<td>53</td>
<td>43</td>
<td>10</td>
<td>81.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. — Correlation between Fas and NF-κB expression in infiltrating ductal breast carcinoma.

<table>
<thead>
<tr>
<th>Fas NF-κB</th>
<th>p value</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>37</td>
<td>32</td>
</tr>
<tr>
<td>Negative</td>
<td>48</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 6. — Correlation between Fas and VEGF-C expression in infiltrating ductal breast carcinoma.

<table>
<thead>
<tr>
<th>Fas VEGF-C</th>
<th>p value</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>64</td>
<td>21</td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 7. — Correlation between NF-κB and VEGF-C expression in infiltrating ductal breast carcinoma.

<table>
<thead>
<tr>
<th>NF-κB VEGF-C</th>
<th>p value</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>48</td>
<td>22</td>
</tr>
<tr>
<td>Negative</td>
<td>39</td>
<td>30</td>
</tr>
</tbody>
</table>

Expression of Fas, 48 (70.6%) were positive for NF-κB expression. Alternatively, of the 69 samples with Fas expression, 37 (53.6%) were negative for NF-κB expression. Spearman correlation analysis (r = -0.175) indicated that expression of Fas and NF-κB were negatively correlated (Table 5). Similar results were found for Fas and VEGF-C expression. Thirteen (9.5%) of 137 samples were negative for both Fas and VEGF-C expression, while 39 (28.5%) were positive for both proteins. However, in the 68 samples lacking Fas expression, VEGF-C was expressed in 55 (80.9%) of them. Furthermore in 69 samples positive for Fas expression, VEGF-C was detected in 39 (56.5%) of them. Thus, expression of Fas was also negatively correlated with VEGF-C expression (r = -0.262) (Table 6).
Finally, 22/137 (16.1%) samples were negative for both NF-κB and VEGF-C expression, while 64/137 (46.7%) were positive for both proteins. In samples negative for NF-κB, 57.7% (30/52) expressed VEGF-C; in samples positive for NF-κB, 75.3% (64/85) also expressed VEGF-C. Expression of NF-κB and VEGF-C were positively correlated ($r = 0.184$) (Table 7).

Correlation between expression of Fas, NF-κB, and VEGF-C and infiltrating ductal carcinoma prognosis

To determine whether expression of these three potential markers of invasive breast cancer correlated with disease outcomes, the authors assessed five-year follow-up data for the patients. Of the 137 cases of infiltrating ductal carcinoma, 18 patients were lost to follow-up and five patients died of accidental causes, leaving 114 cases completing follow-up. By December 31, 2009, 33 patients had died from breast cancer recurrence or metastasis; 81 patients had survived. The five-year survival rate was 71.1%, with a mean survival period of 51.47 ± 2.01 months. Kaplan-Meier survival analysis revealed that five-year survival was significantly higher for patients with Fas-positive tumor samples. Further, log-rank test showed a significant difference in five-year survival rates between patients with Fas-positive and Fas-negative samples ($\chi^2 = 4.448$, $p = 0.035$), indicating that Fas expression was positively correlated with five-year survival. In contrast, NF-κB expression resulted in a significantly decreased five-year survival rate, but log-rank test found no statistical difference in five-year survival rates between patients with and without NF-κB expression ($\chi^2$...
Expression of the apoptosis-inducing Fas antigen is reportedly down-regulated or lost in breast cancer, liver cancer, ovarian cancer, and other tumor cells, and, indeed, Fas expression is more likely to decrease with increasing degree of malignancy [22-24]; the present results corroborate these observations. It was also found that Fas expression was highest in infiltrating ductal carcinoma of the lowest malignancy grade, with significantly decreasing expression rates from grade I to grade III tissues. Additionally, Fas negative expression was positively correlated with tumor diameter and TNM stage of infiltrating ductal carcinoma, and patients with Fas-negative samples had a lower five-year survival rate than those with Fas-positive samples, consistent with previous reports [25, 26]. Thus, down-regulation of Fas expression in malignant tumors may promote tumor progression. Decreased Fas expression may allow tumor cells to escape immune surveillance, and during subsequent tumor progression, selective proliferation of cells may occur: that is, breast cancer cells may make the immune system attack tumors cells with a lower malignancy degree and positive Fas expression through selective cell apoptosis, while tumor cells with a higher malignancy degree and negative Fas expression survive, lead to further development of tumors. Loss of Fas expression in cancer cells may prevent attack by tumor-infiltrating lymphocytes (TIL) and cytotoxic T lymphocytes (CTL), which are usually initiated by FasL expression. When FasL is expressed in cancer cells, apoptosis signaling is initiated through the Fas system, allowing TIL and CTL to kill tumor cells. When Fas expression is lost in tumors, apoptosis is evaded. Therefore, tumor cells lacking Fas expression are more likely to grow, leading to larger carcinoma diameter and higher malignancy degree, making loss of Fas expression an important potential cause of tumorigenesis [27, 28].

NF-κB refers to the most abundantly expressed p50/RelA (p50/p65) heterodimer in the Rel/NF-κB family. The protein is a core regulation and control member in the apoptotic pathway, controlled by death receptor signaling and able to regulate downstream anti-apoptotic genes via trans-activation [29]. NF-κB is constitutively activated in many tumors. As a transcription factor, constitutive expression of NF-κB can lead to increased transcriptional activity, particularly of downstream gene products like VEGF (including VEGF-C). Increased VEGF activity can result in increased tumor vascularization and promote infiltration of surrounding tissues [30].

The authors found that NF-κB expression was higher in infiltrating ductal carcinoma than in intraductal carcinoma and normal breast tissues. Furthermore, NF-κB expression was correlated to carcinoma diameter, histological grade, and lymph node metastasis. Higher NF-κB expression has been reported for patients with lymph node metastasis compared to those without lymph node metastasis [31]. A previous study [32] suggested that NF-κB is related to cell apoptosis induced by DNA damage that is caused by chemotherapy; indeed, NF-κB expression is not only related to occurrence and development of tumors, but also influences the sensitivity of tumor cells to chemotherapy and disease prognosis. However, the present study did not find a difference in five-year survival for patients with and without NF-κB expression in their tumors. Further studies are needed to find the cause of these differences.

Current hypotheses [33] suggest that abnormal activation of NF-κB leads to uncontrolled regulation of the cell cycle, manifested as unlimited cell proliferation and division, and tumor formation. NF-κB dysfunction can not only initiate the development of breast cancer, but may also influence the invasion, growth, and progression of breast cancer. NF-κB may regulate cellular adhesions and induce transcription of other proteins to promote tumor invasion and metastasis. Thus, NF-κB may represent an appropriate clinical target for tumor therapy.

VEGF-C, a member of the VEGF family of growth factors, is expressed in both embryos and mature tissues. In adults, VEGF-C is mainly expressed in heart, placenta, ovary, small intestine, and thyroid. During embryonic development, VEGF-C is involved in formation of the lymphatic network. However, VEGF-C is also expressed in malignant tumors, and tumor cells with VEGF-C expression have a higher rate of local lymph node metastasis [34]. In the present study VEGF-C was detected more commonly in infiltrating ductal carcinoma than in intraductal carcinoma or normal breast tissues. Additionally, expression was correlated with lymph node metastasis and TNM stage. Five-year survival was also reduced in patients with VEGF-C-positive samples, suggesting that VEGF-C expression may predict breast cancer recurrence, consistent with the literature [35, 36].

VEGF-C expression can promote growth, invasion, and metastasis of tumors. Over-expression in tumors can induce lymphatic endothelial cell proliferation through its receptor, VEGFR-3, located in lymphatic endothelial cells. Additionally, lymph-vessel extension, development
of lymph networks, and formation of lymph vessels around the tumors can be induced. Meanwhile, existing lymph vessels can also proliferate and increase in diameter, merge with newly-formed lymph vessels, and transfer tumor cells into lymph nodes, causing lymph node metastasis [36, 37]. Lymph node metastasis is the most common method by which breast cancer spreads. Thus, VEGF-C, by reflecting the degree of lymph node involvement, may help determine staging of breast cancer and its treatment options.

While many studies have focused on the pairwise correlations between Fas, NF-kB [38], and VEGF-C, few have focused on the correlation among all three, particularly in breast cancer. The present results suggest that a possible pathway to promote tumor lymph node metastasis may contain Fas, NF-kB, and VEGF-C. This mechanism would involve loss of Fas expression, subsequent NF-kB activation, and downstream activation of VEGF-C, promoting lymphangiogenesis and lymphatic metastasis.

Briefly, in infiltrating ductal breast carcinoma, Fas expression was negatively correlated with the expression of both NF-kB and VEGF-C. A loss of Fas expression was correlated to increasing tumor severity, while NF-kB and VEGF-C expression reflected more severe disease. Additionally, loss of Fas but positive expression of NF-kB and VEGF-C were each correlated with lower five-year survival rates. Thus, loss of Fas and increased expression of NF-kB and VEGF-C can promote breast cancer progression. NF-kB and VEGF-C may serve as both diagnostic and prognostic indicators of invasive breast cancer.

References

Correlated expression of Fas, NF-κB, and VEGF-C in infiltrating ductal carcinoma of the breast


Address reprint requests to:
X.L. DAI, M.D.
Department of Medical Technology
Yancheng Health Vocational and Technical College
Jiefangnan Road 263
Yancheng City 224006
Jiangsu Province (P. R. China)
e-mail: xiaolidai2012@126.com
Prognostic value of lymph node status and number of removed nodes in patients with squamous cell carcinoma of the vulva treated with modified radical vulvectomy and inguinal-femoral lymphadenectomy

A. Gadducci¹, A. Ferrero², R. Tana¹, M.G. Fabrini³, P. Modaffari², A. Fanucchi¹, C. Vignati¹, P. Zola²

¹Department of Procreative Medicine, Division of Gynecology and Obstetrics, University of Pisa, Pisa
²Department of Gynecology and Obstetrics, University of Turin, Mauriziano Hospital, Turin
³Department of Oncology, Division of Radiation Oncology, University of Pisa, Pisa (Italy)

Summary

**Purpose of investigation:** To assess the outcome of patients with squamous cell vulvar carcinoma treated with deep partial or total vulvectomy and inguinal-femoral lymphadenectomy. **Materials and Methods:** The authors assessed 87 patients who underwent primary surgery. **Results:** Tumor recurred in 34 patients, and the first relapse was local in 19, inguinal in ten, and distant in five. Five-year disease-free survival was 56.7% and was related to Stage (p < 0.0001), grade (p = 0.023), and node status (p < 0.0001). Groin failure occurred in 4.9% of node-negative patients compared with 29.6% of node-positive patients (p = 0.0096). Distant recurrences only developed in women with positive nodes. Among the 47 patients who underwent bilateral lymphadenectomy and who had negative nodes, groin recurrence occurred in 12% of those who had ≤15 nodes removed and 0% of those who had >15 nodes removed. **Conclusions:** Stage and node status were the most important prognostic variables. There was a trend favoring a better groin control in patients with node-negative disease who underwent extensive lymphadenectomy.

Key words: Vulvar carcinoma; Prognosis; Recurrence; Survival; Inguinal-femoral lymphadenectomy.

Introduction

Radical vulvectomy with bilateral inguinal-femoral lymphadenectomy by an en-bloc excision has been long-considered as the standard surgical therapy for squamous cell carcinoma of the vulva [1-4]. Five-year overall survival (OS) ranged from 70% – 93% for patients with negative nodes to 25% – 41% for those with positive nodes, and morbidity was high, with frequent extensive wound breakdown, sexual dysfunction, lymphocele, and leg edema. To decrease surgical-related complications, the current approach is aimed to reduce and to tailor the extension of vulvar resection and to perform node dissection with separated inguinal incisions [4-8]. Furthermore, recent data suggest that sentinel lymph node mapping is a safe procedure in selected early-stage cases [9]. Node status is the strongest prognostic factor, and the incidence of positive groin nodes is mainly related to tumor size, depth of stromal invasion, tumor grade, and lymph vascular space involvement [1, 2, 4, 10-14]. Among the lymph node-related variables, the number of positive nodes, the presence or absence of extra-capsular spread, and the size of nodal metastasis are independent predictors in several papers [1, 12-19]. On the other hand, limited data are currently available in the literature regarding the prognostic relevance of the extension of inguinal-femoral lymphadenectomy in patients with histologically proven negative nodes [20-22]. The aim of this retrospective study was to assess the clinical outcome of patients with squamous cell carcinoma of the vulva treated with deep partial or total vulvectomy and inguinal-femoral lymphadenectomy with separate incision technique and the prognostic value of the number of removed nodes in patients with node-negative disease.

Materials and Methods

This retrospective study was conducted on 87 patients with squamous cell carcinoma of the vulva who underwent primary deep partial or total vulvectomy and inguinal-femoral lymphadenectomy at the Departments of Gynecology and Obstetrics of the University of Pisa and Mauriziano Hospital of the University of Turin between August 1995 and July 2010. Patients who had surgery without lymphadenectomy because of Stage IA1 disease or poor performance status, as well as those who received primary chemo-radiation followed by individualized surgery for locally-advanced disease, were not included in the present analysis. Surgical treatment of the vulva was classified according to the glossary of terminology proposed by Micheletti et al. [23]. The surgical excision encompassed the lesion with a free margin of at least a one cm of clinically normal skin, and removed a portion of the vulva in all its thickness from the surface to the urogenital diaphragm. Deep partial vulvectomy indicated that the vulvar excision was limited to a portion of the vulva (anterior vulvectomy, posterior vulvectomy, hemivulvectomy), whereas deep total vulvectomy denoted the removal of the entire vulva. Deep partial vulvectomy was usually performed in patients with T1 disease when the lesion was unifocal and the remainder of the vulva was normal. Deep total vulvectomy was the standard treatment for patients with T1 tumor and with multifocal disease, widespread intraepithelial vulvar neoplasia, or lichen sclerosis, as well as for those with more advanced tumor. Unilateral or bilateral inguinal-femoral
lymphadenectomy were always performed with surgical incisions separated from vulvar incision. Lymph node resection was usually unilateral in patients with well-lateralized T1 lesion, if ipsilateral groin was free of disease, otherwise bilateral lymphadenectomy was used. The tumor Stage of each case was determined according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) 1988 [13]. Postoperative management was individually established on the basis of histological findings on surgical specimens, patient age and general conditions, after an exhaustive discussion directly with the patient. Patients with histologically proven positive nodes usually underwent adjuvant inguinal-pelvic radiotherapy. Irradiation field encompassed both groins, obturator, external and internal iliac areas, and sometimes the primary tumor bed, and patients received a total dose of 45 – 50 Gy in five – 6.5 weeks. Personalized adjuvant radiotherapy in the vulvar area was sometimes only used in patients with negative nodes but with close surgical margins. The median follow-up of the survivors was 61.4 months.

Statistical methods

The time from surgery to recurrence was defined as disease-free survival (DFS), and the time from surgery to death or last observation was the OS. Stage, grade, size, node status, and number and laterality of positive nodes were related to DFS and OS. The number of removed nodes in patients who underwent bilateral lymphadenectomy and who had histologically negative nodes was related to groin failure rate.

The cumulative probability of DFS and OS was estimated by the product-limit method. The log-rank test was used to compare the homogeneity of DFS and OS functions across strata defined by categories of prognostic variables.

Results

The median age of women was 72 years (range 32 to 87). The surgical treatment of the vulva consisted of deep partial vulvectomy in 23 (26.4%) patients and deep total vulvectomy in 64 (73.6%) patients. Inguinal-femoral lymphadenectomy was bilateral in 65 patients (74.7%) and unilateral in 22 (25.3%) patients. As far as plastic reconstructive procedures are concerned, local fasciocutaneous skin-flaps, and regional myocutaneous skin-flaps were used in 26 (29.9%) and four (4.6%) cases, respectively. FIGO Stage was I in 35 women (40.2%), II in 24 (27.6%), III in 20 (23.0%), and IV in eight (9.2%). Tumor grade was well-differentiated in 25 (28.7%), moderately-differentiated in 43 (49.4%), and poorly-differentiated in 19 (21.8%). Tumor size was ≤ 2 cm in 34 (39.1%), > 2 – 3 cm in 26 (29.9%), > 3 – 4 cm in 12 (13.8%), and > 4 cm in 15 (17.2%). Histologically assessed groin nodes were positive in 26 (29.9%) and negative in 61 (70.1%) patients, respectively. Among the 26 patients with metastatic nodes, ten (38.5%) had one positive node and 16 (61.5%) had two or more positive nodes. Metastatic nodes were unilateral in 18 (69.2%) and bilateral in eight (30.8%) women, respectively.

Postoperative complications were as follows: wound breakdown in 22 (25.3%) patients, moderate to severe groin lymphocele in 14 (16.1%), moderate to severe lymphoedema in 13 (14.9%), deep venous thrombosis in two (2.3%), cellulitis in one (1.1%), and urinary incontinence in one (1.1%).

Nineteen patients (21.8%) underwent adjuvant inguinal-pelvic irradiation, and two patients (2.3%) received adjuvant irradiation in the vulvar area only. Cisplatin-based concurrent chemotherapy was added to radiotherapy in three cases.

Thirty-four (39.1%) patients developed recurrent disease. The first relapse was local in 19 (55.9%) patients, inguinal in ten (29.4%) (associated with local recurrence in one case), and distant in five (14.7%) (associated with groin recurrence in one case). Median time to recurrence was 35 months (range 5 to 134) for local failure, six months (range 3 to 68) for groin failure, and seven months (range 1 to 9) for distant failure.

Table 1 shows the recurrence rate by prognostic variables. It is noteworthy that groin failure occurred in three out of 61 (4.9%) patients with negative nodes compared with seven out of 26 (26.9%) with positive nodes (p = 0.0096, two-tailed Fisher’s exact test). Distant recurrences developed in none of the former and five (19.2%) of the latter (p = 0.0018), and in particular in one out of ten (10%) patients with one positive node and in four out of 16 (25.0%) patients with two or more positive nodes.

The median number of removed nodes in 47 patients who underwent bilateral inguinal-femoral lymphadenectomy and who had histologically-proven negative nodes was 15. Groin recurrence occurred in three out of the 25 (12%) patients who had ≤ 15 removed nodes compared to none of the 22 (0%) patients who had > 15 nodes removed (p = 0.2368).

DFS was significantly related to Stage (p < 0.0001), grade (p = 0.023), node status (p < 0.0001), and laterality of positive nodes (p = 0.034) (Table 2).

Treatment at recurrence was as follows: as far as the 19 women with local failure are concerned, 15 underwent surgery and four underwent radiotherapy. Of the former, seven are alive with no evidence of disease after a median follow-up of 39 months from recurrence, seven died of disease after a median time of 12 months, and one died of intercurrent disease with no evidence of tumor after 47 months. Three of the four patients with local recurrence treated with radiotherapy died of disease after eight, ten, and 12 months, respectively, and one was lost to follow-up after completion of radiotherapy. Regarding the ten women with groin failure, four patients underwent surgery (followed by radiotherapy in one case), one patient received radiotherapy, and five patients had no further treatment. Of the four surgically-treated women, one is alive with no evidence of disease after 19 months, two died of disease after two and seven months, and one died of intercurrent disease with no evidence of tumor after four months. The other six patients with groin failure died of disease after a median time of two months. Of the five women with distant failure, three patients received chemotherapy (cisplatin plus paclitaxel in two cases and single-agent paclitaxel in one case) and died of disease after four, five, and six months, respectively, and two patients underwent no further treatment and died after three and four months, respectively. OS was related to Stage (p < 0.0001) and node status (p < 0.0001) (Table 3).
Discussion

Stage [1, 11, 13, 16, 24] and node status [1, 2, 4, 10-14] are the most important prognostic factors for squamous cell carcinoma of the vulva. The number and characteristics of nodal metastases (i.e., size and extra-capsular spread) are taken into consideration in the new 2009 FIGO staging system which provides a better reflection of prognosis than the former 1988 staging system [25]. On the other hand, the prognostic relevance of tumor grade and size are uncertain. Grade had no prognostic value in several series [12, 16, 18], whereas it correlated with the clinical outcome in the study of Podratz \textit{et al.} [1] and in that of Lavie \textit{et al.} [24]. Tumor size has been reported to be a prognostic variable by some papers [1, 11, 16]. Conversely, Raspagliesi \textit{et al.} [18] found that tumor diameter (< 2 cm vs 2 – 4 cm vs > 4 cm) was not related to OS, both in the whole series and in the subset of patients with nodal metastases. In the present study, tumor recurred in more than one-third of the women, and the first relapse was local in 55.9% of the cases, inguinal in 29.4%, and distant in 14.7%. DFS was related to Stage ($p < 0.0001$), grade ($p = 0.023$), node status ($p < 0.0001$), but not with tumor size. Groin failure occurred in 4.9% of patients with negative nodes compared with 26.9% of those with positive nodes ($p = 0.0096$). Distant recurrences developed only in patients with positive nodes, and in particular in 10% of those with one positive node and 25% of those with two or more positive nodes. The prognostic relevance of the number of removed groin nodes is still debated [20-22]. A retrospective study, including 85 patients who underwent radical vulvectomy and bilateral inguinal-femoral lymphadenectomy, revealed that a total number of removed nodes < 10 was the only independent predictor of shorter time to first progression (hazard ratio [HR] = 12.88, 95% confidence interval [CI] = 1.47 – 112.89, $p = 0.021$) and shorter disease-specific survival (HR = 11.41, 95% CI = 2.21 – 58.86, $p = 0.004$) [20].

