european journal of gynaecological oncology
an International Journal

Founding Editor
A. Onnis
Montréal (Canada)

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

Associate Editor
T. Maggino
Padua (Italy)

Assistant Editor
J. Wilson
San Diego - CA (USA)

Editorial Board

Allen H.H., London, Ontario (Canada)
Ayhan A., Ankara (Turkey)
Balat O., Graziantep (Turkey)
Balega J., Birmingham, England (UK)
Bânceanu G., Bucharest (Romania)
Basta A., Krakow (Poland)
Bender H.C., Dusseldorf (Germany)
Charkviani T., Tbilisi (Georgia)
Chiarelli S., Padua (Italy)
De Oliveira C.F., Coimbra (Portugal)
Dexeus S. Jr., Barcelona (Spain)
Di Paola G.R., Buenos Aires (Argentina)
Di Re F., Milan (Italy)
Di Saia P., Orange, CA (USA)
Elit L., Hamilton (Canada)
Friedrich M., Krefeld (Germany)
Geisler H.E., Indianapolis, IN (USA)
Gorins A., Paris (France)
Heintz A.P.M., Utrecht (The Netherlands)
Ioannidou-Mouzaka L., Athens (Greece)
Jordan J.A., Birmingham, England (UK)
Klastersky J., Bruxelles (Belgium)
Kubista E., Vienna (Austria)
Lee Y.S., Daegu (South Korea)
Markowska J., Poznan (Poland)
Marth C., Innsbruck (Austria)
Massuger Leon F.A.G., Nijmegen (The Netherlands)
Menczer J., Savyon (Israel)
Monsonego J., Paris (France)
Pálfalvi L., Budapest, (Hungary)
Piura B., Beer Sheva (Israel)
Piver S.M., Buffalo, NY (USA)
Rakar S., Ljubljana (Slovenia)
Shepherd J.H., London, England (UK)
Siklòs P., Budapest (Hungary)
Smit B.J., Tygerberg (South Africa)
Stelmachów J., Warsaw (Poland)
Syrijänen K., Turku (Finland)
Ungá L., Budapest (Hungary)
Vermorken J.B., Edegem (Belgium)
Wang P.H., Taipei (Taiwan)
Winter R., Graz (Austria)
Yokoyama Y., Hiroaki (Japan)

Publishing Organization (M. Morsani):
I.R.O.G. CANADA, Inc. - 4900 Côte St-Luc - Apt # 212 - Montréal, Qué. H3W 2H3 (Canada)
Tel. +514-4893242 - Fax +514-4854513 - E-mail: canlux@mgroup-online.com - www.irog.net

Editorial Office (M. Critelli):
Galleria Storione, 2/A - 35123 Padua (Italy) - Tel. (39) 049 8756900 - Fax (39) 049 8752018

EUROPEAN JOURNAL OF GYNAECOLOGICAL ONCOLOGY (ISSN 0392-2936) publishes original peer reviewed works in the fields of female genital cancers and related subjects and also proceedings of gynecologic oncology society meetings all over the world. The Journal is covered by CURRENT CONTENTS, SCISEARCH, RESEARCH ALERT, INDEX MEDICUS, MEDLINE, EMBASE/Excerpta Medica, CURRENT ADVANCES IN CANCER RESEARCH, BIOSIS.
A prospective randomised phase II trial of thalidomide with carboplatin compared with carboplatin alone as a first-line therapy in women with ovarian cancer, with evaluation of potential surrogate markers of angiogenesis


The combination of thalidomide and carboplatin, used as first-line chemotherapy in ovarian cancer, is safe, well tolerated, and may increase the efficacy compared with carboplatin alone.

Nodal metastasis in endometrial cancer

G. Willis, J.E. Misas, W. Byrne, E. Podczaski - Harrisburg (PA), USA

Patient survival and disease characteristics in endometrial cancer patients with nodal spread.