### Table 1. Recurrence rates by prognostic variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>Local (n.)</th>
<th>Groin (n.)</th>
<th>Distant (n.)</th>
<th>Overall (n. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO Stage I-II</td>
<td>59</td>
<td>14</td>
<td>3</td>
<td>0</td>
<td>17 (28.8%)</td>
</tr>
<tr>
<td>III-IV</td>
<td>28</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>17 (60.7%)</td>
</tr>
<tr>
<td>Tumor grade Well-differentiated</td>
<td>25</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>6 (24.0%)</td>
</tr>
<tr>
<td>Moderately/poorly-differentiated</td>
<td>62</td>
<td>14</td>
<td>10</td>
<td>4</td>
<td>28 (45.2%)</td>
</tr>
<tr>
<td>Tumor size ≤ 4 cm</td>
<td>72</td>
<td>16</td>
<td>6</td>
<td>4</td>
<td>26 (36.1%)</td>
</tr>
<tr>
<td>&gt; 4 cm</td>
<td>15</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>8 (53.3%)</td>
</tr>
<tr>
<td>Lymph node status Positive</td>
<td>26</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>16 (61.5%)</td>
</tr>
<tr>
<td>Negative</td>
<td>61</td>
<td>15</td>
<td>3</td>
<td>0</td>
<td>18 (29.5%)</td>
</tr>
<tr>
<td>Numbers of positive nodes 1</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>5 (50.0%)</td>
</tr>
<tr>
<td>≥ 2</td>
<td>16</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>11 (68.8%)</td>
</tr>
<tr>
<td>Laterality of positive nodes Unilateral</td>
<td>18</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>9 (50.0%)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>8</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>7 (87.5%)</td>
</tr>
</tbody>
</table>

### Table 2. DFS in patients with squamous cell carcinoma of the vulva.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>Two-year DFS</th>
<th>Five-year DFS</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire series</td>
<td>87</td>
<td>75.4%</td>
<td>56.7%</td>
<td>–</td>
</tr>
<tr>
<td>FIGO Stage I-II</td>
<td>59</td>
<td>90.5%</td>
<td>71.7%</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>III-IV</td>
<td>28</td>
<td>42.1%</td>
<td>20.1%</td>
<td>–</td>
</tr>
<tr>
<td>Tumor grade Well-differentiated</td>
<td>25</td>
<td>86.0%</td>
<td>75.5%</td>
<td>0.023</td>
</tr>
<tr>
<td>Moderately/poorly-differentiated</td>
<td>62</td>
<td>66.5%</td>
<td>48.6%</td>
<td>–</td>
</tr>
<tr>
<td>Tumor size ≤ 4 cm</td>
<td>72</td>
<td>78.3%</td>
<td>59.8%</td>
<td>0.205</td>
</tr>
<tr>
<td>&gt; 4 cm</td>
<td>15</td>
<td>63.7%</td>
<td>42.5%</td>
<td>–</td>
</tr>
<tr>
<td>Lymph node status Negative</td>
<td>61</td>
<td>90.9%</td>
<td>68.9%</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Positive</td>
<td>26</td>
<td>35.0%</td>
<td>25.5%</td>
<td>–</td>
</tr>
<tr>
<td>Number of positive nodes 1</td>
<td>10</td>
<td>46.3%</td>
<td>28.1%</td>
<td>0.118</td>
</tr>
<tr>
<td>≥ 2</td>
<td>16</td>
<td>27.5%</td>
<td>27.5%</td>
<td>–</td>
</tr>
<tr>
<td>Laterality of positive nodes Unilateral</td>
<td>18</td>
<td>44.7%</td>
<td>36.5%</td>
<td>0.034</td>
</tr>
<tr>
<td>Bilateral</td>
<td>8</td>
<td>11.0%</td>
<td>0%</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table 3. OS in patients with squamous cell carcinoma of the vulva.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>Two-year DFS</th>
<th>Five-year DFS</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire series</td>
<td>87</td>
<td>81.0%</td>
<td>70.7%</td>
<td>–</td>
</tr>
<tr>
<td>FIGO Stage I-II</td>
<td>59</td>
<td>98.1%</td>
<td>89.3%</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>III-IV</td>
<td>28</td>
<td>42.1%</td>
<td>22.5%</td>
<td>–</td>
</tr>
<tr>
<td>Tumor grade Well-differentiated</td>
<td>25</td>
<td>88.0%</td>
<td>78.2%</td>
<td>0.648</td>
</tr>
<tr>
<td>Moderately/poorly-differentiated</td>
<td>62</td>
<td>77.9%</td>
<td>67.5%</td>
<td>–</td>
</tr>
<tr>
<td>Tumor size ≤ 4 cm</td>
<td>72</td>
<td>84.6%</td>
<td>74.7%</td>
<td>0.201</td>
</tr>
<tr>
<td>&gt; 4 cm</td>
<td>15</td>
<td>65.7%</td>
<td>54.8%</td>
<td>–</td>
</tr>
<tr>
<td>Lymph node status Negative</td>
<td>61</td>
<td>98.0%</td>
<td>84.8%</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Positive</td>
<td>26</td>
<td>38.0%</td>
<td>38.0%</td>
<td>–</td>
</tr>
<tr>
<td>Number of positive nodes 1</td>
<td>10</td>
<td>50.0%</td>
<td>50.0%</td>
<td>0.092</td>
</tr>
<tr>
<td>≥ 2</td>
<td>16</td>
<td>28.7%</td>
<td>28.7%</td>
<td>–</td>
</tr>
<tr>
<td>Laterality of positive nodes Unilateral</td>
<td>18</td>
<td>47.5%</td>
<td>47.5%</td>
<td>0.110</td>
</tr>
<tr>
<td>Bilateral</td>
<td>8</td>
<td>22.2%</td>
<td>0%</td>
<td>–</td>
</tr>
</tbody>
</table>
not. The analysis of 164 patients with clinical Stage III disease and histologically-proven negative groin nodes identified from the Surveillance, Epidemiology and End Results (SEER) program showed that five-year disease-specific survival was 85% for those who had > ten removed nodes vs 60% (p = 0.02) for those who had ≤ ten removed nodes [22]. In the present series, among the 47 women who underwent bilateral inguinal-femoral lymphadenectomy and who had histologically proven negative nodes, groin recurrence occurred in 12% of the patients who had ≤ 15 removed nodes compared to none of those with > 15 removed nodes.

Conclusions

Stage and node status are the most important prognostic variables for squamous cell carcinoma of the vulva. Although the difference was not statistically significant because of the low number of cases, the authors found a trend favoring a better groin control in patients with node-negative disease who underwent a more extensive lymphadenectomy. An adequate management of the groin is critical for the clinical outcome, since the prognosis of patients with inguinal failure is unfavorable [11, 21]. In the present series, only one of the ten women with groin recurrence was still alive with no evidence of disease after 19 months from salvage surgery. A thorough inguinal-femoral lymph node dissection should be the goal in the primary treatment of this malignancy, and the benefit from this surgical procedure could be due to the removal of undetected micrometastases [22]. The use of sentinel node mapping should be reserved to selected patients with early-stage disease.

Acknowledgement

The authors are very grateful to Dr. Franco Perrone [3] for providing support in the statistical evaluation of the data.

References

Factors affecting response of chemotherapy in women with ovarian cancer

J. Lubin1, A. Markowska2, P. Knapp3

1Clinic of Oncology in Poznań, Department of Gynecology, Poznań
2Institute of Gynecology and Obstetrics, Poznań; 3University Hospital in Białystok, Department of Gynecology, Białystok (Poland)

Introduction

The aim of chemotherapy is to exert a lethal effect on cancer cells via damage to structures (mainly DNA) crucial for their growth and division. Efficacy of this therapeutic method depends on many factors, including molecular – held responsible for the malignant phenotype associated with, among others, resistance to cytostatics. Advances in molecular biology present clinical implications for the introduction of targeted therapy, which causes damage to the cancer cell and eventually its death with little or no side-effects to healthy tissues and organs of the patient treated. Selected factors, mainly genes and proteins, are presented below that comprise prognostic factors related to the response chemotherapy.

E-cadhedrin (Endothelial cadhedrin)

E-cadhedrin belongs to the type I cadhedrins – adhesion molecules responsible for Ca2+ - dependent cell-cell adhesion in epithelial tissue. Cadhedrins, transmembrane proteins are linked to the actin cytoskeleton of a neighboring cell via catenins (another type of adhesion molecules), thus forming a tightly adherent complex.

Cell adhesion plays a key role in the regulation of cell growth, migration, and apoptosis – processes essential for cancer development [1]. Decreased expression of adhesion molecules is related to cell motility, which, in the case of cancer, leads to dissemination and metastasis. Sawada et al. [2] revealed that E-cadhedrin is also active in combination with other adhesion molecules; inhibition of E-cadhedrin function induces α5-integrin, which via the MAPK pathway mediates cancer progression. Ho et al. [3] in the study of 61 ovarian clear cell adenocarcinoma patients at Stages IIC to IV after cytoreductive surgery discovered that E-cadhedrin expression in more than 75% of patients. Patients receiving paclitaxel-based chemotherapy (paclitaxel/paclitaxel-cisplatin) with positive E-cadhedrin expression demonstrated a significantly better five-year overall survival than those receiving cisplatin. Positive E-cadhedrin expression and paclitaxel-based chemotherapy are a positive prognostic factor in ovarian clear cell carcinoma.

Hypoxia inducible factor α (HIF-1α)

HIF-1 is a protein which, under normoxic conditions (oxygen concentration in cells about 7%), undergoes rapid proteasomal degradation after binding to ubiquitin molecules. Under hypoxic conditions however, it pairs with the HIF-1β subunit to form a heterodimer, which activates expression of various hypoxia-responsive elements (HRES), e.g. VEGF, EPO, LEP, NOS, GLUT1 and CXCR4, which then release a range of substances that stimulate new vessels to form within the tumor, as well as cell migration to new sites – causing cancer dissemination [4, 5]. Abnormal cancer vessels restrain blood supply (as well as penetration of cytostatics) to the tumor, a malignant phenotype of neoplasm develops, followed by chemo- and radioresistant metastases. HIF-1α overexpression correlates with P-gp (P-glycoprotein) overexpression, which is a product of the MDR1 gene (multidrug resistant gene) and with the cell cycle arrest at G0/G1 phase. This leads to reduced intracellular drug accumulation and presence of large quantities of cells phase-insensitive to cytostatics, which explains the paclitaxel-resistance mechanism [6, 7]. Follow-up of patients after adjuvant therapy in 66 ovarian cancer patients revealed that HIF-1α overexpression, as well as FIGO stage, were independent negative prognostic factors of overall survival [8].

Survivin (IAP-4, inhibitor of apoptosis protein)

Survivin is one of the eight proteins that regulates the cell cycle and via the BIR (baculovirus IAP repeat) domain, which enables binding with caspases (blockade...
of caspase-7 and activation of procaspase-9) inhibits apoptosis [9, 10].

Survivin is not present in normal tissues of adult organisms; it is expressed in small amounts only in proliferating tissues (e.g., endometrial). However it is virtually ubiquitous in all types of cancer tissue (with different variants in cytoplasm and nucleus).

The review by Urbaniak [11] analyzed survivin expression in many types of neoplasms and weighed opinion as to survivin’s utility as a diagnostic/prognostic marker and its possible therapeutic use.

Recently, research results have been presented on survivin and TP53 expression in 453 ovarian cancer patients treated with PC (platinum and cyclophosphamide) and TP (taxol and platinum) therapy. Nuclear survivin expression was found in 92% of cancer tissue and cytoplasmic in 74%. High expression of nuclear survivin in patients treated with TP regimen yielded a significantly lower risk of disease recurrence and death with an accompanying increase in platinum sensitivity, however only in TP53 positive patients – which identifies survivin as one of the prognostic factors in ovarian cancer [12].

**COX-2 (Cyclooxygenase-2)**

COX-2 is an enzyme, one of the two products of the cyclooxygenase gene (COX), that catalyzes the conversion of cell membrane phospholipids to prostanooids (prostaglandin, thromboxane, prostacyclin), and is activated mainly in inflammatory processes and immunological response. Under physiological conditions, it is present in the brain, ovaries, and testicles; however its expression changes with cancer conditions – it participates in angiogenesis and cell migration, which contributes to cancer growth and metastases [13]. Some studies revealed its tight correlation with VEGF and p53 expression, clinicopathologic factors, and non-mucinous histologic subtype of ovarian cancer [14, 15].

In the analysis of women with cancer, COX-2 expression was found to be of prognostic value in ovarian cancer. However in one study (160 female patients) positive expression correlated with longer survival, whereas in the other (183 female patients), positive COX-2 expression was associated with shorter survival [16, 17].

Ferrandina et al. [18] investigated clinical outcomes of 87 patients with ovarian cancer and indicated that positive COX-2 expression correlates with shorter time to recurrence and shorter overall survival due to resistance to platinum derivatives. According to the authors, ovarian cancer patients with COX-2 overexpression should be considered candidates for individualized treatment.

**Clusterin (Apolipoprotein J)**

Clusterin is a glycoprotein expressed in many human tissues, contained in body fluids, and involved in processes relevant to physiology, as well as cancer development, such as cell growth and apoptosis, cell cycle regulation, adhesion, and DNA repair [19].

Clusterin overexpression was confirmed in over 40% of ovarian cancer patients and correlated with FIGO stage and histological type. Clusterin overexpression also conferred shorter survival [20].

Wei et al. [21] in their analysis of ovarian cancer cell lines revealed that clusterin location (cytoplasmic or nuclear), as well as its different isoforms, mediate different biological effects, e.g. cisplatin resistance. Retrospective immunohistochemical study of clusterin expression in 62 patients with grade III serous ovarian cancer revealed that its overexpression correlates with increase in paclitaxel resistance due to interaction between clusterin and paclitaxel [22, 23].

**BRCA1**

BRCA1 is a suppressor gene localized on the 17q21 chromosome, and its product, BRCA1 protein, is involved in the repair of damaged DNA strand preventing by the same loss of control over the cell. It is also associated with transcription regulation and cell division [24].

The present state of knowledge allows to confirm that firstly, the evaluation of expression levels and secondly, the evaluation of BRCA1 gene mutation may serve as predictive factors for ovarian cancer patients: an altered response to the chemotherapy applied, especially in treatment with taxanes and platinum derivatives, renders this statement true [25, 26]. The relationship was demonstrated between BRCA1 mRNA expression and survival after chemotherapy treatment: patients with lower expression levels had a better response to platinum-based therapy, whereas patients with higher expression responded better to taxanes and the overall survival rates for patients with higher mRNA BRCA1 expression increased in the group chemotherapeutically treated with taxanes. BRCA1 induces over 1,000-fold sensitivity to chemical factors that cause damage to the mitotic spindle.

In light of recent findings, it is implicated that treatment with platinum and new drugs, namely poly ADP-ribose polymerase (PARP) inhibitors, which are presently under clinical trials, may be in the nearest future the recommended therapy for patients with diagnosed mutation in the BRCA1 gene [27].

**TP53**

Mutations in the suppressor gene p53 resulting in the formation of an abnormal protein TP53 are present in a significant percentage of ovarian cancer patients and are associated with worse prognosis; they also contribute to increase in cell sensitivity to taxanes [28, 29]. A meta-analysis of 62 published studies was released in 2009 compiling data from 9,948 ovarian cancer patients concerned with the correlation between the p53 status and patient survival, as well as clinicopathologic factors and response to the treatment [30]. Despite large heterogeneity of the trials, a statistically worse prognosis was reported for patients with abnormal p53 gene. Some of the studies implied correlations between the p53 status and
response to platinum-based treatment, while treatment with taxanes showed great disparity. In the meta-analysis under discussion, the biochemical marker that is the mutated p53 gene, was not considered a useful prognostic factor in the clinical practice.

YY1

The YY1 protein was discovered in 1991 by Shi et al. in adenovirus cells [31]. It is present in both normal and cancer cells.

Matsumura’s analysis [32] provided evidence of strong positive correlation between YY1 expression, the YY1 encoding gene, and the E2F transcription factor in serous ovarian cancer cells. High YY1/E2F activity correlates with survival in patients receiving paclitaxel treatment. Increased sensitivity to taxane therapy, not observed in treatment with platinum derivatives, was characteristic for NCI60 cell line, on which the study was performed. Reduction of YY1 expression resulted in decreased cell growth and proliferation, but also in increased taxane-resistance along with lack of cisplatin resistance.

MDR - multidrug resistance proteins

Resistance to chemotherapy in ovarian cancer patients is associated with multi-drug resistance proteins, which comprise: MRP1 protein – product of the ABC2 gene, M10 protein (also called lung resistance related protein, LRP), BCRP protein (breast cancer related protein) – product of the ABCG2 gene and P-glycoprotein (P-gp) - product of the MDR1 gene, also known as ABCB1 [33-36]. All mentioned substances are transport proteins, including P-gp, a transmembrane transporter, and are present in both normal and cancer cells. MRP1 expression in ovarian cancer cells is associated with resistance to anthracycline, vinca alkaloids, and etoposide [34, 37-40]. Detection of M10 in cancer cells correlates with resistance to doxorubicin, vinca alkaloids, and platinum derivatives (cisplatin and carboplatin) [41], whereas presence of BCRP – to anthracyclin, mitoxantrone, and topotecan [42, 43]. P-gp expression was found to be associated with resistance to anthracyclin, vinca alkaloids, actinomycin, taxol, etoposide, cisplatin, mitomycin-C, topotecan, and colchicines [44].

It seems valid to state that the resistance to ovarian cancer treatment is acquired via activation of the MDR1 gene, which is linked to a higher P-gp expression in cancer cells in patients with neoplastic recurrence in comparison to expression in primary tumor cells, as it was made evident in the work of Van der Zee et al. [45], where the P-gp presence was noted in 15% of cancer cells obtained from primary tumor and in 47% of cells from patients with neoplastic recurrence. A possibly efficient and safe gene therapy with the use of adenoviruses may be applied in the future for ovarian cancer patients with a diagnosed MDR1-mediated resistance to chemotherapy [46].

Interleukin 8

Interleukin-8 (IL-8) is a cytokine produced by monocytes, neutrophils, fibroblasts, epithelial, endothelial, and mesothelial cells as a response to inflammation as well as to tumor cells [47]. It plays an important function in inflammatory processes, and its role in the promotion of cancer cell growth, pro-angiogenic activity in many neoplasms, and involvement in metastasis is currently under analysis [47, 48]. High protein expression is associated with more advanced stages of cancer and higher differentiation of ovarian cancer cells which portends worse survival for patients, as reported in the research by Merritt et al. [49]. The analysis of 102 patients demonstrated high levels of IL-8 in a group of 43 subjects, and from this group 42 were in Stages III or IV of advanced cancer; all of the 43 patients still showed low cancer cell differentiation (grade 3), contrary to the patients with low IL-8 expression. Comparison of median survival in both groups is 1.62 and 3.79 years, respectively. Both endogenous as well as exogenous IL-8 was reported to induce cisplatin and paclitaxel resistance in non-IL8-expressing A2870 cell line cells, whereas deletion of the endogenous IL-8 in IL-8-overexpressing SKOV-3 cell lines resulted in sensitivity to antineoplastic drugs [50]. For this reason, modulation of IL-8 expression or IL-8 signaling pathway may be a promising method of sensitizing cells to chemotherapy in drug-resistant ovarian cancer.

References

Factors affecting response of chemotherapy in women with ovarian cancer


Case Reports

A case of granulosa cell tumor of the ovary detected from metastatic foci


Department of Obstetrics and Gynecology, Hirosaki University Graduate School of Medicine, Hirosaki, Aomori (Japan)

Summary

The authors report a case of granulosa cell tumor of the ovary that followed a rare clinical course, where the primary focus did not appear as a mass, and disseminated foci grew in the abdominal cavity. In 2008, a 70-year-old patient, gravida 6 and para 3, was diagnosed with a perihepatic mass, peritoneal dissemination, and an abdominal wall mass as confirmed by computed tomography (CT) scanning. There was no mass lesion in the pelvis. The pathological diagnosis based on the resected mass in the abdominal wall was malignant mesothelioma. During follow-up, abdominal bloating developed from April 2009. CT scans indicated growth of the intraperitoneal lesions. Therefore, the patient received two cycles of combination therapy with cisplatin and pemetrexed. The treatment was discontinued due to lack of efficacy. The intraperitoneal lesions grew but the clinical course was slow and inconsistent with that of malignant mesothelioma. Central pathological review was requested in April 2011, and a granulosa cell tumor was diagnosed. The patient was referred to the department for detailed examination and treatment. The patient underwent incision of the intraperitoneal lesions. Therefore, the patient was started on a treatment regimen for malignant mesothelioma. This consisted of two cycles of combination therapy with cisplatin and pemetrexed. The treatment was discontinued due to lack of efficacy. Right lower quadrant abdominal pain developed from the Spring of 2010. Simultaneously, enlargement of the intraperitoneal lesions were identified by a CT scan. However, the clinical course was not consistent with that of malignant mesothelioma, which is often rapidly fatal. The histopathology was re-examined. As a result of the central pathologic review, a granulosa cell tumor was diagnosed (Figure 1A). The patient visited our department for detailed examination and treatment in April 2011. The patient was to undergo surgery for adult-type granulosa tumor, although the primary lesion was unidentified. The levels of tumor markers were within the normal range, except for a slightly elevated CA-125 of 69 U/ml. The estradiol level was 38.4 pg/ml, considered high for her age. No abnormalities were detected in other blood data. A transvaginal ultrasound (TVUS) at the initial consultation did not identify endometrial thickening or ovarian enlargement. As ascites retention was detected in Douglas’ pouch, aspiration biopsy cytology

Key words: Granulosa cell tumor; A rare clinical course; Debulking surgery.