Metastasis gene expression analyses of choriocarcinoma and the effect of silencing metastasis-associated genes on metastatic ability of choriocarcinoma cells

L. Huining, C. Jingting, H. Keren - Guangzhou, People’s Republic of China

This article discovered choriocarcinoma metastasis-associated genes, and proved that CAV-1 and VEGF-B play an important role in metastatic capability of human choriocarcinoma.

Safety and efficacy of a splenectomy during debulking surgery for Müllerian carcinoma


Splenectomy can be performed safely and effectively during debulking surgery for appropriately selected patients with primary or recurrent Müllerian carcinoma.

XRCC1 Arg399Gln polymorphism and risk for cervical cancer development in Argentine women


A case-control study of 217 Argentine women revealed reduced risk of cervical cancer development associated with XRCC1 Arg399Gln genotypes and 194Arg-399Gln haplotype.

Compliance to adjuvant therapy in breast cancer patients

C. Dittmer, K. Roeder, F. Hoellen, D. Salehin, M. Thill, D. Fischer - Luebeck, Germany

An evaluation of breast cancer patients regarding their adherence to radiotherapy, chemotherapy and endocrine therapy is provided.

Discrepancy of pre- and postoperative grades of patients with endometrial carcinoma

A. Karateke, N. Tug, C. Cam, S. Selcuk, M.R. Asoglu, S. Cakir - Istanbul, Turkey

This study highlights the clinical fact that endometrial cancer patients with preoperative low grades have a significant potential to upgrade, thus the management of these patients peroperatively according to their preoperative grades could be inappropriate, at least for some.

Diagnostic test for ovarian cancer composed of ovarian cancer symptom index, menopausal status and ovarian cancer antigen CA125

R. Macuks, I. Baidekalna, S. Donina - Riga, Latvia

The ovarian cancer symptom index could be used as a first step screening tool in combination with serum biomarkers.

Ovarian germ cell tumors in children: a 20-year retrospective study in a single institution

Chao Yang, Shan Wang, Chang-Chun Li, Jun Zhang, Xiang-ru Kong, Jun Ouyang - Chongqing, China

A retrospective study to guide the evaluation and surgical management of ovarian tumors in children was conducted.
Specific downregulation of death-associated protein kinase enhances Fas-mediated apoptosis in the human differentiated endometrial adenocarcinoma cell line, HHUA

T. Tanaka, T. Bai, K. Yukawa - Wakayama, JAPAN

Death-associated protein kinase negatively regulates Fas-mediated apoptosis in human differentiated endometrial adenocarcinoma cells.

Immunological evaluation of vaginal secretion in patients with high-grade cervical intraepithelial neoplasia treated with intralesional interferon α-2b

M.C. Mardegan, M.C. Ramos, S.J. Adad, M.A. Michelin, D. Shimba, E.F.C. Murta - Uberaba (MG), BRAZIL

Levels of more inflammatory cytokines may correlate with intralesional IFNα-2b treatment failure of CIN2-3.

Importance of office hysteroscopy screening to diagnose endometrial carcinoma in menopausal women

A. Tripodi, C. De Salvo, C. Ermio, D. Manuzio, G. Romeo, P. Vadala - Reggio Calabria, ITALY

Among different diagnostic procedures, office hysteroscopy is a valid instrument to detect endometrial pre cancer and clearly neoplastic lesions especially in menopausal women.

Copper and zinc concentrations in Nigerian women with breast cancer

G.O. Ajayi - Lagos, NIGERIA

The concentrations of copper and zinc are altered in Nigerian females with breast cancer.

p16 and retinoblastoma protein expression in endometrial carcinoma and clinical significance

V. Mue Koh, Y.X. Shi, Q.H. Tang - Yaounde, CAMEROUN

Decreased p16 expression is a significant event in endometrial carcinoma pathogenesis and is inversely correlated to tumor cell grade.