Introduction

Granulosa cell tumors of the ovary account for 1.9% of primary ovarian tumors and 6.0% of malignant ovarian tumors [1, 2]. The prognosis in general is considered good in the early stages, however, there are some studies reporting that late recurrence more than ten years after surgery is not uncommon [3-5]. Postoperative follow-up is necessary for a long period of time. Metastases outside the ovary usually remain within the pelvis, with distant metastases that are apparently rare [6]. The authors report a case of granulosa cell tumor of the ovary with an unusual clinical course, where the primary focus did not appear as a mass, and disseminated foci alone grew in the abdominal cavity.

Case report

The patient was a 70-year-old female, gravida 6 and para 3, who underwent menopause at the age of 50 years. The patient had undergone an appendectomy at the age of 20 years and had goiter surgery at 30 years. The patient noticed an abdominal mass in 2008 and consulted a medical practitioner. A perihepatic mass, peritoneal dissemination, and an abdominal wall mass were detected on CT scans. A pelvic mass was not noted at that point. The patient was referred to the department of gastroenterological surgery at a general hospital for detailed examination. The mass in the abdominal wall was resected for diagnostic purposes. Subsequent histopathology findings indicated a diagnosis of malignant mesothelioma. On this basis, the patient was to be followed without postoperative treatment. In 2009, abdominal bloating developed. Another CT scan confirmed an apparently enlarged perihepatic lesion along with the peritoneal dissemination. The patient requested chemotherapy and other treatment and visited the department of medical oncology at our hospital for consultation in April 2009. The patient was to be followed as an outpatient as progression of the disease was slow. Periodic CT scans identified further enlargement of the intraperitoneal lesions. Accordingly, the patient was started on a treatment regimen for malignant mesothelioma. This consisted of two cycles of combination therapy with cisplatin and pemetrexed. However, the treatment was discontinued due to lack of efficacy. Right lower quadrant abdominal pain developed from the Spring of 2010. Simultaneously, enlargement of the intraperitoneal lesions were identified by a CT scan. However, the clinical course was not consistent with that of malignant mesothelioma, which is often rapidly fatal. The histopathology was re-examined. As a result of the central pathologic review, a granulosa cell tumor was diagnosed (Figure 1A). The patient visited our department for detailed examination and treatment in April 2011. The patient was to undergo surgery for adult-type granulosa tumor, although the primary lesion was unidentified. The levels of tumor markers were within the normal range, except for a slightly elevated CA-125 of 69 U/ml. The estradiol level was 38.4 pg/ml, considered high for her age. No abnormalities were detected in other blood data. A transvaginal ultrasound (TVUS) at the initial consultation did not identify endometrial thickening or ovarian enlargement. As ascites retention was detected in Douglas’ pouch, aspiration biopsy cytology.
A case of granulosa cell tumor of the ovary detected from metastatic foci

Figure 1. — A) Pathological findings in the abdominal wall mass. B) Pathological findings in the left ovarian lesion. The tumor cells are relatively large, and near-circular coffee-bean nuclei are found. Call-Exner body-like structures can be identified in any specimen.

Figure 2. — A) Metastatic peritoneal mass near the ileocecal region. B) Macroscopic finding on the resected surface of the mass. It is solid, yellow, and fragile mass.

Figure 3. — Macroscopic findings in the internal genitalia. An apparent mass lesion was not detected, however, a yellowish lesion growing outward from the left ovary can be seen.
was performed. Atypical cells were not detected. A comparison of the images from an abdominopelvic CT scan performed in 2008 and in 2011 prior to surgery suggested that the masses under the right diaphragm, in the ileocecal region, and left lower abdominal quadrant, tended to enlarge and increase in number; however, progression of the mass was slow. No apparent enlargement in the ovaries was seen in any CT scans.

Laparotomy was performed in July 2011. A peritoneal mass around the ileocecal region (Figure 2A), a peritoneal mass under the right diaphragm, and an omental mass were found. The resected surfaces showed that all were solid, yellow, and fragile masses (Figure 2B). There were no apparent mass lesions in the internal genitalia, however, a yellowish lesion growing outward from the left ovary was detected (Figure 3). Incision of the intraperitoneal tumors, a simple total hysterectomy, bilateral salpingo-oophorectomy, and omentectomy were performed. Complete debulking surgery without any residual mass was achieved.

Pathological findings were that Call-Exner body-like structures were detected in the specimens of the abdominal wall mass (Figure 1A) and left ovarian lesion (Figure 1B), and in the mass cells relatively large and near-circular coffee-bean nuclei were detected. Immunostaining showed positive results for α-inhibin and calretinin. The lesion was considered to be an adult-type granulosa cell tumor originating from the left ovary.

The postoperative course was good. The patient was discharged 18 days after surgery. The postoperative estradiol level one month after surgery dropped below five pg/ml, lower than the measurement sensitivity level. The case was diagnosed as Stage IV granulosa cell tumor of the ovary. Complete surgical resection was achieved and the patient did not require additional treatment. The estradiol level remained low, and no recurrence or metastases were clinically found as of March 2012.

Discussion

The authors dealt with a case of granulosa cell tumor of the ovary with a clinical course that was quite rare in terms of the following: a granulosa cell tumor was diagnosed from the metastatic focus, while the disseminated foci were growing in the abdominal cavity and the primary focus did not appear as a mass.

Granulosa cell tumors are categorized as gonadostromal borderline malignancies. These tumors are characterized by slow progression and have a relatively good prognosis. However, recurrence more than ten years after surgery has been reported [3]. In rare cases, malignant courses such as intraperitoneal dissemination and hepatic metastases have also been reported [6]. Ninety percent of the patients with granulosa cell tumor present with a Stage I tumor. Approximately 95% of cases are unilateral [7].

First-line therapy is surgical treatment. The same treatment for malignant ovarian tumors is adopted in most cases. Simple total hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and staging laparotomy are recommended for the initial operation [8]. For patients in which fertility must be preserved and the lesion is confined to the ovaries, unilateral salpingo-oophorectomy alone will be performed. In such a case, patients must be placed under strict postoperative management [9]. More than 95% of young patients are in Stage IA, and unilateral salpingo-oophorectomy is selected for such patients.

The five-year survival rate in Stage I and II is good, at 95%, while that in Stages III and IV is not as good, at 59% [9]. If an appropriate staging is achieved, surgical treatment alone may be sufficient for initial treatment in Stage I. As in the presented case, however, recurrence occurs in 20% to 30% of adult-type granulosa cell tumors. A ruptured tumor, Stage IC, low differentiation and tumors not less than 10 cm to 15 cm in diameter are considered risk factors for recurrence in Stage I. Postoperative treatment such as chemotherapy is recommended for Stages IC, II and more advanced stages [10]. Chemotherapy regimens such as BEP (bleomycin, etoposide, and cisplatin) therapy, PVB (cisplatin, vinblastine, and bleomycin) therapy, and TC (paclitaxel and carboplatin) therapy demonstrate relatively high response rates [11, 12]. However, phase III clinical trials of chemotherapy for the treatment of granulosa cell tumors have not been conducted, and effective therapies have not yet been established. Where there are residual lesions or the patient is at high-risk of recurrence or where recurrence has already occurred, chemotherapy, including use of platinum-containing drugs, is considered to be indicated.

The patient in this case was diagnosed with a Stage IV granulosa cell tumor. Since the surgery in this case was performed successfully, leaving no residual tumor, additional treatment, such as postoperative chemotherapy, was not necessary. The association between the presence or absence of residual lesions and patient prognosis has been recognized in multiple studies [13-16], although multivariate analysis is not often performed. It has been suggested that total resection in debulking surgery significantly prolongs disease-free survival in cases of recurrence or metastasis. In cases such as this with a slow clinical course, disease-free status may be maintained for a long period, even in Stage IV, as long as a complete surgical resection is achieved. The patient in this case did not appear to have clinical signs of recurrence or metastasis, but a long follow-up period is considered necessary, due to the very slow tumor progression, insufficient data on prognosis, and the unusual clinical course.

References

A case of granulosa cell tumor of the ovary detected from metastatic foci


Address reprint requests to:
Y. YOKOYAMA, M.D.
Department of Obstetrics and Gynecology
Hirosaki University Graduate School of Medicine
5-Zaifu-cho, Hirosaki
Aomori 036-8562, (Japan)
e-mail: yokoyama@cc.hirosaki-u.ac.jp
Micro-metastases into the uterine leiomyoma from invasive ductal breast cancer under adjuvant tamoxifen therapy: case report

A. Dirican¹, Y. Kucukzeybek¹, I. Somali¹, C. Erten¹, L. Demir¹, A. Can¹, I.V. Bayoglu¹, S.C. Yigit², F.C. Unay², M.H. Yetimalar³, M.O. Tarhan¹

¹Medical Oncology Clinic, ²Pathology Clinic, ³Obstetrics and Gynecology Clinic
Izmir Ataturk Training and Research Hospital, Izmir (Turkey)

Summary
Metastasis of breast cancer to the uterus is extremely rare. However, breast cancer is the leading tumor metastasizing from extra-genital organs to the uterus. The most common signs of uterine metastasis are bleeding and mass effect. Tamoxifen use is known to increase risk of endometrial cancer. Immunohistochemical staining with GCDFP can be useful in differentiating primary uterine tumors from breast cancer metastasis. Metastasis to the uterus has been reported to worsen the prognosis. Although hysterectomy has been effective on survival, treatment modality to be used in the presence of other systemic metastases is not clear. Locoregional treatments can be used in oligometastatic cases. In addition, removal of solitary organ metastasis together with bone metastasis provides improvement in survival.

Key words: Uterine leiomyoma; Micrometastasis; Breast cancer.

Introduction
More than one million women are diagnosed with breast cancer annually [1]. Less than 10% of women diagnosed with breast cancer have Stage IV disease. Despite the absence of data obtained from prospective randomized studies, removal of the primary tumor or isolated metastatic lesions can be an attractive treatment strategy to improve survival in these patient subgroups [2]. Risk of endometrial cancer is increased in breast cancer patients receiving premenopausal and post-menopausal tamoxifen treatment. Therefore, regular gynecologic follow-up is recommended in women using tamoxifen. This is the presentation of a case diagnosed with breast cancer which metastasized into uterine leiomyoma while using tamoxifen.

Case Report
A 47-year-old premenopausal female patient underwent mammography and fine needle aspiration biopsy following breast ultrasonography (US) because of a breast mass. Partial mastectomy and axillary dissection were performed with the diagnosis of breast cancer. Surgical material was diagnosed to be invasive ductal carcinoma following histopathologic examination. Following immunohistochemical examination, progesterone receptor (PR) was 30% (++), estrogen receptor (ER) and Cerb B-2 were negative (Figure 1 A/a). Metastasis was not found in systemic screening and the patient was staged as T2N0M0. A chemotherapy regimen consisting of cyclophosphamide 500 mg/m², epirubicin 100 mg/m², 5-fluorouracil 500 mg/m² was administered for a total of six sessions in 21 days. Following this, adjuvant radiotherapy was applied. Tamoxifen was started for adjuvant hormone therapy. Gynecologic examination and pelvic US revealed a larger than normal cervix and uterus, and probe curettage which was performed twice, and cervical smear yielded benign findings. In the 46th month of follow-up and 38th month of tamoxifen treatment, whole body bone scintigraphy carried out because of elevated serum CA 15-3 levels in routine work up together with back pain was suspicious of bone metastases and lumbar magnetic resonance imaging (MRI) revealed diffuse infiltrative vertebral bone metastases (Figure 2). Palliative radiotherapy was applied to the painful lumbar vertebral region. Abdominal computed tomography (CT) revealed a solid mass measuring 8-8.5 mm in the long axis with heterogeneouse density and suggesting uterine myometasis extending to the adnexa and Douglas pouch in the right side and with borders not separated from the uterus-cervix borders. Although our patient underwent probe curettage for the third time and cervical smear, no malignancy was found. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. Histopathologic examination of the surgical material revealed carcinoma metastasis in the form of microscopic foci originating from the breast within the leiomyoma (Figure 1 B/b). For example, immunohistochemical staining was positive for GCDFP-15 (Figure 1 C), 30% positive for pan-keratin (Figure 1 D) and negative for smooth muscle actin, CerbB2 and ER. These findings led to the conclusion that metastatic foci within the uterine leiomyoma belonged to breast carcinoma. It was planned to administer a chemotherapy regimen with docetaxel 100 mg/m² every 21 days and zole-dronic acid 4 mg treatment every 21 days with the diagnosis of metastatic breast cancer. At the time of case presentation, CA 15-3 levels of the patient who underwent a third session of chemotherapy and biphosphonate treatment decreased by 50%. For two months, follow-up without progression has been ongoing.
Discussion

Breast cancer most commonly metastasizes to the liver, bones and lungs. Among tumors which metastasize from extragenital organs to the uterus are the breast, colon, stomach, pancreas, gallbladder, lung, cutaneous melanoma, urinary bladder, and thyroid tumors. Breast cancer is among the leading cancers. In three separate studies, breast cancer rates of tumors metastasizing from extragenital organs to the uterus were 56%, 60% and 42.9% [3-5]. When the literature is searched, metastasis of breast cancer to the uterus is frequently seen to be in the histological subtype of invasive lobular carcinoma [3, 6-8]. On the other hand, Kondo et al. reported that in 11 cases of breast cancer metastasizing to the uterus nine had the histological subtype of invasive ductal carcinoma (breast cancer) [9]. Mean survival in metastatic breast cancer has been reported to be 18-24 months [10]. Case reports have shown uterine metastasis of breast cancer to be an indicator of poor prognosis. Again in these reports, hysterectomy was reported to provide improvement in survival but oophorectomy did not affect survival. It should be kept in mind that these studies are case reports and not prospective studies. In cases of metastases such as bone metastases which are not visceral metastases, the effect of hysterectomy is not known. According to multivariable analyses, it has been reported that in breast cancer with solitary metastasis local treatments (only surgery or radiotherapy combined with surgery) lead to improvement in survival or disease-free survival [2, 11]. When resection of liver metastasis associated with bone metastasis is performed in breast cancer patients, long-term results have not shown a difference with the long-term results of metastasectomy in patients with only solitary liver metastases [12, 13]. However, the effect of surgical resection of metastatic regions outside the liver with bone metastasis on survival is not known.

Figure 1. — A) Microscopic appearance of the invasive ductal carcinoma of the breast (HE x 44); a) Cellular details of the carcinoma of the breast (HE x 440). B) Microscopic image of breast cancer cells infiltrating into the uterine leiomyoma (HE x 44); b) Cellular details of the metastatic tumor which has a similar appearance to the primary breast carcinoma (HE x 440). C) Positive staining for GCDFP-15 by immunohistochemistry (IHC) (DAB x 220). D) Strong positivity for pan CK by IHC (DABX 44).
Tamoxifen use increases the risk of endometrial carcinoma due to its partially agonistic effect on endometrium [14, 15] and most commonly presents with vaginal bleeding [16]. When case reports are analyzed, uterine metastases present most commonly with vaginal bleeding. The second most common presentation is with a uterine mass. Our case presented with a uterine mass. In breast cancer, GCDFP IHC stain is positive at a rate of 65%-80% and is relatively specific for breast cancer [17-19]. GCDFP can be used in distinguishing a primary uterine malignancy from a breast cancer metastasis particularly detected during tamoxifen use. General survival in five cases administered chemotherapy following hysterectomy has been reported between four months and four years. Axillary lymph node metastasis has been found in two patients, visceral metastases such as brain and liver metastases in two patients and bone metastases in two patients [20-24]. The number of cases are too small to allow an evaluation of the effect of chemotherapy on survival.

One case had a diagnosis of invasive lobular carcinoma metastasizing to the bone and into a uterine leiomyoma during tamoxifen treatment. The patient presented with pain due to the bone metastasis and a uterine mass. Due to the absence of visceral metastases in abdominal and thoracic CT screening and presence of only bone metastases, diagnostic total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. Micro-metastases within the leiomyoma were positive for GCDFP which is relatively specific for breast cancer. In patients with metastases limited to the bone with no life risk, surgical removal of other solitary metastatic areas is important to increase quality of life, and to improve symptoms and survival. Furthermore, total abdominal hysterectomy with bilateral salpingo-oophorectomy may be useful in distinguishing primary uterine cancer from metastasis of breast cancer to the uterus in cases with abnormal vaginal bleeding or uterine masses particularly during tamoxifen treatment. Even in cases with diffuse metastatic disease, hysterectomy can be performed to alleviate symptoms. In this case, there are no sufficient data on the effect of chemotherapy in addition to surgery on survival.

It should be kept in mind that in breast cancer patients receiving adjuvant tamoxifen, in addition to the possibility of endometrial cancer, uterine metastases can also occur. Larger series are needed to evaluate the contribution of hysterectomy on survival.

References


Figure 2. — Bone metastases in lumbar and sacral vertebrae, particularly in L4.
Micro-metastases into the uterine leiomyoma from invasive ductal breast cancer under adjuvant tamoxifen therapy: case report


Address reprint requests to:
M.O. TARHAN, M.D.
Izmir Ataturk Training and Research Hospital
Medical Oncology Clinic
35360 Izmir (Turkey)
e-mail: motarhan@yahoo.com
Leiomyosarcoma after hysteroscopic myomectomy: a case report

G. Carta, P. Palermo, R. Di Ramio, V. De Lellis, A. Carta, F. Patacchiola

Department of Surgical Sciences, University of L’Aquila (Italy)

Summary

Objectives: The aim of this study was to illustrate the importance of hysteroscopy in the evolution of mitotically active leiomyoma to leiomyosarcoma (LMS). Uterine sarcomas are rare tumors. The three microscopic criteria are: 1) the presence of coagulative tumor necrosis, 2) high mitotic index (exceeding 15 × 10) catabolite gene activator (CGA) and 3) occurrence of moderate to severe cytologic atypia. The authors report a case of a 52-year-old nulliparous woman with a LMS detected two months after a hysteroscopic resection of a mitotically active leiomyoma. After the first hysteroscopic resection the diagnosis was atypical leiomyoma with a mitotic index of two per ten high-power field (hpf) in the absence of coagulation necrosis. After two months, a new myoma was detected and another hysteroscopic resection was performed: the microscopic diagnosis was LMS and a total abdominal hysterectomy with bilateral salpingo-oophorectomy (BSO) was performed. Conclusion: The patient must undergo close clinical and instrumental follow-up procedures. Hysteroscopy plays an important role in the evaluation and evolution of both recurrent and de novo disease.

Key words: Leiomyosarcoma; Hysteroscopy.

Introduction

Uterine sarcomas are rare tumors that originate from mesenchymal cells of the uterine body. They represent only 8.4% of uterine cancers [1], but are aggressive; the five-year survival rate is 15% to 25% [2]. Leiomyosarcomas (LMS) are a sub-group of uterine sarcomas that arise from the smooth muscle of the uterus and are composed of spindle cells. LMS accounts for 1% to 2% of uterine sarcomas. The three microscopic criteria to diagnose leiomyosarcoma are:

1) presence of coagulative tumor necrosis
2) high mitotic index (exceeding 15 × 10) catabolite gene activator (CGA)
3) occurrence of moderate to severe cytologic atypia [3, 4].

Generally, this tumor occurs in women during the post-menopausal phase with a peak incidence between 50 and 65 years of age. The most frequent symptom of LMS is abnormal uterine bleeding that can also be associated with pelvic pain. Often, the disease is diagnosed after surgery. There are no screening techniques for LMS, nor can a clear preoperative diagnosis be made. Recently, a study reported a significant usefulness of an endometrial biopsy: the biopsy was positive in 12 of 21 cases [5]. The LMS staging was published by the International Federation of Gynecology and Obstetrics (FIGO) in 2009 (Table 1) [6]. Optimal treatment is the surgical removal of the LMS through total abdominal hysterectomy with bilateral salpingo-oophorectomy (BSO) [7].

Case Report

A 52-year-old nulliparous woman with a LMS detected two months after a hysteroscopic resection of a submucous uterine myoma is the subject of this report. The patient arrived at the Department of Obstetrics and Gynecology of the University of L’Aquila in May 2011 with the diagnosis of recurrent abnormal uterine bleeding (AUB) during post-menopause. During a bimanual examination the uterus was found enlarged and annexes were not palpable. Transvaginal ultrasound (TVUS) identified a submucosal myoma measuring 3.7 × 2.5 × 1.4 cm and the diagnostic hysteroscopy confirmed it. There was a type 0 myoma (completely within the endometrial cavity), of hard consistency and which was located in the medial third part of the uterine cavity. An endometrial biopsy was performed with negative result. During the same month, the patient underwent hysteroscopic myomectomy using a Hamou 26Fr resectoscope (Storz, Tuttlingen, Germany) and sorbitol-Manitol for distension media with Hysteromat Hamou (Storz). There were no intraoperative complications. The tissue fragments were examined and the diagnosis was atypical leiomyoma with mitotic index of 2/10 high-power field (hpf) in the absence of coagulation necrosis.