Recombinant human endostatin, Endostar, enhances the effects of chemo-radiotherapy in a mouse cervical cancer xenograft model

Y. Jia, M. Liu, L. Cao, X. Zhao, J. Wu, F. Lu, Y. Li, Y. He, S. Ren, Y. Ju, Y. Wang, Z. Li - Hebei, CHINA

Endostar enhanced the anti-cancer effect of chemo-radiotherapy in a mouse xenograft model of cervical cancer.

CASE REPORTS

Lymphoepithelial-like carcinoma of the uterine cervix; a case report


A case report of lymphoepithelial-like carcinoma of the uterine cervix in a 52-year-old woman is presented.

Laparoscopic total fallopian tube removal at the time of bilateral salpingo-oophorectomy in BRCA2 positive women

J. Wabersich, G. Artioli, R. Giordano, F. De Lorenzi, G. Azzarello, F. Garbin - Mirano, ITALY

A case of a BRCA 2 positive woman who underwent laparoscopic surgery with removal of all tubes for pathological analysis without hysterectomy is presented.

Virilizing ovarian Krukenberg tumor in a 27-year-old pregnant woman. A case report and literature review


A case of virilizing ovarian Krukenberg tumor in a 27-year-old pregnant woman is reported.

Unexpected synchronous follicular lymphoma of paraaortic and pelvic lymph nodes in a patient with endometrial carcinoma: a case report


Non-Hodgkin’s lymphoma was discovered unexpectedly during the staging operation of endometrial carcinoma.

Leiomyosarcoma of ovarian vein compression as a cause of hydronephrosis

S.H. Yang, J.C.W. Chien, C.L. Chen, W.P. Chan - Taiwan, REPUBLIC OF CHINA

MRI can clearly define the relationships of the ureter, ovarian vein and psoas muscle in diagnosing leiomyosarcoma of the ovarian vein.

Proximal-type epithelioid sarcoma of the mons pubis: report of a case

A. Andrisani, A. Serena, G. Ambrosini, G. Capobianco, S. Chiarelli - Padua, ITALY

Gynecologists should not undervalue all soft-tissue masses of the genital area. PES is a rare aggressive soft tissue tumor with a deceitful behavior.
Off-midline retroperitoneal choriocarcinoma presenting as neurologic symptoms
J.C.W. Chien, Y.L. Hsiao, S.E. Lin, W.P. Chan - Taiwan, REPUBLIC OF CHINA
A child-bearing woman who presents with a cerebral hemorrhagic mass with rapid growth choriocarcinoma should be considered.

Conservative management of decidualized ovarian endometriotic cyst during pregnancy mimicking malignancy: case report and a review of the literature
T. Tohya, T. Tajima - Kumamoto, JAPAN
Conservative management of a decidualized ovarian endometriotic cyst during pregnancy mimicking malignancy: Case report and a review of the literature.

Primary squamous cell carcinoma of the endometrium: a case report
Histogenesis, diagnosis and treatment of primary endometrial squamous cell carcinoma – a rare neoplasm without characteristic symptoms and predisposing factors.

Angiomyofibroblastoma of the vulva: a clinicopathological and immunohistochemical analysis of a rare benign mesenchymal tumor
Morphological characteristics of a rare case of angiomyofibroblastoma which developed in the vulva.

Krukenberg tumor of gastric origin in pregnancy with dismal outcome
J. Stojnic, A. Stefanovic, K. Jeremic, S. Kadija, M. Jefovic, J. Jeremic - Belgrade, SERBIA
A 31-year-old female in the 27th week of pregnancy treated for ovarian krukenberg tumor metastasis resulted in a fatal outcome for both mother and newborn.

Alveolar soft part sarcoma of the uterine cervix in a woman presenting with postmenopausal bleeding: a case report and literature review
W.D. Kang, S.H. Heo, Y.D. Choi, H.S. Choi, S.M. Kim - Gwangju, KOREA
The good prognosis of cervical ASPS is related to early clinical detection, small size, resectability, and demarcation of the tumor.