After two months the patient underwent a diagnostic hysteroscopy as a follow-up procedure. Subsequently, another myoma approximately 3.5 cm, with a soft consistency was found within the uterine cavity, occupying the medial third part of the cavity. Following this diagnosis a second operative hysteroscopy was performed in August 2009. The microscopic diagnosis of the fragments of the second surgical procedure was: “fragments of new formation consisting of intersecting bundles of fusiform elements with moderate eosinophilic cytoplasm characterized by hyperchromatic nuclei, some of which are markedly pleomorphic and with a karyokinetic index equal to 3/10 hpf mitoses; there are also multifocal areas of coagulative tumor necrosis. Final diagnosis: leiomyosarcoma”.

After the appropriate and comprehensive patient counseling, a total abdominal hysterectomy was performed with BSO. The instrumental clinical assessment for the preoperative staging showed a LMS Stage I according to FIGO classification (2009).
Leiomyosarcoma after hysteroscopic myomectomy: a case report

A macroscopic examination of the uterus showed a fundic subserous neoformation (2.4 x 2 x 1.5 cm), right broad ligament neoformation (2.7 x 1.5 x 1.2 cm) and submucosal neoformation (2.8 x 2 x 1 cm). The adnexes were in standard type and size. There were no obvious signs of pelvic leakage or abdominal metastases. Microscopic examination showed usual leiomyomas (fundic subserous and right broad ligament fibroid). The submucosal neoformation consisted of mainly fusiform cell elements arranged in intersecting bundles with some areas of epithelioid-type with trabecular or insular pattern. Hyalinization and coagulative necrosis (the latter in the submucosa) were also evident, with aspects of vascular invasion and immunophenotypic profile of smooth muscle derivation: alpha-smooth actin (+), HHF-35 (+), caldesmon (+), desmin (+), CD10 (-), AE1/AE3, and EMA (-). Final diagnosis: LMS of low-grade and well-differentiated. Subsequent clinical and instrumental examinations showed that the patient is still disease-free.

Discussion

The mitotically active leiomyoma is generally characterized by an increased mitotic count of 5-20 mitotic figures per 10 hpf, the absence of tumoral coagulative necrosis or mitosis, and atypical benign course. However, the mitotically active leiomyoma is generally characterized by an increased mitotic count of 5-20 mitotic figures per 10 hpf, the absence of tumoral coagulative necrosis or mitosis, and atypical benign course. However, these tumors have a malignant potential and can recur locally [8]. Forty-seven percent of women with recurrent disease manifests LMS with an average of 1.3 years. Fourteen percent of LMS remain at the site of presentation [9]. Many factors have been shown predictive of aggressiveness and low survival rates: advanced stage, high-grade, and mitotic index, invasion of vascular spaces, lack of primary surgery, older age, and Afro-American race [10, 11]. The surgical treatment recommended for the LMS is a total hysterectomy with BSO. However, it has not demonstrated a significant reduction in the incidence of recurrence after BSO [10].

Table 1. — FIGO 2009 staging system for LMS and endometrial stromal sarcoma.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tumor limited to the uterus</td>
</tr>
<tr>
<td>IA</td>
<td>≤ 5 cm</td>
</tr>
<tr>
<td>IB</td>
<td>&gt; 5 cm</td>
</tr>
<tr>
<td>II</td>
<td>Tumor extends beyond the uterus, within the pelvis</td>
</tr>
<tr>
<td>IIA</td>
<td>Adnexal involvement</td>
</tr>
<tr>
<td>IIB</td>
<td>Involvement of other pelvic tissues</td>
</tr>
<tr>
<td>III</td>
<td>Tumor invades abdominal tissues (not just protruding into the abdomen)</td>
</tr>
<tr>
<td>IIIA</td>
<td>1 site</td>
</tr>
<tr>
<td>IIIB</td>
<td>&gt; 1 site</td>
</tr>
<tr>
<td>IIIC</td>
<td>Metastasis to pelvis and/or para-aortic lymph nodes</td>
</tr>
<tr>
<td>IV</td>
<td>Tumor invades bladder and/or rectum</td>
</tr>
<tr>
<td>IVA</td>
<td>Tumor invades bladder and/or rectum</td>
</tr>
<tr>
<td>IVB</td>
<td>Tumor invades bladder and/or rectum</td>
</tr>
</tbody>
</table>

Concluding, the mitotically-active leiomyoma is a uterine neoformation at risk of malignancy with a short interval between diagnosis and the development of a LMS. Given this behaviour, it is necessary that the patient undergoes a close clinical instrumental follow-up care. Diagnostic hysteroscopy plays an important role in the evaluation of both recurrent and de novo disease while the operative hysteroscopy allows the microscopic confirmation and the possible evolution of the pathology.

References


Address reprint requests to:
G. CARTA, M.D.
Department of Surgical Sciences
University of L’Aquila (Italy)
e-mail: gaspare.carta@cc.univaq.it, gasparecarter@libero.it
Aggressive deep angiomyxoma - a case report and review of the literature

P. Kaščák1,2, M. Zámečník3
1Faculty of Health, Alexander Dubcek University, Trenčín, 2Department of Obstetrics and Gynecology, Regional Hospital, Trenčín
3Medicyt, s. r. o., Department of Pathology, Trenčín (Slovak Republic)

Summary
Background: Aggressive deep angiomyxoma (AA) is locally infiltrative, non-metastasizing neoplasm that typically occurs in the pelvis and perineum in women of the reproductive age. Local recurrence is high despite apparently complete surgical resection. Case: The authors describe a case of AA in the pelvis in a 27-year-old woman. She underwent one invasive diagnostic procedure and three surgical interventions during four months, due to diagnostic problems and very early recurrence of the disease. Follow-up one year later revealed no recurrence. Conclusion: The present case confirms that AA are locally aggressive and notorious for local recurrence. Such an early recurrence of AA has not been described in available literature.

Key words: Aggressive angiomyxoma; Surgery; Recurrence.

Introduction
Aggressive (deep) angiomyxoma (AA) was described for the first time in 1983 as an aggressive myxoid mesenchymal benign tumor that affects adult women and involves mostly pelvic and vulvoperineal area [1]. Occurrence of the tumor is rare [2]. Typically, the tumor grows slowly, and it has potential for local infiltration, which is associated with frequent local recurrence. The primary treatment is surgical resection. Even in the case of radical intervention, the risk of recurrence is high [2].

Case study
In November 2010, the patient, a 27-year-old nullipara, underwent in another facility laparoscopy due to suspicion of a tumor of the right adnexa. A tumor was detected during preventive gynecological examination. Upon examination the patient was complaining of mild pain and edema in the right gluteal area. During laparoscopy, no significant findings were revealed in the inner genitalia or abdomen, and therefore the procedure did not continue further. However, follow-up ultrasound confirmed the presence of isoechogenic and slightly hypoechogetic homogeneous mass with poorly-defined margins, with dimensions 11 cm by 6 cm. Examination with computed tomography (CT) was recommended. CT scan confirmed presence of oval, septal, cystoid lesion with maximum size of 12 cm by 6 cm. CT showed postcontrast enhanced image, the tumor was pressing on the bladder, uterus, and rectosigmoid colon. Radiologist recommended biopsy and histological examination of the tumor. During the CT-guided biopsy (Figure 1) in November 2010, only small fragments of normal smooth muscle were obtained. After the procedure, the patient was sent to the department for consultation and more testing. Ultrasound examination confirmed presence of tumor in the pelvis; the blood flow characteristics were typical for benign tumor. On the basis of the CT findings and previous sonographic results, the diagnosis of retroperitoneal tumor was established. The second laparoscopy was suggested and surgery was carried out in December 2010. The tumor was located in the retroperitoneal area below the level of iliac blood vessels. Its direction was toward the obturator foramen and toward the pelvic bottom. It was irregular in shape, soft and rubbery in consistency. The tumor’s cut surface was homogeneous, glassy, and yellow-white, and it lacked any necrosis or hemorrhage. On slicing the specimen, the tumor tissue stuck tenaciously to the knife. The tumor was removed completely, and it was sent for pathologic examination (Figure 2). Through inspection of the abdominal cavity, the finding was normal. Histological examination (Figure 3) showed mesenchymal tumor composed of bland-appearing, spindle-shaped, and stellate cells embedded in abundant myxoid stroma. In addition, the lesion contained a prominent vascular component with numerous vessels displaying often a medial hypertrophy and vascular grouping. Nuclear atypia and mitoses were absent. In the tumor margin, infiltration of the pelvic skeletal muscle tissue was visible focally. Immunohistochemically, the tumor cells expressed vimentin, desmin, alpha-smooth muscle actin, estrogen receptor, and progesterone receptor. Based on these findings, a diagnosis of AA was rendered. The high-risk of recurrence of the tumor, even after its radical removal, was explained to the patient. During the first follow-up exam, one month after surgery, the patient complained of pressure pain while in a sitting position, otherwise she denied other discomfort. Ultrasound showed early recurrence of the disease. It confirmed the presence of another tumor sized 9 cm x 6 cm. The condition and the methods of treatment were explained to the patient and the patient agreed to undergo another surgical intervention. The third laparoscopy was performed in February 2011. During surgery, an artificial lesion on the bladder was detected. The tumor was found to be located deep in the pelvis bottom. Due to anatomical changes caused by the first surgery and pathologic findings and intraoperative complications, the intervention was converted into laparotomy. The tumor was situated in the paravesical direction, toward the pelvis bottom. It had spread into rectovaginal space, almost to the anal canal. The tumor had size of 11 cm x 5 cm x 3 cm, it was excised radically, and intraoperatively the diagnosis of AA with the typical histological structure was confirmed. In the margins of the excision, infiltration of the tumor into skeletal...
Aggressive deep angiomyxoma - a case report and review of the literature

One article reports a gluteal AA reaching the size of 60 cm by 40 cm [5]. The authors from Bratislava described a case in which AA of the pelvis and vulva reached the size of 34 cm x 14 cm x 10 cm and weight of 2,040 grams [6]. Patients suffering from the disease report localized pain, dyspareunia, and sensation of pressure. Although it is generally understood that the tumor does not represent potential for metastases, development of metastases in lungs was reported in two cases [7, 8]. Recent reports describe rare, uncommon localization of the primary tumor [9-12]. Diagnosis of suspicious AA is generally established with difficulties due to the fact that this lesion occurs rarely. An appearance of AA by clinical examination is polypoid and cystic. Usually different types of lesions are considered, such as Bartholin’s gland cyst, hernia, pelvic cyst, lipoma or fibroma. Ultrasound shows muscle and fatty tissue was observed. At present, one year after the last surgery, the patient is free of the disease. However, she still reports mild discomfort in the lesser pelvis. She describes this discomfort as a pressure pain which comes and goes.

Discussion

AA is a mesenchymal tumor occurring mostly in pelvis, retroperitoneum, vagina, vulva, and gluteal region [3]. Etiopathogenesis of the lesion is unknown. According to available data, the disease occurs in patients from the age of six to 77 years, and its occurrence culminates during the fourth decade of a woman’s life [4]. The occurrence of AA was reported also in male patients; however, in females it is seven times more frequent [4]. Because of the slow growth and slow infiltration rate, in most patients, the disease is asymptomatic and the tumor may reach the size of more than ten cm [3]. One article reports a gluteal AA reaching the size of 60 cm by 40 cm [5]. The authors from Bratislava described a case in which AA of the pelvis and vulva reached the size of 34 cm x 14 cm x 10 cm and weight of 2,040 grams [6]. Patients suffering from the disease report localized pain, dyspareunia, and sensation of pressure. Although it is generally understood that the tumor does not represent potential for metastases, development of metastases in lungs was reported in two cases [7, 8]. Recent reports describe rare, uncommon localization of the primary tumor [9-12]. Diagnosis of suspicious AA is generally established with difficulties due to the fact that this lesion occurs rarely. An appearance of AA by clinical examination is polypoid and cystic. Usually different types of lesions are considered, such as Bartholin’s gland cyst, hernia, pelvic cyst, lipoma or fibroma. Ultrasound shows
the homogenous hypechohcogenic mass, however, it usually underestimates the actual size of the tumor. Examination by CT or by magnetic resonance imaging (MRI) is more useful, since it provides information about the translevator spread of the tumor, and its distance from the anal sphincter, urethra, and the wall of the urinary bladder [2]. Correct diagnosis can be established only on the basis of results of histological examination [2, 6]. The typical morphology of the lesion includes bland-appearing, spindle or stellate cells lying in abundant myxoid matrix, and prominent vascular component with numerous abnormal vessels [1]. Immunohistochemically, the tumor is positive for desmin, and often for alpha-smooth muscle actin and CD34 (smooth muscle and myofibroblastic markers). Immunohistochemical reactivity for estrogen and progesterone receptors is frequent, and, in some male cases, expression of androgen receptor was observed [13]. Molecular genetic studies revealed that rearrangement of transcription factor HMGA-2 on chromosome 12q15 appears to be typical for AA [13]. Imaging methods are important also for planning of surgical management of the disease. Even with adequate surgical treatment, in 30% - 70% of the patients, there is the risk of recurrence of the tumor at different time intervals. Salman reports recurrence of vulvar AA eight years after the primary therapy [3]. Resection with wide margin of normal tissue was a preferable means of treatment in the past. According to current reports, the risk of the recurrence in females with negative resection margins is statistically the same as in females with the tumor cells on the edge of the resection [12, 14]. Authors from the Netherlands described seven cases treated in one facility within 20 years, three of them were pregnant at the time of diagnosis [15] and all of them were treated surgically. Excision with positive margin was performed in five patients. Recurrence of the tumor within two to ten years was reported in three patients. These patients were treated by selective embolization and by surgical reinterventions. No recurrence of the tumor was reported within two to 20 years after this treatment. Angiographic embolization is not considered to be effective, since the blood supply to the tumor is provided by numerous small blood vessels and imaging techniques usually fail to determine which blood vessel is the “primary” [2]. There are no reports available which evaluated the relation between the size of the primary tumor and the risk of recurrence. Nowadays, in cases with high-risk of perioperative complications, partial surgical therapy is considered to be acceptable means of management of the tumor [2]. It is difficult to achieve a negative margin of the surgical resection line because of the aggressive infiltration growth of the tumor and the lack of the tumor encapsulation. Haldar achieved a negative resection line in the group of seven patients only in one case, and recurrence of the tumor was reported in two females with the positive resection line [2]. AA originates from sex-steroid hormone dependent cells of the pelvisoperineal soft tissue, as indicated by its frequent positivity for estrogen and progesterone receptors (including this case). It was proved that in selected cases, hormonal therapy can be effective [2, 3, 5]. Flores described the case of the patient with a vulvar AA sized 15 cm x 10 cm [16]. Since the histological findings of the resection line were positive, the patient was treated with radiation therapy, and for six months she received hormonal therapy with gonadoliberin agonists (aGnRH). Three years after the primary therapy, there was no recurrence of the tumor in this patient. Effectiveness of the therapy with aGnRH was described in the patient with AA of the vulva, in which the tumor residuals disappeared completely after this therapy had been applied for postoperative residuals or for secondary recurrence of the tumor [17, 18]. In the case of the presented patient, positive hormonal receptors were also found, and therefore, in the case of another recurrence of the tumor, therapy with aGnRH is planned. When the hormonal receptors are found to be positive, therapy with tamoxifen, raloxifen or with aromatase inhibitors may be considered [13, 19]. To lower the risks of recurrence of the tumor, it is not recommended to apply radiation therapy or chemotherapy postoperatively because AA is a neoplasm with a low proliferation rate [3]. Nowadays long-term follow-up without adjuvant therapy is the preferred means of postoperative management of the disease. Unfortunately, it is not possible to plan the strategy of treatment on the basis of evidence-based medicine, since the current literature presents only case reports or studies of small series of cases [2, 5, 6].

Conclusion

AA is a rare benign tumor invading soft tissues. Primary treatment is surgical resection without subsequent treatment. Due to the high-risk of recurrence of the tumor, long-term follow-up is required. Preoperatively, it is recommended to use imaging diagnostic methods, since the tumor may occupy large pelvic space, and it may invade surrounding anatomical structures. The patient must be informed about the risks of the radical surgical intervention. This report presents a case of the patient who experienced recurrence of the AA in the retroperitoneal area of the pelvis one month after the surgical intervention. The authors believe that such an early recurrence of AA has not been described in available literature.

References

Aggressive deep angiomyxoma - a case report and review of the literature


Address reprint requests to:
P. KAŠČÁK, M.D., Ph.D.
Faculty of Health
Alexander Dubcek University
Studentáška 2
911 50 Trenčín (Slovak Republic)
e-mail: pkascak@gmail.com
Can malignant transformation in mature cystic teratoma be preoperatively predicted?

M. Futagami1, Y. Yokoyama1, H. Mizukami2, T. Shigeto1, H. Mizunuma1

1Department of Obstetrics and Gynecology, 2Department of Pathology and Molecular Medicine, Hirosaki University Graduate School of Medicine, Hirosaki, Aomori (Japan)

Summary

Purpose of investigation: The study aimed to determine whether malignant transformation of mature cystic teratoma (MCT) can be preoperatively predicted by presenting two cases of MCT with malignant transformation and comparing their clinical factors with those of benign MCT encountered at around the same time. Materials and Methods: Age, maximum tumor diameter, tumor marker levels (serum squamous cell carcinoma (SCC) and carbohydrate antigen (CA) 19-9), the presence of solid tumor masses, and the presence or absence of contrast enhancement in pelvic magnetic resonance imaging (MRI) were investigated in two cases of MCT with malignant transformation and 76 cases of benign MCT in which surgery was performed and a pathological diagnosis given by the department from 2004 to 2010. Results: The mean ages of the two cases with malignant transformation and the cases of benign MCT were 42.5 years and 34.2 years, respectively. The mean maximum diameter of the two tumors with malignant transformation and the cases of benign MCT were 130 mm and 73.6 mm, respectively. The mean serum levels of SCC in the two cases with malignant transformation and the cases of benign MCT were 31.5 ng/ml and 0.92 ng/ml, respectively. Contrast enhancement and the presence of solid masses in images of MCT with malignant transformation were apparent. Conclusion: In order to accurately detect malignant transformation of MCT, the authors found it to be important to determine whether tumors larger than 100 mm in diameter were present and to check for the presence of solid masses enhanced in pelvic MRI examination, as well as to measure at least serum SCC and CA19-9 even in relatively young patients.

Key words: Mature cystic teratoma; Malignant transformation; Solid mass enhancement; Serum SCC level.

Introduction

Mature cystic teratoma (MCT) that transforms to malignancy is rare. The authors investigated whether malignant transformation of mature cystic teratoma can be predicted prior to surgery by clinically comparing two cases of MCT that transformed to malignancy with other MCT cases. The authors report the results of this investigation along with a discussion of the literature.

Case Report

Case 1

A 49-year-old patient, gravida 2 and para 2, with no relevant family or medical history, was diagnosed with an ovarian tumor and referred to the department in 2007. Pelvic magnetic resonance imaging (MRI) identified a tumor 14 cm in diameter in the pelvis. MCT accompanied by malignant transformation was suspected because solid masses, which were partially enhanced, were detected (Figure 1), although fat was also seen, and her serum squamous cell carcinoma (SCC) level was 50.6 ng/ml. There were no abnormalities found for peripheral blood, biochemistry or coagulation testing. The tumor marker levels were high with SCC of 50.6 ng/ml, CA125 of 86 U/ml, CA19-9 of 699 U/ml and carcinoembryonic antigen (CEA) of 19.7 ng/ml. Although the initial pathological diagnosis was struma ovarii, simple total hysterectomy, bilateral salpingo-oophorectomy, and omentectomy were performed due to the patient’s age and suspected malignant transformation. No apparent dissemination was found in the greater omentum or abdominal cavity. Macroscopic findings were that the tumors were bilateral and that solid masses, as well as fat and hair were recognized (Figure 1). Histopathological findings indicated that most of the right ovarian tumor was MCT, and adenosquamous cancer was partially detected (Figure 2). Microscopic dissemination was seen in the greater omentum, and was Stage IIA according to the FIGO classification. The left ovarian tumor was a MCT without malignant transformation. Postoperative chemoradiation was performed, with a total of 45 Gy administered over the entire pelvis. With the radiation therapy, 30 mg/m² of cisplatin (CDDP) was administered once weekly for five times. The serum SCC level normalized after treatment and recurrence was not found during five years follow-up after the initial surgery.

Case 2

A 36-year-old patient, gravida 1 and para 1, with no relevant family or medical history, was diagnosed with an ovarian tumor during a gynecological examination in December 2009. Laparoscopic surgery had been performed for the 12 cm diameter ovarian MTC at a previous hospital and, due to a solid mass within the tumor suggestive of malignancy found during this surgery, right salpingo-oophorectomy had been performed. The tumor had ruptured during surgery, and after a postoperative pathological examination indicated that the solid mass was squamous cell cancer, the patient was referred to the department. There were no abnormalities detected on hematology, peripheral blood, biochemistry or coagulation testing. Tumor marker levels were as follows: CA125 25.2 U/ml, CA19-9 58.6 U/ml, and serum SCC was high at 12.3 ng/ml. Careful observation of the images from preoperative pelvic MRI indicated that the 12-cm MCT was accompanied by a solid mass measuring six cm diameter above the tumor. Macroscopic findings were...
Can malignant transformation in mature cystic teratoma be preoperatively predicted?

Materials and Methods

Age, maximum tumor diameters, tumor marker levels (serum SCC and CA19-9), the presence of solid tumor masses, and the presence or absence of contrast enhancement in pelvic MRI were investigated in two cases of MCT with malignant transformation and 76 cases of benign MCT in which surgery was performed, and a pathological diagnosis was given by the department during 2004 to 2010.

Figure 1. — A: The resected ovarian tumor; B: The resected surface. Solid masses along with hair and fat are found in the multilocular mass.

Figure 2. — Pathological findings from the ovarian tumor. A: Squamous cell cancer; B: adenocarcinoma.

Figure 3. — The resected ovarian tumor. The tumor is comprised mostly of fat and hair, with a partial solid mass (black circle).

that the MCT was comprised mostly of fat and hair but there was also a partial solid mass (Figure 3). On the basis of the pathological findings, the lesion was diagnosed as a transitional cell cancer accompanied by squamous metaplasia (Figure 4). Our department additionally performed staging laparotomy, which gave a staging of Stage IC according to the FIGO classification. Postoperative treatment comprised of three cycles of combined paclitaxel and carboplatin therapy. During three years follow-up, no recurrence was detected.
Results

A summary is shown in Table 1. The mean ages of the two cases with malignant transformation and the cases of benign MCT were 42.5 years and 34.2 years, respectively. The mean maximum tumor diameter in the two cases with malignant transformation and the cases of benign MCT were 130 mm and 73.6 mm, respectively. The mean levels of serum SCC in the two cases with malignant transformation and the cases of benign MCT were 31.5 ng/ml and 0.92 ng/ml, respectively, with mean levels of CA19-9 of 377.8 U/ml and 69.8 U/ml, respectively. The number of cases that showed enhancement in pelvic MRI in the two cases with malignant transformation and the cases of benign MCT were 1/1 case (100%) and 11/76 cases (14.7%) respectively, with solid masses detected in two cases (100%) and 2/76 cases (2.6%), respectively. Thickening of the tumor wall and a septal wall were enhanced in the contrasted benign MCT images.

Discussion

MCT accounts for 10% to 20% of ovarian tumors. However, malignant transformation is thought to occur in only 1% to 2% of MCT cases [1, 2]. Histologically, SCC accounts for the largest percentage of cases where malignant transformation occurs at 75% [3].

As malignant transformation of MCT is rare and there are only a few reports that cover a large number of cases, while many individual cases have been reported. Reports by single institutions that have relatively large numbers of patients and reports by multiple institutions working together cover several dozens of cases (7 to 37 cases) [3-5]. Three reviews on malignant transformation in MCT have been published so far [6-8]. These reviews are the accumulation of 30 years of experience. Therapies and surgical methods have inevitably changed during these 30 years. Therefore these reviews lack sufficient data to establish methods for the prediction and treatment of MCT that transform into malignancy.

As the authors had only two cases of MCT with malignant transformation in their department, the data may again be insufficient for establishing a definitive method. However, by comparing these cases with those of benign MCT, the authors were able to establish the following: (1) patients with MCT with malignant transformation were not particularly old but did appear to have a mean age higher than that of cases of benign MCT; (2) the diameter of MCT that transformed into malignancy was greater than that of benign MCT; (3) serum SCC may be an indicator of malignancy, whereas CA19-9 varies widely and may be elevated in both malignant and benign MCT; (4) While benign MCT rarely shows enhancement, malignant transformation may be characterized by enhanced solid masses.

The authors looked at previously published reports to further investigate these four points. First, concerning age, Hackethal et al. [7] reported a mean age of 55 years, with other reports having similar results. In these reports, the ages when MCT transformed into malignancy ranged from 19 to 87 years, with a standard deviation of 14.1 years. These findings suggest that careful consultation is required for patients aged 30 years and older. One patient treated in the department was 36 years old. It may be assumed that malignant transformation of MCT occurs in elderly patients, but this is not always true.

Second, concerning tumor diameter, Hackethal et al. [7] reported that 70% of cases of MCT with malignant transformation had tumors 100 mm or larger in diameter. Chen et al. [8] reported a diameter range of 137 mm ± 57 mm. Both cases treated in the department had tumors with a diameter of at least 100 mm, which is consistent with the report by Kikkawa et al. [4] that by assuming the cutoff value for tumor diameter at 99 mm, sensitivity is 86%, specificity 74%, and diagnostic yield 64%. However, prediction based on the tumor diameter alone may be difficult, as MCT with a tumor diameter exceeding 100 mm is not frequently seen in clinical practice. The mean tumor diameter in cases with benign MCT in this department was 73.6 mm ± 34.6 mm. Tumor diameter of at least 100 mm was observed in 10/76 cases (13.2%).

Which tumor marker should be measured when MCT is suspected in the ultrasound exam? The most likely choic-
es would be CA19-9 and CA125. SCC is unlikely to be routinely measured due to economic considerations. In the review by Hackethal et al. [7], SCC was measured only in 52 cases (18.8%), CA125 in 51 cases (18.4%), CA19-9 in 39 cases (14.1%), and CEA in 24 cases (8.7%) among a total of 277 cases. In order to justify measurement with these markers, malignant transformation of MTC should be suspected. Tumor images, discussed later, tumor diameter, and patient age should be carefully considered before measurement.

What is the best tumor marker to predict MCT that will transform into malignancy? When considering that SCC accounts for 75% of MCT with malignant transformation, SCC is considered the best choice. Kikkawa et al. [4] reported that when MCT with malignant transformation was screened using SCC (< 2.0 ng/ml), CA125 (< 35 U/ml), CA19-9 (< 37 U/ml), and CEA (< 5.0 ng/ml), diagnostic yields were 63%, 50%, 28%, and 45%, respectively. The sensitivity of CEA was low at 45%, but its specificity was 100%. Based on the previous statement, SCC and CEA are considered appropriate to screen MCT for malignant transformation.

The last point for discussion is whether pelvic MRI can identify MCT that has transformed into malignancy? Only a limited number of reports on this issue are available [9]. Most medical institutions perform preoperative pelvic MRI, and laparoscopic surgery is widely performed in cases where MCT is suspected. Not many institutions, however, evaluate MCT using contrast agents. In case 2 the authors reported a patient that did not undergo contrast studies. Contrast agents, of course, will rarely be used when malignant transformation is not suspected. However, if the presence of solid masses is confirmed by pelvic MRI, re-examination, such as performing tumor marker tests and contrast studies, will be performed. If there is strong suspicion of malignant transformation, laparotomy rather than laparoscopy, will be performed. The order of these procedures is considered ideal at present [8]. This present investigation suggests it important to focus on the presence or absence of solid masses, on the basis of the finding that among cases of benign MCT, only 2.6% had solid masses, and only 14.7% showed contrast enhancement on pelvic MRI.

The way to detect all cases of malignant transformation of MCT is to perform pelvic MRI to check for the presence of solid masses in patients 30 years and older and with a tumor at least 100 mm in diameter. If solid masses are enhanced, at least serum SCC and CEA should be measured. The findings of this investigation, along with the existing literature, suggest that in order to avoid missing cases of malignant transformation, that these procedures are considered prudent.

Conclusion

It must be noted that it is difficult to honestly answer the question of whether malignant transformation of MCT can be predicted preoperatively on the basis of the results of this investigation. Prognosis, which has not been discussed in this paper, is particularly unfavorable in many advanced cases. A treatment strategy has not been established for malignant transformation of MCT, and a relevant therapy is likely to be found by accumulating data on cases of MCT with malignant transformation.

References

Synchronous squamous cell carcinoma of the endometrium and endometrioid adenocarcinoma of the ovary

C. Grigoriadis1, G. Androutsopoulos2, D. Zygouris1, N. Arnogiannaki3, E. Terzakis1

1Second Department of Gynaecology, St. Savvas Anticancer-Oncologic Hospital, Athens
2Department of Obstetrics and Gynecology, University of Patras, Medical School, Rion
3Department of Pathology, St. Savvas Anticancer-Oncologic Hospital, Athens (Greece)

Summary

Background: Synchronous primary endometrial and ovarian cancers are relatively uncommon in general population. The etiology and pathogenesis of this phenomenon remains unclear. The authors’ aim was to present a case of synchronous squamous cell carcinoma of the endometrium and endometrioid adenocarcinoma of the ovary and review current literature. Case: The patient, a 64-year-old, nulliparous postmenopausal Greek woman presented with a complaint of abdominal pain and abnormal uterine bleeding. Preoperative computer tomography (CT) of the abdomen and pelvis, and abdominal ultrasound (U/S) revealed an intra-abdominal three cm mass with solid components between the left ovary and small bowel. The patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH+BS), total omentectomy, pelvic and para-aortic lymph node dissection, and removal of the implant at the serosa of small bowel. Histopathology revealed Stage IA endometrial cancer squamous type and Stage IIIC ovarian cancer of endometrioid-type. Postoperatively the patient underwent adjuvant chemotherapy and radiotherapy. Follow-up of 22 months after initial surgery revealed no evidence of recurrence. Conclusion: The reason for better median overall survival of patients with synchronous primary endometrial and ovarian cancers is not intuitively obvious. Perhaps favourable clinical outcome may be related with the detection of patients at early stage and low-grade disease with an indolent growth rate.

Key words: Squamous cell carcinoma of the endometrium; Endometrial cancer; Ovarian cancer; Synchronous primary cancers.

Introduction

Synchronous primary cancers are relatively uncommon in general population. About 1-6% of women with gynecological malignancies have synchronous primary cancers of the female genital tract [1, 2]. Synchronous primary endometrial and ovarian cancers are the most common combination [1, 2].

The etiology and pathogenesis of this phenomenon remains unclear [3, 4]. It has been postulated that embryologically similar tissues, when simultaneously exposed to hormonal influences or to carcinogens, may develop synchronous cancers [3, 4].

The authors’ aim was to present a case of synchronous squamous cell carcinoma of the endometrium and endometrioid adenocarcinoma of the ovary and review current literature.

Case Report

The patient, a 64-year-old, nulliparous postmenopausal Greek woman presented at the Second Department of Gynaecology of St. Savvas Anticancer-Oncologic Hospital, with a complaint of abdominal pain and abnormal uterine bleeding. She had surgical history of appendectomy. Her family history revealed no evidence of cancer among the first-degree relatives.

During gynecologic examination there was a palpable pelvic mass. There were no palpable inguinal lymph nodes and the remaining pelvic examination was normal.

Preoperative computer tomography (CT) of the abdomen and pelvis, and abdominal ultrasound (U/S) revealed an intra-abdominal mass three cm with solid components between left ovary and small bowel. CT of the chest, chest X-ray, intravenous pyelography (IVP), colonoscopy, and urethrocystoscopy were normal. Preoperative CA-125 and CA 19-9 were elevated at 561 U/ml and 672 U/ml, respectively.

On exploratory laparotomy, the left ovary was normal in size, with a papillary exophytic appearance. An implant of three cm was found at the serosa of small bowel. Frozen section showed malignancy and the patient underwent TAH+BS, total omentectomy, pelvic and para-aortic lymph node dissection, and removal of the implant at the serosa of small bowel.

Histopathology revealed synchronous primary cancers of the endometrium and left ovary. The endometrial tumor invaded less than one-half of the myometrium (Figure 1). The ovarian tumor invaded and ruptured the capsule of the left ovary, invaded left Fallopian tube, extending to the right ovary, and to the serosa of small bowel (Figure 2). The omentum and 22 totally removed pelvic and para-aortic lymph nodes were negative for metastatic disease. The peritoneal washing smear was negative for malignant cells. The final diagnosis was Stage IA endometrial cancer squamous type and Stage IIIC ovarian cancer of endometrioid type.

The patient underwent postoperative adjuvant chemotherapy. She received six courses of carboplatinum (AUC 4) and paclitaxel (175 mg/m²). The patient also underwent postoperative adjuvant radiotherapy. She received 5000 cGy of external pelvic radiotherapy and 2000 cGy of intravaginal brachytherapy.

Follow-up at 22 months after initial surgery, with CT of the chest, abdomen, and pelvis, abdominal U/S, chest X-ray, IVP, colonoscopy, and urethrocystoscopy, revealed no evidence of recurrence.

Revised manuscript accepted for publication May 5, 2012
Synchronous squamous cell carcinoma of the endometrium and endometrioid adenocarcinoma of the ovary

Discussion

Synchronous primary cancers are relatively uncommon in general population. About 1-6% of women with gynecological malignancies have synchronous primary cancers of the female genital tract [1, 2]. Synchronous primary endometrial and ovarian cancers are the most common combination [1, 2]. However, primary squamous cell carcinoma of the endometrium is a very rare neoplasm with a prevalence estimated at 0.15 - 0.5% [5, 6]. Only sporadic cases of primary squamous cell carcinoma of the endometrium have been published in the literature. The patient in this study had an extremely rare combination of synchronous squamous cell carcinoma of the endometrium and endometrioid adenocarcinoma of the ovary.

The theory of the “secondary Müllerian system” was proposed to explain the observation of multiple similar cancers in the female genital tract [4, 7]. According to this theory, epithelia of the cervix, uterus, Fallopian tubes, ovaries, and peritoneal surfaces simultaneously respond to a carcinogenic stimulus [4, 7]. Shared hormonal (estrogen) receptors may be responsible for the development of multiple primary malignancies in predisposed tissue [3, 8].

Squamous cell carcinoma of the endometrium are possibly related to bi-directional differentiation of pluripotent endometrial precursor cells, heterotopic cervical tissue or squamous metaplasia (chronic inflammation, pyometra, and previous radiation) [9-12].

It is also possible that the synchronous presence of these cancers is an indicator of an etiologically distinct condition [13]. Perhaps patients have a more fragile genome and prior genetic damage may predispose to synchronous cancers [13-15]. Thus, embryologic, hormonal, or other phenomena may be associated with the development of malignancies arising simultaneously in genital tissues [3, 4, 8-13 15].

Patients with synchronous primary endometrial and ovarian cancers tended to be 10-20 years younger than their counterparts with endometrial or ovarian cancer [16]. The median age at diagnosis was 50 years [17, 18]. They had distinct clinical characteristics including: young age, obesity, premenopausal status, and nulliparity [17]. The most common presenting symptoms and signs were: abnormal uterine bleeding (46%), abdominal/pelvic pain (17%), and abdominal/pelvic mass (13%) [17]. The patient in this study was 64-year-old, postmenopausal, nulliparous, with normal body mass index (BMI), and the main presenting symptoms were abdominal pain and abnormal uterine bleeding.

Synchronous primary endometrial and ovarian cancers may have a similar appearance or may be of different histologic types [16, 18]. The distinction between metastatic and synchronous primary cancers is relatively simple, when they have different histologic types. However, the distinction is relatively difficult when they share the same histologic features [19, 20]. According to the empirical criteria described in detail by Scully et al., the patient presented in this study had synchronous primary endometrial and ovarian cancers [20].

Diagnosis in patients with squamous cell carcinoma of the endometrium is especially based on strict pathologic criteria [21]. According to the empirical criteria described in detail by Fluhmann, the patient in this study had squamous cell carcinoma of the endometrium [21].

Treatment of choice in early-stage patients with synchronous primary endometrial and ovarian cancers is: TAH+BS, total omentectomy, appendectomy, and pelvic lymph node dissection [18]. Advanced-stage patients, required more aggressive management with postoperative adjuvant chemotherapy and/or radiotherapy [22-24]. According to current treatment strategies, the described patient underwent radical surgery and received postoperative adjuvant chemotherapy and radiotherapy, as she was diagnosed with advanced-stage disease.
Prognostic factors for synchronous primary endometrial and ovarian cancers are: age, stage of ovarian cancer, grade of endometrial cancer, and adjuvant therapy [25]. Patients with synchronous primary endometrial and ovarian cancers of endometrioid-type have a better median overall survival compared with non-endometrioid or mixed histologic subtypes [17]. Also, patients with synchronous primary endometrial and ovarian cancers have overall five-year survival in 85.9% and ten-year survival in 80.3% [18]. The patient in this study had synchronous primary endometrial and ovarian cancer of different histologic type; however, 22 months after initial surgery, she is in good condition with no evidence of relapse.

Conclusion
The reason for better median overall survival of patients with synchronous primary endometrial and ovarian cancers is not intuitively obvious [18]. Perhaps favorable clinical outcome may be related with the detection of patients at early-stage and low-grade disease [14, 15, 23, 26, 27]. Usually endometrial cancer produces earlier symptoms, so synchronous ovarian cancer may be detected at an earlier stage [14, 15, 23, 26, 27].

References

Address reprint requests to:
G. ANDROUTSOPOULOS, M.D.
Nikolaou Apostoli 21
26532 Patra (Greece)
e-mail: androustopoulosgeorgios@hotmail.com
Aggressive angiomyxoma of the vaginal wall at the initial stage: a case report

G. Carta¹, V. Parisse¹, V. Accurti¹, L. Sollima², L. Di Stefano¹, A. D’Alfonso¹, F. Patacchiola¹
¹Department of Surgical Sciences, ²Department of Health Sciences, University of L’Aquila (Italy)

Summary

Aggressive angiomyxoma (AA) is a rare mesenchymal tumor usually located in the pelvic and perineal region. Less than 30 cases of aggressive angiomyxoma with vaginal location have been reported in the literature up to this date. The authors report the case of a 50-year-old female patient diagnosed with vaginal AA whose characteristics at its initial stage were macroscopically indistinguishable from those of a polypoid lesion. Therefore this case suggests that this type of tumor should be considered as part of the differential diagnosis of vaginal polypoid lesions.

Key words: Aggressive angiomyxoma; Polyp; Vagina.

Introduction

Aggressive angiomyxoma (AA) is an uncommon, benign, and mesenchymal tumor of unknown etiology with a high-risk of infiltrative growth and local recurrence, occurring in the vulvo-vaginal region, pelvis, and perineum of women during their reproductive years. AA was first described in 1983 by Steeper and Rosai [1] and around 250 cases have been reported in world medical literature since then [2]. Ninety percent of women presenting this tumor are in their reproductive years with a peak incidence in the fourth decade of life [3]. Similar lesions have been described in men, postmenopausal women, and in children. AA typically displays slow growth and has a gelatinous appearance; it is not encapsulated and has the same consistency as the surrounding connective tissue. Due to these characteristics, as well as its anatomical position, surgical excision is difficult and the tumor recurs following incomplete excision. Two cases of systemic metastasis have been reported [4, 5].

Materials and Methods

A 50-year-old woman with endometrial hyperplasia was admitted to San Salvatore Hospital in L’Aquila for a hysteroscopy and an endometrial biopsy. The patient was a smoker with a family history of breast cancer and a light, regular menstrual cycle. The patient had suffered a miscarriage and delivered two children, one by natural delivery and the other by Caesarean. She had been suffering from sideropenic anaemia in the months prior to her admission and was followed regularly by a gynaecologist: a pap test carried out in 2009 was negative while a scan performed in September 2009, revealed endometrial thickening (12 mm) that was not associated with her menstrual cycle. On examination, a polypoid mass was found in the right lateral wall of the vagina. This mass was one cm in diameter and soft in consistency. Routine blood tests were normal: haemoglobin of 12.9 g/dl and a white blood cell count of 5.47 mm³: neutrophils (64.8%), lymphocytes (24.9%), eosinophils (1.7%), monocytes (6.1%), and basophils (0.3%). Liver and renal function tests were normal. The preoperative clinical diagnosis was vaginal polyp. A hysteroscopy and endometrial biopsy were performed under general anaesthetic; the lesion was excised with a sufficient margin at the site of the stalk and was reconstructed with a flap.

Results

The pathological diagnosis was “endometrial simple hyperplasia without atypia and aggressive angiomyxoma of the vagina”. The surgical borders of the specimen were negative for tumor. Microscopically, the neoplasm was paucicellular and contained stellate and spindle cells embedded in an abundant myxoid stroma with some collagen formation and numerous blood vessels of varying calibre, open, thick-walled, and often hyalinized. Smooth muscle cells were observed in loose clusters or around blood vessels (Figure 1). Tumor cells had ovoid nuclei with dispersed chromatin and eosinophilic cytoplasm. Mitotic activity and nuclear atypia were absent. Immunohistochemically the cells showed positive reactivity for desmin, vimentin, CD34, and estrogen and progesterone receptors (Figure 2 and Figure 3). The patient was advised to return for a follow-up in two years, in view of the high rate of recurrence.

Discussion

The term ‘aggressive angiomyxoma’ was adopted by Steeper to underline the neoplastic nature of the blood vessels and its locally infiltrative and recurrent nature [1]. The World Health Organization describes AA as a “soft tissue neoplasm of uncertain differentiation” [6]. The tumor has been found in the pelvis and perineum, the vulvar region being the most common site [7]. Vaginal location has only been described in approximately 30 cases in literature. AA has been associated with a clonal chromosomal translocation in chromosome 12 (T(8,12), (p12;q15)) [8], that results in an aberrant expression of the HMGA2 protein (previously known as HMGIC), a
and sarcoma botryoides, a myxoid variant of malignant fibrous hystiocytoma. Distinguishing this disease from angiomyofibroblastoma (AMF) is very important. Both are rare mesenchymal tumors which were first compared by Fletcher [11]. AMF is characteristically a well-circumscribed and superficial lesion and is typically less than five cm in diameter; histologically it is composed of alternating hypocellular and hypercellular areas with plumper and round-shaped cells in clusters or in a linear array around small capillary-sized vessels. Tumor cells are typically immunoreactive to vimentin and often to α-SMA; there is also immunoreactivity to estrogen and progesterone receptors. AMF demonstrates hypointense and hyperintense signals on T1-weighted and T2-weighted sequences respectively, and has a strong and homogeneous uptake in gadolinium-enhanced T1-weighted sequences [10]. AMF is a benign and non-recurring tumor and local surgical excision with clear margins is adequate treatment. AA, on the other hand, is a low-cellularity neoplasm containing small bland ovoid, spindled or occasionally stellate-shaped cells embedded in an abundant myxoid matrix. Mitotic activity and nuclear atypia are absent. Blood vessels are medium to large sized with thick, muscularized, and hyalinized walls [1]. Immunohistochemical stains show cells positive for vimentina, desmin, smooth muscle-actin (SMA), CD34, estrogen and progesterone receptors, and negative for S-

DNA architectural factor which plays an important role in transcriptional regulation. The over-expression of HMGA2 may be useful as a marker when used in the immunoperoxidase technique. A recent study has shown that the HMGA2 protein can be used after the initial diagnosis to evaluate margins and for identifying foci of residual or recurrent tumor [9]. However the pathogenetic mechanism involved is still the subject of study. Macroscopically, AA is typically deep, poorly-circumscribed, of a gelatinous, myxoid or occasionally fibrous consistency, and larger than ten cm in diameter. On slicing, the tumor often sticks tenaciously to the knife. Clinically it may be mistaken for Bartolini’s cyst, Gartner’s duct cyst, vaginal prolapsed, a vulvar abscess, a vaginal mass, a polyp, hernia or lipoma. The tumor appears as a hypoechoic mass similar to a cyst in an ultrasound scan, however, magnetic resonance imaging (MRI) is the gold standard for diagnosis: the tumor appears hypo-isointense with the muscle on T1-weighted images and hyperintense on T2-weighted sequences and shows a characteristic swirled intense pattern in gadolinium-enhanced T1-weighted sequences [10].

No radiological investigations were carried out as the clinical appearance of the tumor was that of a benign polyp. The differential diagnosis of this unusual tumor takes into account angiomyofibroblastoma, intramuscular myxoma, myxoid liposarcoma, myxoid neurofibroma, and sarcoma botryoides, a myxoid variant of malignant fibrous hystiocytoma. Distinguishing this disease from angiomyofibroblastoma (AMF) is very important. Both are rare mesenchymal tumors which were first compared by Fletcher [11]. AMF is characteristically a well-circumscribed and superficial lesion and is typically less than five cm in diameter; histologically it is composed of alternating hypocellular and hypercellular areas with plumper and round-shaped cells in clusters or in a linear array around small capillary-sized vessels. Tumor cells are typically immunoreactive to vimentin and often to α-SMA; there is also immunoreactivity to estrogen and progesterone receptors. AMF demonstrates hypointense and hyperintense signals on T1-weighted and T2-weighted sequences respectively, and has a strong and homogeneous uptake in gadolinium-enhanced T1-weighted sequences [10]. AMF is a benign and non-recurring tumor and local surgical excision with clear margins is adequate treatment. AA, on the other hand, is a low-cellularity neoplasm containing small bland ovoid, spindled or occasionally stellate-shaped cells embedded in an abundant myxoid matrix. Mitotic activity and nuclear atypia are absent. Blood vessels are medium to large sized with thick, muscularized, and hyalinized walls [1]. Immunohistochemical stains show cells positive for vimentina, desmin, smooth muscle-actin (SMA), CD34, estrogen and progesterone receptors, and negative for S-

Figure 1. — Aggressive angiomyxoma: the typical morphology with spindle and stellate cells in a hypocellular myxoid stroma containing large thick open-walled blood vessels.

Figure 2. — Vimentin positive cells: tumor cells are typically immunoreactive to vimentin.

Figure 3. — Immunohistochemical staining: positive staining of stromal cells for the estrogen receptor.
100 [12]; androgen receptor positivity has been described in cases in men. The preoperative diagnosis is challenging because patients are asymptomatic. A correct differential diagnosis is necessary for appropriate treatment and when AA is suspected, peripheral tissue should be resected to prevent recurrence. However only a microscopic examination, with immunohistochemical staining can confirm the diagnosis. Treatment usually consists in local surgical excision; however hormone-receptive patients have been treated with SERM (tamoxifen and raloxifen), aromatase inhibitors and GnRH analogues. Embolization and chemoembolization have also been used as treatment [13] while prophylactic ovariectomy is still the subject of study. Chemotherapy and radiotherapy are not used because the tumor has low mitotic activity. The local recurrence rate of AA is 9-72% [12] in the five years after surgical excision: approximately 70% of these within the first three years but late recurrences of up to 14 years have been reported [2]. Recurrence frequently occurs at the resection margins and is usually the result of insufficient primary excision, due to the fact that the tumor is non-encapsulated and has the same consistency as that of the surrounding connective tissue. The ischiorectal fossa, perineum, pelvis, and retropertioneum are the most common sites of recurrence documented in literature. In these cases surgical excision, radiotherapy and/or chemotherapy have been performed with some success. Follow-up should be clinical and radiological using MRI.

In the literature, angiomyxomas are consistently described as sizeable masses usually located elsewhere; however this presented case highlights the incidental finding of a neoplasia at an initial stage. This report details the initial growth phase of the neoplasia, before it acquires the characteristic features and dimensions that would lead a gynecologist to diagnose an angiomyxoma.

Indeed, pre-excision examination revealed a diameter of a few millimeters, a pink color that exactly matches the color of the vaginal mucosa, and the absence of abrased, bruised, or necrotic areas on the surface or the narrow base of implantation. These characteristics favored a preoperative diagnosis of pedunculated polypoid lesion of the lateral vaginal wall, and thus photographic evidence of the lesion, which was under hysteroscopic monitoring after the lateral vaginal wall, and thus photographic evidence of the lesion, which was under hysteroscopic monitoring and/or chemotherapy have been performed with some success. Follow-up should be clinical and radiological using MRI.

References

Address reprint requests to:
G. CARTA, M.D.
Department of Surgical Sciences
University of L’Aquila
Viale San Salvatore Edificio 6 ingresso A
67100 Coppito, L’Aquila (AQ) Italy
e-mail: gasparecarta@libero.it
clinicaostgjin@libero.it
Carney complex and teratoma maturum ovarii - a case report

L. Nejkovic, V. Pazin, S. Dragojevic-Dikic
“Narodni front” Clinic of Gynecology and Obstetrics, University of Belgrade, School of Medicine, Belgrade (Serbia)

Summary
This is a case report of an extremely rare Carney complex (CNC) syndrome in a 17-year-old patient. After the decision made by a team of cancer specialists, the patient was admitted to the hospital for surgery because of adnexal tumor associated with ascites and increased Ca 125 tumor marker level. The patient underwent cardiac surgery twice. Adnexal mass and ascites, revealed by transvaginal ultrasound (TVUS) and confirmed by magnetic resonance imagings (MRI), indicated the malignant alteration. Surgery was performed and surgical pathological staging was refined according to the FIGO guideline and included a staging laparotomy. After surgery, general condition of the patient was good, without ascites and pain, with Ca 125 marker levels within reference ranges.

Key words: Carney complex; Teratoma ovarii maturum.

Introduction
Carney complex is a syndrome named after A.J. Carney who first described it in 1985. According to NIH - Mayo Clinic and Cochin Centre data, there are approximately 500 cases who have undergone surgery to date. The latest research findings have proved that certain genetic changes may cause this syndrome and confirmed that malignant diseases and cell proliferation within the body are genetically induced [1-3].

This syndrome is characterized by tumor formation affecting different organs, such as: heart, breasts, endocrine organs including pituitary and thyroid glands, ovaries, gonads, adrenal glands, and includes skin and mucous membrane alterations [4].

It is an autosomal dominantly inherited syndrome, with overall penetrance of up to 70%. There are two types of chromosome mutations – type I mutation of the PRKAR 1A gene coding for the regulatory type I-α subunit of protein kinase A (PKA), located in 17q22q24; type II mutation located in chromosome 2 [5-7].

Two subtypes – type I and type II contain NAME – nevus, heart myxomas, myxoid neurofibromas and ephelide, and LAMB – (lentigines, atrial myxomas, mucocutaneous myxomas, and blue nevi syndrome). Mucosal and skin lesions (spotty pigmentation) occur in the face, hands, and lips [8, 9].

Tumors affecting endocrine system, heart, and skin are primarily heart myxomas which, due to their enhancement, lead to changes in heart functioning, embolisation, and strokes [10]. Myxoma symptoms are similar to fever – temperature accompanied by arthralgia and rash, and followed by increase in temperature. Changes in gland function can cause some ovarian disorders followed by enlarged adnexal mass and ascites, which can be of malignant nature [11-14].

Case Report
The authors report a 17-year-old patient with the diagnosis of CNC who was admitted to hospital for the surgery of malignant ovaries, after it had been decided by a team of cancer specialists.

She underwent heart surgeries in 2006 and in 2007. Since then she has had regular gynecological, endocrinological, ophthalmologic, neurological, and cardiovascular check-ups. She had been first hospitalized for cerebrovascular insult and it was then that the heart tumor was confirmed.

Genetic test results, confirmed by the Institute of Health Bethesda (USA), proved that she was the carrier of de novo mutation c418-419 delCa in egzon 4 of PRKAR 1A gene, which is characteristic of CNC syndrome and associated diseases [5, 6, 15].

Neurologist described left hemipareses including increased tone in the left arm, diminished tone in the left leg, negative Romberg test, Babinski’s sign was present on the left, meningeal signs were negative, brisk deep tendon reflexes, reduced power of the left arm, and weakness in the left leg. The patient was recommended spa rehabilitation. She also suffered from severe headaches and was treated with magnesium and zinc.

Laboratory and biochemical tests were performed as preoperative preparation and resulted within the reference ranges.

Abdominal ultrasound findings revealed that the liver, spleen, and pancreas were normal without focal lesions.

Furthermore the TVUS revealed substantial amount of fluid in the abdomen, heterogeneous mass (74 x 56 x 52 mm) in the left ovary and a right ovary with tumoral aspect (43 x 34 x 40 mm). The MRI imaging showed normal urinary bladder and ureters. Uterus corresponded to the patient's age. There was a lesion, over 100 mm in diameter, arising from the left ovary above the uterus and vesica urinaria. Tumor mass was heterogeneous and cystic in shape. The right ovary was 30 mm in diameter, its structure similar to tumor mass. No signs of retroperitoneal lymphadenopathy. Color doppler chest X-ray ultrasound of breast and thyroid and echocardiogram (ECG) were normal.

The management of the case was discussed with a multidisciplinary team.

The diagnosis was the following: obesity; malignant ovarian
neoplasia with ascites; left lateral hemiparesis and naevus pigmentosus.

The patient underwent exploratory laparotomy with aspiration approximately three liters of fluid, ovariectomy sin, appendectomy, multiple biopsies of the peritoneum and omentum.

Staging was done according to FIGO guidelines for ovarian carcinoma, ascites sample was sent to cytological analysis, as well as smears from paracolic gutter and subphrenic space with results that showed no malignant cells.

Ovariectomy was performed on the left side and tissue was sent to frozen histopathological analysis. The findings showed benign alteration on the left ovary – teratoma solidum maturum ovarii (Figure 1).

Appendectomy was performed and was normal. Multiple peritoneal biopsy revealed – hyperplasia of the mesothelium. Partial resection of omentum was carried out and no disease was found. The surgery was completed and the pathology report then showed benign nature of the removed tissue – teratoma maturum ovarii, in a usual manner (Figure 2).

The post-operative course was uneventful. Bowel peristalsis was normal and the patient was discharged five days after surgery. Ca 125 tumor marker level was within reference ranges one month after the surgery. Abdominal postoperative ultrasound findings were normal.

Discussion

Carney complex is an extremely rare syndrome and so far there have been 500 reported cases of this syndrome in the world. According to the latest data there are 160 cases [16]. It is a genetically determined syndrome, although it can appear as a de novo mutation on PRKAR1A gene in up to 30% [16, 17].

The diagnosis of CNC and de novo mutation c 418-419 delCa in egzon 4 PRKAR1A had just been confirmed in the patient reported.

This syndrome may include tumors of the central nervous system (CNS), endocrine organs, and heart, as well as spotty pigmentation of skin and mucous membranes and bone tumors [1, 4, 9, 10]. Myxoma cordis had been confirmed and the patient had undergone surgery twice.

About 7% of heart tumors, myxoma cordis, which were confirmed by ultrasound images, coexist with CNC [10]. Myxoma cordis had been confirmed and the patient had undergone surgery twice.

About 7% of heart tumors, myxoma cordis, which were confirmed by ultrasound images, coexist with CNC [10]. Myxoma cordis had been confirmed and the patient had undergone surgery twice.

Cases of bone tumors, osteochondromyxoma, coexisting with CNC have rarely been described in literature, only 1% [10]. There is evidence on coexistence of bone tumors and CNC in up to 10% of cases [16]. In certain cases ovarian cysts can become malignant [12, 14].

Cysts on a bizarre shape ovaries are usually followed by ascites and increased Ca 125 tumor marker level, which indicates to ovarian malignant cancer.
The patient presented symptoms of ascites and adnexal tumor. The CNC patients are usually related and their average age is 20 years [10]. The patient reported is 17-years-old and the diagnosis was confirmed when she was 12.

In order to confirm genetic mutation and the diagnosis, PRKAR1A gene mutation must be checked which can be altered in up to 50% of all the patients [10].

Surgery is to be performed according to the guidelines for ovarian cancers. Regardless of the symptoms prevailing in the patient, the authors decided to perform staging according to FIGO guidelines and preserve fertility after ex-tempore histopathological evaluation had confirmed benign nature of disease.

There is almost no evidence in literature on coexistence of ovarian cancers with CNC [13, 14, 18, 19]. In most cases serous and mucinous cystadenomas and simple ovarian cysts can be reported. There have been some cases of serous papillary ovarian carcinoma coexisting with CNC [11, 12]. There are no data on mature teratoma coexisting with CNC, as in our case.

Ascites evacuation, pain disappearance, opisthotonos and nausea with bloating, followed by laboratory and biochemical results within reference ranges, and subsequent return of Ca 125 tumor marker levels to reference ranges as well, were the result of well-performed operation.

Preoperative diagnostics - TVUS and MRI findings, together with the increase in tumor marker, had indicated a malignant nature of the tumor, but was then proved wrong. The only confirmation may come from intraoperative histopathological examination that is mandatory in these cases in order to preserve fertility in young patients.

The patients are advised to have regular check-ups every three months by a neurologist, cardiologist, and endocrinologist together with ultrasound examination of every three months by a neurologist, cardiologist, and endocrinologist together with ultrasound examination of every three months by a neurologist, cardiologist, and endocrinologist together with ultrasound examination of every three months by a neurologist, cardiologist, and endocrinologist together with ultrasound examination of every three months.

References

Covariates of high-risk human papillomavirus (HPV) infections are distinct for incident CIN1, CIN2 and CIN3 as disclosed by competing-risks regression models - K. Syrjänen, I. Shabalova, L. Sarian, P. Naud, A. Longatto-Filho, S. Derchain, V. Kozachenko, S. Tatti, M. Branovskaja, M. Branca, V. Grunjberga, M. Eržen, A. Juschenko, L. Serpa Hammes, J. Podistov, S. Costa, S. Syrjänen, the NIS* and the LAMS** study research groups ...

Expression of beclin 1, an autophagy-related protein, in human cervical carcinoma and its clinical significance - H.Y. Cheng, Y.N. Zhang, Q.L. Wu, X.M. Sun, J.R. Sun, X. Huang ...

Comparison of nerve content in removed parametrial tissue after classic radical hysterectomy and nerve-sparing radical hysterectomy - histologic evaluation - M. Barbič, S. Rakar, A. Levičnik, A.B. Di Stefano ...

Comparison of tumor markers and clinicopathological features in serous and mucinous borderline ovarian tumors - I. Alanbay, E. Aktürk, H. Coksuer, C.M. Erçan, E. Karaşahin, M. Dede, M.C. Yenen, H. Ozan, S. Dilek ...

Is magnetic resonance imaging useful in early evaluation of women on neoadjuvant chemotherapy for locally advanced cervical cancer? - P. Sala, P. Marchiolè, G. Cittadini, M. Valenzano Menada, M. Moioli, S. Mammoliti, S. Costantini ...

3D optical coherence tomography of cervical intraepithelial neoplasia - early experience and some pitfalls - J. Gallwas, R. Gaschler, H. Stepp, K. Friese, C. Dannecker ...

Combination of fertility preservation strategies in young women with recently diagnosed cancer - M. Huser, J. Zakova, L. Smardova, I. Crha, P. Janku, R. Hudecek, P. Ventruba ...

Mammographic features in infertile women as a potential risk for breast cancer: a preliminary study - M.L. Meggiorini, V. Cipolla, F. Rech, L. Labi, A. Vesti, C. de Felice ...

Prognosis of primary peritoneal carcinoma: effect of cytoreductive surgery combined with neoadjuvant chemotherapy after laparoscopic diagnosis and evaluation: a multi-center trial - F. Yang, J. Wang, H. Li, X. Tong ...

Could endometrial cytology be helpful in detecting endometrial malignancies? - U. Indraccolo, C. Bracalenti, R. Di Torto, S.R. Indraccolo ...

Evaluation of treatment results and prognostic factors in early-stage cervical carcinoma patients treated with postoperative radiotherapy or radiochemotherapy - S. Demirci, Z. Ozsaran, A. Ozsaran, F. Yavas, B. Demircioglu, M. Hanhan, Y. Dikmen, A.B. Aras ...

Small cell neuroendocrine carcinoma of the cervix: analysis of the prognosis and role of radiation therapy for 43 cases - L. Lan-Fang, S. Hai-Yan, Y. Zuo-Ming, Z. Jian-Qing, C. Ya-Qing ...

Possible effects of insulin-like growth factor-I, IGF-binding protein-3 and IGF-1/IGFBP-3 molar ratio on mammographic density: a cross-sectional study - M.L. Meggiorini, V. Cipolla, G. Borgoni, I. Nofroni, A. Pala, C. de Felice ...

Alterations in the mortality and growth cycle of cervical cancer cells treated with electroporation at different electric strengths - X.Y. Liu, Z.A. Xiong, H.S. Li, C.X. Li ...

Retrospective study comparing irinotecan and pegylated liposomal doxorubicin in treatment of recurrent platinum-refractory/resistant epithelial ovarian cancer - H. Nomura, H. Tsuda, F. Kataoka, T. Chiyoda, W. Yamagami, E. Tominaga, N. Susumu, D. Aoki ...


### CASE REPORTS

**Desmoplastic small round cell tumor (DSRCT) arising in the ovary: report of a case diagnosed at an early stage and review of the literature** - G. D’Ippolito, M.T. Huizing, W.A.A. Tjalma  

**Peritoneal mesotheliomas mimicking adnexal tumors. Clinicopathological characteristics of four cases and a short literature review** - D. Dellaportas, E. Kairi-Vassilatou, P. Lykoudis, P. Mavrigiannaki, S. Mellou, C.K. Kleanthis, A. Kondi-Pafiti  


**Primary gynaecological tumours mistaken for metastases: report of two cases with review of literature** - P.A. Menon, G. Kousparos, G.A. Culora  

**Rare metastases of pancreatic tail carcinoma in female genital organs** - M. Zamurovic, I. Pesic-Stevanovic  

**Atypical polypoid adenomyoma of the uterus. A case report and a review of the literature** - A. Zizi-Sermpetzoglou, E. Moustou, N. Petrakopoulou, E. Arkoumani, N. Tepelenis, V. Savvaidou


**Prognostic importance of selected molecular immunohistochemical markers and DNA ploidy in endometrial carcinoma** - M. Kudela, R. Pilka, M. Lubsky, P. Hejtmank, P. Dzubak, S. Brychtova


**Correlation between pre-operative endometrial sampling and final endometrial cancer histology** - O. Sany, K. Singh, S. Iha  

**The association between polymorphisms of the RAD51-G135C, XRCC2-Arg188His and XRCC3-Thr241Met genes and clinico-pathologic features in breast cancer in Poland** - H. Romanowicz-Makowska, B. Smolarz, M. Zadrożny, B. Westfal, J. Baszyczyński, G. Kokolażwili, M. Burzynski, I. Polač, S. Sporny  

**Sentinel node dissection in the treatment of early stages of vulvar cancer** - A. García-Iglesias, M.O. Rodríguez-Martín, R. Ruano, D. Beltrán, L. Péñalosa, B. Hernández-Barreiro, A. Martín de Arriba, J.L. Lanchares

**Expression of survivin and VEGF-C in breast cancer tissue and its relation to lymphatic metastasis** - Xiangqi Li, Xiangguo Dang, Xibo Sun


**Impact of sampling origin on molecular detection of high-risk human papillomavirus and oncogene expression** - S. Kahla, M. Achour, S. Oueslati, L. Kochbati, M.B. Chanoufi, M. Maalej, R. Oueslati
Index - Volume XXXIII, 2012

The impact of presurgical magnetic resonance in early breast cancer: an observational study - C. De Felice, V. Cipolla, A. Stagnitti, A. Marinii, E. Pasqualitto, M.L. Meggiorini.

Radical abdominal trachelectomy is a safe and fertility preserving option for women with early stage cervical cancer - A. Karateke, C. Kabaca.

Distribution of human papillomavirus types in Turkish women - Z.S. Tuncer, G. Boyraz, N. Sahin, A. Alp.

Do high levels of CA 19-9 in women with mature cystic teratomas of the ovary warrant further evaluation? - M.G. Ugur, E. Ozturk, O. Balat, E. Dikensoy, S. Teke, A. Aydin.


Peritonitis due to iatrogenic colpotomy after large loop excision of the transformation zone (LLETZ) in a patient with cervical intraepithelial neoplasia III: our experience of a rare case with review of the literature - M. Varras, C. Akrivis, A. Anastasiadis, G. Lekkas, G. Diakakis.


Tumor of the mesosalpinx: case report of a female adnexal tumor of probable Wolffian origin - X.U. Tianmin, Chang Weiqim, Cui Manhua, Li Xiaocui, Gao Hongwen, Yao Min.


Effect of cryotherapy and povidone-iodine preparation on eradication of DNA corresponding to highly oncogenic HPV in women with lesions in the uterine cervix - J. Markowska.


Comparison of the efficacy and complications of different surgical methods for cervical intraepithelial neoplasia - S.Y. Zeng, M.R. Liang, L.Y. Li, Y.Y. Wu.


Expression of tumor associated antigens CA 15-3 and CA 19-9 in trophoblast of the normal human placenta - Ž. Bojić-Trbojević, M. Jovanović Krivokuća, S. Vrzić-Petronijević, M. Petronijević, L. Vićovac

Regulation of radiosensitivity by HDAC inhibitor trichostatin A in the human cervical carcinoma cell line Hela - J. Yu, J. Mi, Y. Wang, A. Wang, X. Tian


Evaluation of endometrium by transvaginal ultrasonography and Doppler in tamoxifen-treated women with breast cancer - I. Bezircigolu, A. Baloglu, M.O. Tarhan, E. Oziz, S. Yigit

Status quo and prevention of overtreatment in cervical diseases - Xu Tianmin, Chang Weiqin, Cui Manhua, Li Yang, Si Libui, Wei Tianshu, Li Xiaocui


CASE REPORTS


Bilateral juvenile fibroadenosis of the breast: management with subcutaneous mastectomy and silicone implant placement - Z. Mátrai, G. Gulyás, G. Tizedes, L. Tóth, Z. Langmár, M. Kásler

Primary ovarian small cell carcinoma of pulmonary type with enlarged paraaortic lymph node masses: a case report and review of the literature - D. Tsolakidis, A. Papanikolaou, K. Ktenidis, S. Pervana


Long-term disease-free survival in three ovarian cancer patients with a single relapse - C.Y. Chen, H.P. Chang, K.K. Ng, C.C. Wang, C.H. Lai, A. Chao

Endometrioid ovarian cancer arising from an endometriotic cyst in a young patient - D. Zygouris, V. Leontara, G.M. Makris, C. Chrelias, E. Trakakis, Ch. Christoudoulaki, P. Panagopoulos

Removal of a vaginal leiomymoma presenting as tumor previum allowing vaginal birth - V. Boškovic, S. Vrzić-Petronijević, M. Petronijević, J. Atanackovic, D. Bratic


Primary ovarian leiomyosarcoma - D. Zygouris, G. Androutsopoulos, C. Grigoriadis, N. Arnogiannaki, E. Terzakis

Primary retroperitoneal mucinous cystadenoma adjacent to the kidney: report of two cases and review - S. Cheng, Y. Chen, L. Xu, Z. Zhang, G.Q. Ding

Volume XXXIII, n. 4, July-August 2012

DISTINGUISHED EXPERT SERIES

Longitudinal outcomes of high-risk human papillomavirus (HPV) infections as competing-risks events following cervical HPV test at baseline visit in the *NIS-LAMS** cohort - K. Syrjänen, I. Shabalova, L. Sarian, P. Naud, A. Longatto-Filho, S. Derchain, V. Kozachenko, S. Derchain, V. Kozachenko, C. Roteli-Martins, R. Nerovjna, L. Kljukina, S. Tatti, M. Branovskaja, M. Branca, V. Grunjberga, M. Eržen, A. Juschenko, L. Serpa Hames, J. Podistov, S. Costa, S. Syrjänen and the NIS* and LAMS** Study Research Groups

ORIGINAL ARTICLES


Expression of inflammatory cytokines by adipose tissue from patients with endometrial cancer - A. Zemlyak, J. Zakhaleva, M. Pearl, I. Mileva, M. Gelato, D. Mynarčík, M. McNurlan

Wortmannin inhibits proliferation and induces apoptosis of MCF-7 breast cancer cells - J. Yun, Y.G. Lv, Q. Yao, L. Wang, Y.P. Li, J. Yi


Laparoscopic versus laparotomic approach to endometrial cancer - A.M. Perrone, B. Di Marcoberardino, M. Rossi, F. Pozzati, A. Pellegrini, M. Procacci, D. Santini, P. De Iaco

Human epididymal protein 4 (HE4) is a novel biomarker and a promising prognostic factor in ovarian cancer patients - A. Chudecka-Głaz, I. Rzepka-Górska, I. Wojciechowska

Model for predicting the burden and cost of treatment in cervical cancer and HPV-related diseases in Thailand - W. Terungruanglert, P. Havanond, N. Khemapech, S. Lertmaharit, S. Pongpanich, P. Pijakorchaipong, S. Kitsiripornchai, S. Taneapanichskul

Laparoscopic surgery compared to traditional abdominal surgery in the management of early-stage cervical cancer - T. Simsek, M. Ozekinci, Z. Saruhan, B. Sever, E. Pestereli

Labeling of microvessel density, lyphatic vessel density and potential role of proangiogenic and lymphangiogenic factors as a predictive/prognostic factors after radiotherapy in patients with cervical cancer - M. Biedka, R. Makarewicz, A. Marszałek, J. Sir, H. Kardymowicz, A. Goralewska

A single nucleotide polymorphism in the 5' untranslated region of RAD51 and ovarian cancer risk in Polish women - H. Romanowicz-Makowska, B. Smolarz, D. Samulak, M. Michalska, J. Lewy, M. Burzyński, G. Kokofaszwili

CASE REPORTS


Cisplatin-gemcitabine as palliative chemotherapy in advanced squamous vulvar carcinoma: report of two cases - D.A. Santeufemia, G. Capobianco, G. Lo Re, G.M. Miolo, G.M. Fadda, P.L. Cherchi, S. Tumolo

A case of extramedullary solitary plasmacytoma arising at the uterine cervix - N. Sun, L. Wang, W. Li

Primary endometrial natural killer (NK)/T cell lymphoma: case report and review of literature - J. Wei, H. Wu, M. Sun, W. Liu, L. Meng


A rare case of umbilical and vaginal metastasis from endometrial cancer - review of the literature - A. Danilidis, A. Pantelis, K. Lathouras, F. Carcea, O. Papathanasiou, A. Loufopoulos

Hydatidiform mole in a perimenopausal and primary infertility patient: case report - M. Li, M.Y. Wu, Y. Han, R. Li

An apparently benign vulvar mass: possibly a rare malignancy - E.M. Messalli, M.L. D’Aponte, R. Luise, L. Rosselio, M. Rotondi, P. De Franciscis

Volume XXXIII, n. 5, September-October 2012

ORIGINAL ARTICLES

Clinical significance of Mena and Her-2 expression in breast cancer - J.W. Du, K.Y. Xu, L.Y. Fang, X.L. Qi


Role of the association of high-risk HPV identified by real-time PCR in cervical preneoplastic lesions - G. Balbi, E. Seguino, A. Napolitano, F. Giordano, S. Capuano, M.A. Manganaro, L. Di Martino, D. Fusco, F. Grauso.


CD4+CD25+Foxp3+ Treg and TGF-β play important roles in pathogenesis of Uygur cervical carcinoma - Z.F. Chen, Q. Xu, J.B. Ding, Y. Zhang, R. Du, Y. Ding.


Correlation of human papilloma virus infection with cytology, colposcopy and histopathological examination of the biotic tissue in low- and high-grade intraepithelial lesions - A. Mitrovic Jovanovic, S. Dragojevic, V. Kalampokas, A. Dragovic, D. Krsic, S. Rakic, B. Stanimirovic.

Diagnostic value of thrombocytosis and high CA 125 level in women with adnexal masses - T. Atacag.


Dermatofibrosarcoma protuberans of the mons pubis - A. Zizi-Sermpetzoglou, V. Savvaidou, S. Fournogerakis, E. Moustou, M. Konstantidelli, N. Vlachakos.

A case of occult bowel perforation after a cycle of chemotherapy for advanced epithelial ovarian carcinoma - X. Zhou, P. Hu, Y. Yue, Z. Duan.


Volume XXXIII, n. 6, November-December 2012

LETTER TO EDITOR

Emerging etiopathogenic connections between Arg399Gln polymorphism and systemic malignancies besides their etiological role in endometrial carcinomas - S. Kapoor.

REVIEW ARTICLE

Brain metastases from cervical carcinoma: overview of pertinent literature - E. Piura, B. Piura.

ORIGINAL ARTICLES


HPV at the time of vaccine: has screening reached its goal? - E. Tartaglia, D. Iafusco, A. Cocca, S. Palomba, M. Rotondi, P. Mastrantonio.


Management of ASCUS findings in Papanicolaou smears. A retrospective study - C. Iavazzo, I. Boutsas, C. Grigoriadis, N. Vrachnis, N. Salakos.


Ten years survival of FIGO Stage IIIC epithelial ovarian cancer cases due to lymph node metastases only - E. Grossi, S. Noli, G. Scarfone, A. Villa, F. Parazzini, S. Cipriani, G. Bolis.


Factors affecting response of chemotherapy in women with ovarian cancer - J. Lubin, A. Markowska, P. Knapp ................................................. 644

**CASE REPORTS**

Leiomyosarcoma after hysteroscopic myomectomy: a case report - G. Carta, P. Palermo, R. Di Ramio, V. De Lellis, A. Carta, F. Patacchiola ................................................. 656
Aggressive deep angiomyxoma - a case report and review of the literature - P. Kaščák, M. Zámečník ............ 658
Can malignant transformation in mature cystic teratoma be preoperatively predicted? - M. Futagami, Y. Yokoyama, H. Mizukami, T. Shigeto, H. Mizunuma ........................................... 662
Synchronous squamous cell carcinoma of the endometrium and endometrioid adenocarcinoma of the ovary - C. Grigoriadis, G. Androutsopoulos, D. Zygouris, N. Arnogiannaki, E. Terzakis .............................. 666
Aggressive angiomyxoma of the vaginal wall at the initial stage: a case report - G. Carta, V. Parisse, V. Accurti, L. Sollima, L. Di Stefano, A. D’Alfonso, F. Patacchiola ................................................. 669
Carney complex and teratoma maturum ovarii - a case report - L. Nejkovic, V. Pazin, S. Dragojevic-Dikic .................... 672
Contents index vol. XXXIII, 2012 ........................................ 675
Authors index vol. XXXIII, 2012 ........................................ 683
Index of Authors
in alphabetical order

Accurti V., 669
Achour M., 187
Adad S.J., 245
Adanir I., 278
Aguilera C., 485
Akrivos C., 214
Aktürk E., 25, 168
Al R., 625
Alanbay I., 25, 168
Ali-Fehmi R., 449
Alp A., 204
Altgassen C., 574
Altinboga O., 459
Altintas A., 493
Al-Wahab Z., 449
Anastasiasis A., 214
Androustopoulos G., 331, 666
Antonopoulou Z., 521
Aoki D., 86
Aoyama T., 579
Arai H., 370
Arakawa A., 138
Aras A.B., 62
Arkoumani E., 118, 230
Arnojanniaki N., 331, 666
Arsenijevic L., 236
Arsic B., 543
Ashkar N., 90
Assorgi C., 164
Atacag T., 517
Atanackovic J., 236, 326
Athanatos D., 174
Audlin K.M., 534
Aydin A., 207
Babarzic E., 134
Baird R.D., 211
Bakalianou K., 328
Balan O., 207
Baltal, F., 467
Baloglu A., 295
Barcat E.C., 155, 498
Barbić M., 211
Barton D.P.J., 211
Başılılı N., 549
Basta Nikolač M., 227
Baszczynski J., 145
Battista Meloni G., 90
Baykal C., 528, 549, 625
Bayoglu I.V., 652
Beltrán D., 151
Bends R., 574
Ben-Harim Z., 419
Besler A., 459

Beyan E., 459
Bezircigluglu I., 295
Biedka M., 399
Bili H., 174
Bojić-Trbojević Ž., 281
Bolis G., 617
Bononi M., 164
Borahay M., 183
Borgoni G., 74
Boškovic V., 326
Bösze P., 129
Botsis D., 508
Boutas I., 605
Boynukalin K., 625
Boyray G., 204, 278
Bracalenti C., 60
Branca M., 5, 341
Branovskaja M., 5, 341
Bratic D., 326
Bruchini L., 419
Brychtiowa S., 159
Burzyski M., 145, 406
Čumonoglu C., 625
Cai Y.L., 584
Can A., 652
Candiani M., 261
Cantú D., 485
Capobianco G., 90, 421, 629
Capuano S., 467
Carcea F., 436
Carminati G., 261
Carriero C., 433
Carta A., 656
Carta G., 656, 669
Cetina L., 485
Chang H.P., 321
Chanouf M.B., 187
Chao A., 321
Chen C.Y., 321
Chen J.G., 105
Chen K., 601
Chen Y., 334
Chen Z.F., 502
Chen Z.H., 105
Cheng B., 93
Cheng H.Y., 15
Cheng S., 334
Cheng Y.X., 93, 291
Cherchi C., 629
Cherchi P.L., 90, 421, 629
Chiyoda T., 86
Chrielas C., 324
Christodoulakis Ch., 324
Chudecka-Glaz A., 382
Chung H.S., 358
Ciardi A., 164
Cipolla V., 51, 74, 193
Cipriani S., 617
Cidadini G., 31
Clementi M., 261
Cocca A., 591
Cokkuer H., 25, 168
Contini M., 629
Cooper J.C., 534
Corrino G., 433
Coronel J., 485
Cossu-Rocca P., 629
Costa M.P., 155

Costa S., 5, 341
Costantini S., 31
Crhá I., 42
Culora G.A., 109
D’Alfonso A., 669
D’Aponte M.L., 441
D’Ippolito G., 96
Dai X.L., 633
Dang Xiangguo, 178
Damilidis A., 436
Dannecker C., 37
De Cesare A., 164
de Felice C., 51, 74, 193
De Franceschi P., 441
De Iaco P., 376
De Lellis V., 656
De Miglio M.R., 629
de Oliveira Filho H.R., 498
Dede M., 25, 168
Dellaportas D., 101
Demir G., 549
Demir L., 652
Demirci S., 62
Demircioglu B., 62
Demirat Y., 459
Decherain S., 5, 341
Dessole F., 90
Dessole M., 629
Dessole, S., 90
Di Iorio R., 60
Di Marcoberardino B., 376
Di Martino L., 467
Di Ramiro R., 656
Di Stefano A.B., 21
Di Stefano L., 669
Diakakis G., 214
Diedrich K., 306, 574
Dikensyo E., 207
Dikmen Y., 62
Dilke S., 25
Dilvio M., 411
Ding G.Q., 334
Ding J.B., 502
Ding Y., 502
Dircan A., 652
Djurdjevic S., 627
Dogusoy G.B., 528, 549
Doussias V., 304
Dragosjevic Dikic S., 512, 672
Du Č.X., 274
Du J.W., 455
Du R., 502
Duan G., 472
Duan P., 472
Duan Z., 540
Dueñas-González A., 485
Dünder I., 528, 549
Dzubak P., 159

Elsaihaik M.A., 449
England J., 183
Enomoto T., 620
Ercan C.M., 25, 168
Ertan A.K., 183
Ertan C., 652
Erzen M., 5, 341
Fabrini M.G., 640
Fadda G.M., 421
Fang L.Y., 455
Famucchi A., 640
Ferreira M.C., 155
Ferrero A., 640
Filassi J.R., 498
Filippidou A., 463
Filippidou-Giannopoulou A., 617
Firfiris N., 230
Fishman A., 419
Fournogerakis S., 537
Freeman D., 183
Frega A., 164
French D., 164
Friedrich M., 306, 574
Friese K., 37
Fujimori K., 223
Fujimoto T., 353
Fujita M., 524
Fukuda T., 252
Furukawa S., 223
Furuya K., 269, 414, 579
Fusco D., 467
Futagami M., 662
Gaca M., 217
Gadducci A., 640
Gallwas J., 37
Garalejic E., 543
García-Arias A., 485
García-Iglesias A., 151
Gaschler R., 37
Geachan N., 304
Gelato M., 363
Gentile M., 164
Giordano F., 467
Gissi F., 433
Göcz P., 134
Godoy H.E., 477
Goralewska A., 399
Goto T., 269, 414, 579
Grammatoglou X., 230
Grauso F., 467
Gregorioiu O., 508
Grigoriadis C., 328, 331, 463, 508, 605, 617, 666
Grigoriadis L., 508
Grisaru D., 265
Grossi E., 617
Grunjberga V., 5, 341
Gulec U.K., 610
Gulyás G., 309
Gumurdulu D., 493, 610
Guzel A.B., 493, 610
Güz K., 480
Hai-Yan S., 68
Han Y., 438
Hanman M., 62
Hashiguchi Y., 252
Hassiaskos D., 463
Hasson J., 265
Haugk C., 306
Havanond P., 391
Hayashi S., 370
Hejtmánek P., 159
Heiler I., 265
Hemmerlein B., 306
<table>
<thead>
<tr>
<th>Index of Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jha S., 142</td>
</tr>
<tr>
<td>Jenei C., 134</td>
</tr>
<tr>
<td>Jeftovic M., 236</td>
</tr>
<tr>
<td>Janku P., 42</td>
</tr>
<tr>
<td>Jang J.Y., 601</td>
</tr>
<tr>
<td>Im D.D., 534</td>
</tr>
<tr>
<td>Indraccolo U., 60</td>
</tr>
<tr>
<td>Ichimura O., 252</td>
</tr>
<tr>
<td>Huizing M.T., 96</td>
</tr>
<tr>
<td>Hua H., 633</td>
</tr>
<tr>
<td>Horányi D., 134</td>
</tr>
<tr>
<td>Hongwen Gao, 233</td>
</tr>
<tr>
<td>Khemapech N., 391</td>
</tr>
<tr>
<td>Khan A.Z., 211</td>
</tr>
<tr>
<td>Kelling K., 574</td>
</tr>
<tr>
<td>Khan A.Z., 211</td>
</tr>
<tr>
<td>Khatib G., 493</td>
</tr>
<tr>
<td>Khemapech N., 391</td>
</tr>
<tr>
<td>Ki E.Y., 151</td>
</tr>
<tr>
<td>Kilic G., 183</td>
</tr>
<tr>
<td>Kim D.H., 358</td>
</tr>
<tr>
<td>Kim T.H., 358</td>
</tr>
<tr>
<td>Kimura T., 316, 524, 620</td>
</tr>
<tr>
<td>Kita T., 269</td>
</tr>
<tr>
<td>Kitada K., 252</td>
</tr>
<tr>
<td>Kitazawa S., 352</td>
</tr>
<tr>
<td>Kitasiripornchai S., 193</td>
</tr>
<tr>
<td>Kianthit C.K., 101, 617</td>
</tr>
<tr>
<td>Klujinina L., 5, 341</td>
</tr>
<tr>
<td>Knapp P., 644</td>
</tr>
<tr>
<td>Kochubi L., 187</td>
</tr>
<tr>
<td>Koiss R., 134</td>
</tr>
<tr>
<td>Kokohaszewil G., 145, 406</td>
</tr>
<tr>
<td>Kono K., 620</td>
</tr>
<tr>
<td>Konstantideli M., 537</td>
</tr>
<tr>
<td>Kopuz A., 459</td>
</tr>
<tr>
<td>Koukouras D., 255</td>
</tr>
<tr>
<td>Kououlolias V., 250</td>
</tr>
<tr>
<td>Kouskos E., 521</td>
</tr>
<tr>
<td>Kousparos G., 109</td>
</tr>
<tr>
<td>Kozachenko V., 5, 341</td>
</tr>
<tr>
<td>Kric V., 312</td>
</tr>
<tr>
<td>Ktenidis K., 312</td>
</tr>
<tr>
<td>Kucukcevki Guce V., 493</td>
</tr>
<tr>
<td>Kucukcevkev G., 652</td>
</tr>
<tr>
<td>Kudela M., 159</td>
</tr>
<tr>
<td>Kudoh K., 269</td>
</tr>
<tr>
<td>Kumagai S., 353</td>
</tr>
<tr>
<td>Kurt S., 459</td>
</tr>
<tr>
<td>Labi L., 51</td>
</tr>
<tr>
<td>Lai C.H., 321</td>
</tr>
<tr>
<td>Lanchares J.L., 151</td>
</tr>
<tr>
<td>Lan-Fung L., 68</td>
</tr>
<tr>
<td>Langmár Z., 309</td>
</tr>
<tr>
<td>Laser B., 449</td>
</tr>
<tr>
<td>Lathouras K., 436</td>
</tr>
<tr>
<td>Lee H., 56, 599</td>
</tr>
<tr>
<td>Lee H.H., 358</td>
</tr>
<tr>
<td>Lee H.N., 526</td>
</tr>
<tr>
<td>Lee K.H., 526</td>
</tr>
<tr>
<td>Lee W.S., 358</td>
</tr>
<tr>
<td>Lekkas G., 214</td>
</tr>
<tr>
<td>Lele S.B., 477</td>
</tr>
<tr>
<td>Leone L., 433</td>
</tr>
<tr>
<td>Leontara V., 324</td>
</tr>
<tr>
<td>Lertmaharit S., 391</td>
</tr>
<tr>
<td>Lessing J.B., 265</td>
</tr>
<tr>
<td>Levčík A., 21</td>
</tr>
<tr>
<td>Lewy J., 406</td>
</tr>
<tr>
<td>Li B.S., 291</td>
</tr>
<tr>
<td>Li C.X., 79</td>
</tr>
<tr>
<td>Li H.S., 79</td>
</tr>
<tr>
<td>Li L., 257</td>
</tr>
<tr>
<td>Li M., 438</td>
</tr>
<tr>
<td>Li R., 438</td>
</tr>
<tr>
<td>Li W., 423</td>
</tr>
<tr>
<td>Li Xiangqi, 178</td>
</tr>
<tr>
<td>Li Y.P., 367</td>
</tr>
<tr>
<td>Liang M.R., 257</td>
</tr>
<tr>
<td>Libii Si, 300</td>
</tr>
<tr>
<td>Liu L., 425</td>
</tr>
<tr>
<td>Liu X.Y., 79</td>
</tr>
<tr>
<td>Liu Y.F., 633</td>
</tr>
<tr>
<td>Liu Y.J., 472</td>
</tr>
<tr>
<td>Lo Re G., 421</td>
</tr>
<tr>
<td>Louzi V., 433</td>
</tr>
<tr>
<td>Lombardi D., 164</td>
</tr>
<tr>
<td>Longatto-Filho A., 5, 341</td>
</tr>
<tr>
<td>Lorenzon L., 164</td>
</tr>
<tr>
<td>Loufopoulos A., 436</td>
</tr>
<tr>
<td>Lu J.Q., 472</td>
</tr>
<tr>
<td>Lu M., 449</td>
</tr>
<tr>
<td>Lu W., 93</td>
</tr>
<tr>
<td>Lubin J., 644</td>
</tr>
<tr>
<td>Lubusky M., 159</td>
</tr>
<tr>
<td>Luise R., 441</td>
</tr>
<tr>
<td>Luo D.L., 105</td>
</tr>
<tr>
<td>Ly.Y.G., 367</td>
</tr>
<tr>
<td>Lykoudis P., 101</td>
</tr>
<tr>
<td>Maalej M., 187</td>
</tr>
<tr>
<td>Mabuchi S., 316, 524, 620</td>
</tr>
<tr>
<td>Makarewicz R., 399</td>
</tr>
<tr>
<td>Makris G.M., 324</td>
</tr>
<tr>
<td>Mammoliti S., 31</td>
</tr>
<tr>
<td>Manganaro M.A., 467</td>
</tr>
<tr>
<td>Manhua Cui, 233, 300</td>
</tr>
<tr>
<td>Manoloudaki K., 230</td>
</tr>
<tr>
<td>Mantus D., 521</td>
</tr>
<tr>
<td>Marchiolì P., 31</td>
</tr>
<tr>
<td>Marini A., 193</td>
</tr>
<tr>
<td>Markopoulos C., 521</td>
</tr>
<tr>
<td>Markouvas A., 644</td>
</tr>
<tr>
<td>Markowska J., 249</td>
</tr>
<tr>
<td>Marras V., 90, 629</td>
</tr>
<tr>
<td>Marszalek A., 399</td>
</tr>
<tr>
<td>Martin de Arriba A., 151</td>
</tr>
<tr>
<td>Mastrantonio P., 591</td>
</tr>
<tr>
<td>Mátrai Z., 309</td>
</tr>
<tr>
<td>Matsumoto Y., 252</td>
</tr>
<tr>
<td>Matsuo K., 534</td>
</tr>
<tr>
<td>Mavigiannaki P., 101</td>
</tr>
<tr>
<td>McNurlan M., 363</td>
</tr>
<tr>
<td>Meggiorini M.L., 51, 74, 193</td>
</tr>
<tr>
<td>Mellou S., 101</td>
</tr>
<tr>
<td>Meng L., 425</td>
</tr>
<tr>
<td>Menon P.A., 109</td>
</tr>
<tr>
<td>Messali E.M., 441</td>
</tr>
<tr>
<td>Mi J., 285</td>
</tr>
<tr>
<td>Michalska M., 406</td>
</tr>
<tr>
<td>Michelin M.A., 598</td>
</tr>
<tr>
<td>Mileva I., 363</td>
</tr>
<tr>
<td>Min Yao, 233</td>
</tr>
<tr>
<td>Mi J., 285</td>
</tr>
<tr>
<td>Misirlioglu S., 610</td>
</tr>
<tr>
<td>Mitrovic Jovanovic A., 512</td>
</tr>
<tr>
<td>Miyamoto M., 269, 414, 579</td>
</tr>
<tr>
<td>Miyazaki K., 546</td>
</tr>
<tr>
<td>Mizukami H., 662</td>
</tr>
<tr>
<td>Mizunuma H., 648, 662</td>
</tr>
<tr>
<td>Mocan Kuzey G., 625</td>
</tr>
<tr>
<td>Mocko Kacanski M., 227</td>
</tr>
<tr>
<td>Modaffari P., 640</td>
</tr>
<tr>
<td>Moioli M., 31</td>
</tr>
<tr>
<td>Moreno V., 211</td>
</tr>
<tr>
<td>Morimoto A., 316</td>
</tr>
<tr>
<td>Moscarini M., 324</td>
</tr>
<tr>
<td>Moustou E., 118, 537</td>
</tr>
<tr>
<td>Mukkarah A., 449</td>
</tr>
<tr>
<td>Murta E.F.C., 245, 598</td>
</tr>
<tr>
<td>Munarcik R., 376</td>
</tr>
<tr>
<td>Naganatsu M., 620</td>
</tr>
<tr>
<td>Nakahara K., 353</td>
</tr>
<tr>
<td>Nakayama K., 546</td>
</tr>
<tr>
<td>Napolitano A., 467</td>
</tr>
<tr>
<td>Narahara H., 219</td>
</tr>
<tr>
<td>Nasu K., 219</td>
</tr>
<tr>
<td>Naud P., 5, 341</td>
</tr>
<tr>
<td>Navrozoglou L., 304</td>
</tr>
<tr>
<td>Nejovic L., 672</td>
</tr>
<tr>
<td>Nerovnya R., 5, 341</td>
</tr>
<tr>
<td>Ng K.K., 321</td>
</tr>
<tr>
<td>Nikolic B., 512</td>
</tr>
<tr>
<td>Nisoli O., 227</td>
</tr>
<tr>
<td>Ninomiya T., 370</td>
</tr>
<tr>
<td>Nishida M., 219</td>
</tr>
<tr>
<td>Nishikawa H., 138</td>
</tr>
<tr>
<td>Nishiyama H., 223, 353</td>
</tr>
<tr>
<td>Nisihyama T., 138</td>
</tr>
<tr>
<td>Nofroni I., 74</td>
</tr>
<tr>
<td>Noli S., 617</td>
</tr>
<tr>
<td>Nomelini R.S., 245</td>
</tr>
<tr>
<td>Nomura H., 86</td>
</tr>
<tr>
<td>Noviita G., 498</td>
</tr>
<tr>
<td>Oduki K., 477</td>
</tr>
<tr>
<td>Okamoto M., 219</td>
</tr>
<tr>
<td>Okazawa M., 620</td>
</tr>
<tr>
<td>Olawase J.B.B., 477</td>
</tr>
<tr>
<td>Ooki S., 370</td>
</tr>
<tr>
<td>Opara T., 217</td>
</tr>
<tr>
<td>Origoni M., 261</td>
</tr>
<tr>
<td>Oueslati R., 187</td>
</tr>
<tr>
<td>Oueslati S., 187</td>
</tr>
<tr>
<td>Ozan H., 25, 168</td>
</tr>
<tr>
<td>Ozekinci M., 395</td>
</tr>
<tr>
<td>Oziz E., 295</td>
</tr>
<tr>
<td>Oszarans A., 62</td>
</tr>
<tr>
<td>Oszarazn Z., 62</td>
</tr>
<tr>
<td>Ozturk E., 207</td>
</tr>
<tr>
<td>Pados G., 174</td>
</tr>
<tr>
<td>Pala A., 74</td>
</tr>
<tr>
<td>Palermo P., 656</td>
</tr>
<tr>
<td>Palomba S., 591</td>
</tr>
<tr>
<td>Panagopoulos P., 324</td>
</tr>
<tr>
<td>Pantelakos P., 411</td>
</tr>
<tr>
<td>Pantelis A., 436</td>
</tr>
<tr>
<td>Papakostantinou E., 617</td>
</tr>
<tr>
<td>Papagnikolaou A., 312</td>
</tr>
<tr>
<td>Papanastassou O., 436</td>
</tr>
<tr>
<td>Parazzini F., 617</td>
</tr>
<tr>
<td>Parisse V., 669</td>
</tr>
<tr>
<td>Park E.S., 358</td>
</tr>
<tr>
<td>Park J.S., 318</td>
</tr>
<tr>
<td>Park T.S., 318</td>
</tr>
<tr>
<td>Park T.C., 526</td>
</tr>
<tr>
<td>Paschosopoulos M., 304</td>
</tr>
<tr>
<td>Pasqualito E., 193</td>
</tr>
<tr>
<td>Patacchiola E., 656, 669</td>
</tr>
<tr>
<td>Paydas S., 493</td>
</tr>
<tr>
<td>Pazin V., 672</td>
</tr>
<tr>
<td>Pearl M., 363</td>
</tr>
<tr>
<td>Pechhivani F., 304</td>
</tr>
<tr>
<td>Pedrazza D., 183</td>
</tr>
<tr>
<td>Pellegrini A., 376</td>
</tr>
<tr>
<td>Pepehlos L., 151</td>
</tr>
<tr>
<td>Pérez D., 485</td>
</tr>
<tr>
<td>Perovic M., 543</td>
</tr>
<tr>
<td>Perrone A.M., 376</td>
</tr>
<tr>
<td>Pervan S., 312</td>
</tr>
<tr>
<td>Pesic-Stevanovic I., 116</td>
</tr>
</tbody>
</table>
Index of Authors

685

Pesterel E., 395
Petrkopoulou N., 118
Petronevijevic M., 281, 326
Pili F., 629
Pilka R., 159
Pincarat O.K.M., 498
Piura B., 567
Piura E., 567
Platoni K., 411
Podistov J., 5, 341
Polaj L., 145
Pongpanich S., 391
Poizatti F., 376
Procaccini M., 376
Putignano G., 433
Qui X.L., 455
Qui X.Y., 291
Qui J., 633
Rahman M., 546
Rahman M.T., 546
Rakar S., 21, 428
Rakic S., 512
Rattan G., 265
Rauh-Hain I.A., 477
Rech F., 51
Ren M.L., 584
Resta L., 433
Ricci M.D., 498
Rivera L., 485
Robbins J.R., 449
Rodriguez-Martin M.O., 151
Rolla S., 261
Romanowicz-Makowska H., 145, 406
Rossi M., 376
Rossiello L., 441
Rotelli-Martins C., 5, 341
Rotondi M., 441, 591
Ruan R., 151
Ruzi C.A., 498
Rzepka-Gorska L., 382
Şahin N., 204, 278
Sala P., 31
Salakos N., 328, 463, 605
Salehin D., 306, 574
Samulak D., 406
Santeufemia D.A., 421, 629
Santini D., 436
Santojic A., 236
Safos T., 304
Step H., 37
Stieken A., 530
Stojanovic S., 227
Stojinic J., 236
Sturc N., 480
Gugino T., 223
Sugiura-Ogasawara M., 138
Sugiyama T., 353
Sumi T., 252
Sun J.R., 15
Sun M., 425
Sun N., 423
Sun X.M., 15
Sun Xibo, 178
Sun Y.C., 489
Susumu N., 86
Suzumori N., 138
Syriakos K., 5, 341
Syriakos S., 5, 341
Takai N., 219
Takano M., 269, 414, 579
Takano T., 223
Takeuchi K., 352
Tamura R., 648
Tan R., 640
Tanevanchaskul S., 391
Taniguchi R., 648
Tarhan M.O., 295, 652
Sever B., 395
Shalabova I., 5, 341
Shahzad M.M.K., 477
Shao X.G., 601
Shavit T., 419
Shen Y., 584
Shi Y.H., 584
Shigeto T., 648, 662
Shin O.R., 526
Shoji T., 353
Siddiqui M.S., 449
Sideri M., 261
Siklos P., 134
Simek T., 395
Singh K., 142
Svecovec J., 428
Sirc J., 399
Skafka E., 230
Slepnev R., 217
Smardova L., 42
Smolarz B., 145, 406
Smlorki S., 428
Snyder R., 183
Soares J.M., 155, 498
Soeda S., 223
Soufodis C., 328, 508
Sollina L., 669
Somali L., 652
Sor T., 428
Soykan C., 555
Sporny S., 145
Spyropoulos C., 255
Stagnitti A., 193
Stanimirovic B., 512
Stanojevic D., 543
Stefanovic A., 236
Stefos T., 304
Step K., 37
Stiekema A., 530
Stojanovic S., 227
Stojnic J., 236
Suer N., 480
Sugino T., 223
Sugiura-Ogasawara M., 138
Sugiyama T., 353
Sumi T., 252
Sun J.R., 15
Sun M., 425
Sun N., 423
Sun X.M., 15
Sun Xibo, 178
Sun Y.C., 489
Susumu N., 86
Suzumori N., 138
Syriakos K., 5, 341
Syriakos S., 5, 341
Takai N., 219
Takano M., 269, 414, 579
Takano T., 223
Takeuchi K., 352
Tamura R., 648
Tan R., 640
Tanevanchaskul S., 391
Taniguchi R., 648
Tarhan M.O., 295, 652
Tarlazis B., 174
Tartaglia E., 591
Tatti S., 5, 341
Teke S., 207
Tepelenis N., 118
Termurunglert W., 391
Terzakis E., 331, 666
Thill M., 306
Tian X., 285
Tianmin Xu, 233, 300
Tianshu Wei, 300
Tizedes G., 309
Tjalma W.A.A., 96
Tolia M., 411
Tominaga E., 86
Tong X., 56
Torrasi M.R., 164
Toth L., 309
Trakakis E., 324
Tsolakidis D., 174, 312
Tsokalas N., 411
Tsuda H., 86
Tsujino T., 552
Tumolo S., 421
Tuncer Z.S., 204, 278
Türkmen I.C., 528, 549
Tzarakosletherakis E., 255
Ueda Y., 620
Ugur M.G., 207, 625
Unay F.C., 652
Usaj S.K., 343
Valenzano Menada M., 31
Vardar M.A., 493, 610
Varras M., 214
Vasilakaki Th., 230
Vasiljevic M., 543
Ventru P., 42
Verheijen R.H.M., 530
Vestri A., 51
Vícovac L., 281
Vignati C., 640
Villa A., 617
Vitifel-Pedersen J., 211
Vlachakos N., 537
Vrachnis N., 615
Vrzić-Petronevijevic S., 281, 326
 Wan X., 93
Wang A., 285
Wang Ch., 321
Wang J., 56
Wang L., 367, 423
Wang Y., 274, 285
Warenik-Szymankiewicz A., 217
Watanabe A., 269, 579
Watanabe T., 223
Wei J., 425
Weijing Chang, 233, 300
Westfall B., 145
William M., 306
Wojciechowska I., 382
Wu H., 425
Wu L.Y., 489
Wu M.Y., 438
Wu Q.L., 15
Wu Y.Y., 257
Xiaocui L., 233, 300
Xie X., 93
Xiong Z.A., 79
Xu K.Y., 455
Xu L., 334
Xu Q., 502
Xu X.X., 291
Yabuta M., 552
Yagasaki N., 353
Yamagami W., 86, 370
Yamashita H., 370
Yamagita T., 648
Yang F., 56
Yang Li, 300
Yao Q., 367
Yap T.A., 211
Ya-Qing C., 68
Yasui T., 252
Yasus F., 62
Yayci E., 480
Ye H., 105
Yenen M.C., 25, 168
Yetimalah M.H., 652
Yi J., 367
Yigit S.C., 295, 652
Yokoyama Y., 353, 648, 662
Yoshida H., 252
Yoshikawa T., 269
Yoshino K., 620
Yoshizaki A., 353
Yu J., 285
Yue Y., 540
Yun J., 367
Zabere Rossi A.G., 155
Zadrozný M., 145
Zakhalava J., 363
Zakharchenko S., 5, 341
Zakova I., 42
Žámečník M., 658
Zamurovic M., 116, 512
Zaramboukas T., 174
Zecic N., 236
Zemlyak A., 363
Zeng S.Y., 257
Zevrakis A., 508
Zervoudis S., 304
Zhang G.Y., 489
Zhang R., 489
Zhang Y., 502
Zhang Y.N., 15
Zhang Y.X., 584
Zheng Z., 334
Zheng B.B., 472
Zhou L.M., 291
Zhou S.L., 633
Zhou X., 540
Zizi-Sermetetzoglou A., 118, 537
Zola F., 640
Zuo J., 489
Zuo-Ming Y., 68
Zygomas A., 255
Zygouris D., 324, 331, 666
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 JACOB (UK)
JACQUES HOECKEL (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
an International Journal
www.irog.net

The Journal publishes original research and clinical contributions, preferably briefly reported, in the fields of Gynaecology, Obstetrics, Foetal Medicine, Gynaecological Endocrinology, Fertility and Sterility, Menopause, Uro-gynaecology, Ultrasound, Sexually transmitted diseases and related subjects, from all over the world.

Founded in 1974 (ISSN 0390 6663) Issued quarterly in English, the Journal is covered by INDEX MEDICUS, MEDLINE (PUBMED), EMBASE/Excerpta Medica, INDEX COPERNICUS.

We hope to have you as a subscriber of our Journal which is improving its scientific and clinical interdisciplinary activity and value and which is approaching its XXXIII year of life.

You can subscribe or renew your subscription by sending us the following form.

Yes, start my subscription.

ISSN: 0390-6663

Published three monthly

Founding Editor
A. Onnis
Montréal (CND)

Editors-in-Chief
M. Marchetti
J.H. Check
Montréal (CND) Camden, NJ (USA)

Assistant Editor
A. Sinopoli
Toronto (CND)

SUBSCRIPTION ORDER CARD 2013

ISSN 0390-6663. Published three monthly. 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.

Please enter my subscription at the rate I've checked:

☐ Institutional: $100 $US
☐ Individual: $90 $US
☐ Booksellers and subscription agencies $270 $US
☐ Please send me a free sample copy

Payment: (U.S. CURRENCY ONLY)

☐ for PDF file: on line through PAY PAL (all credit card)
☐ for hard copy

Credit Card: ☐ Mastercard ☐ American Express ☐ Visa ☐ Diners

Bank transfer: Beneficiary: 7847050 Canada Inc. - 4900 Côte St-Luc, # 212 - Montréal, Québec, Canada H3W 2H3 - Account number 00001 003402-402245 SWIFT ROYCCAT 2

N° ___________________________________________ Exp. Date ________________

Signature _________________________________ Date __________________________

Issues are to be mailed to:

7847050 CANADA, Inc. - 4900 Côte St-Luc - Apt # 212 - Montréal, Qué. H3W 2H3 (Canada)
Tel. +514-4893242 - Fax +514-4854513 - E-mail: canlux@qc.aira.com - www.irog.net
The journal publishes original peer reviewed works, preferably briefly reported, in the fields of female genital cancers and related subjects and also proceedings of gynecologic oncology society meetings all over the world.

Founded in 1980 (ISSN 0392 2936) it is issued bi-monthly in English.

The Journal is covered by CURRENT CONTENTS, SCISEARCH, RESEARCH ALERT, INDEX MEDICUS, MEDLINE (PUBMED), EMBASE/Excerpta Medica, CURRENT ADVANCES IN CANCER RESEARCH, BIOSIS, INDEX COPERNICUS.

We hope to have you as a subscriber of our Journal which is improving its scientific and clinical interdisciplinary contributions on female genital cancer, year by year.

You can subscribe or renew your subscription by sending us the following form.

Yes, start my subscription.

European Journal of Gynaecological Oncology
an International Journal
www.irog.net

ISSN: 0392-2936
Published bimonthly

Founding Editor
A. Onnis
Montréal (Canada)

Editors-in-Chief
M. Marchetti P. Bősze
Montréal (Canada) Budapest (Hungary)

Associate Editor
T. Maggino
Padua (Italy)

Assistant Editor
A. Sinopoli
Toronto (CND)

EUROPEAN JOURNAL
OF GYNAECOLOGICAL ONCOLOGY
an International Journal

SUBSCRIPTION ORDER CARD 2013

ISSN 0392-2936. Published 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.

Please enter my subscription at the rate I’ve checked:

☐ Institutional: 430 $US
☐ Individual: 220 $US
☐ Booksellers and subscription agencies 370 $US
☐ Please send me a free sample copy

Payment: (U.S. CURRENCY ONLY)
☐ for PDF file: on line through PAY PAL (all credit card)
☐ for hard copy
Credit Card: ☐ Mastercard ☐ American Express ☐ Visa ☐ Diners
Bank transfer: Beneficiary: 7847050 Canada Inc. - 4900 Côte St-Luc, # 212 - Montréal, Québec, Canada H3W 2H3 - Account number 00001 003402-402245 SWIFT ROYCCAT 2

N° ___________________________ Exp. Date ___________

Signature ______________________ Date ____________________

Issues are to be mailed to:

7847050 CANADA, Inc. - 4900 Côte St-Luc - Apt # 212 - Montréal, Qué. H3W 2H3 (Canada)
Tel. +514-4893242 - Fax +514-4854513 - E-mail: canlux@qc.aira.com - www.irog.net