Tumor dissemination after laparoscopic surgery for an unsuspected endometrial stromal tumor
K. Pavlakis, I. Messini, C.A. Papadimitriou, F. Zagouri, P. Yiannou, D. Mavrelos, T. Panoskaltis - Athens, GREECE
Laparoscopic excision of an endometrial stromal tumor might result in tumor dissemination into the abdominal cavity. A second careful examination of the abdomen or a radical surgical approach is proposed.

ERRATA - CORRIGE
Role of lymphadenectomy in endometrioid endometrial cancer
Vol. XXXII, n. 1, 2011, page 49
Errata:
A. Martínez
Corrige:
A. Pascual Martínez
A prospective randomised phase II trial of thalidomide with carboplatin compared with carboplatin alone as a first-line therapy in women with ovarian cancer, with evaluation of potential surrogate markers of angiogenesis

S.R. Muthuramalingam¹, J.P. Braybrooke¹, A.D. Blann², S. Madhusudan¹, S. Wilner¹, A. Jenkins³, C. Han¹, K. Kaur¹, T. Perren³, T. S. Ganesan¹

¹Cancer Research UK Medical Oncology Unit, University of Oxford, Churchill Hospital, Oxford
²Haemostasis, Thrombosis and Vascular Biology Laboratory, University Department of Medicine, City Hospital, Birmingham
³Cancer Research UK Clinical Centre, St. James’s University Hospital, Leeds (UK)

Introduction

Ovarian cancer is the fourth most common cancer among women in the UK with around 6,900 new cases diagnosed each year. The majority of patients present with advanced disease (Stage III/IV) and primary treatment is usually optimal debulking surgery followed by chemotherapy with carboplatin ± paclitaxel, which typically produces a response rate of around 60-70% [1, 2]. Despite advances in treatment, most patients die from recurrent disease, with about 30% surviving five years after diagnosis [3]. Hence, there is a major need for new approaches to the treatment of this common gynaecological cancer.

Angiogenesis, the formation of new blood vessels, is required for most tumours to grow beyond 1-2 mm in diameter. The expression of platelet-derived growth factor, an angiogenic agent, was shown to be greater in malignant ovarian tumours than benign tumours (p < 0.001) [4] and increased VEGF expression was observed in 97% of ovarian carcinomas, and strong expression was associated with advanced stage and poorer survival [5]. Tumour derived VEGF is also obligatory for ascites formation in ovarian cancer patients [6]. Elevated pre-treatment serum VEGF levels in ovarian cancer patients have been shown to be associated with poorer disease-free survival and overall survival [7, 8]. Other circulating angiogenic factors like basic fibroblast growth factor (bFGF), E-selectin, von Willebrand factor (vWF) and vascular cell adhesion molecule-1 (VCAM-1) have been found to be elevated in patients with many solid tumours including ovarian cancer [9-12].

Thalidomide is an immunomodulatory agent that inhibits angiogenesis and cytokines such as tumour necrosis factor [13, 14]. In addition it has been shown to modulate cell adhesion [15]. Thalidomide has been demonstrated to have modest activity in several human tumours including ovarian cancer [16]. There was no previous published report of a combination of thalidomide with chemotherapy in ovarian cancer when this trial was designed. Recent studies using single agent thalidomide at the median dose of 200 mg/day in advanced and recurrent ovarian cancer showed the response rate varied from 7.7% to 18% and stable disease in 35%-53.8% of patients [17, 18]. Many phase I and II trials using thalidomide in incremental doses in combination with carboplatin in solid tumours support further investigation of this combination [19, 20].

We initiated an open label, prospective, randomised phase II study of carboplatin and thalidomide in chemotherapy naïve patients with Stage IC-IV epithelial ovarian cancer. The primary objectives were to investi-
gate safety and efficacy of thalidomide in combination with carboplatin and to assess the anti-angiogenic effects by surrogate markers. The effective dose of thalidomide in solid tumours is not known. It is not clear if a definite dose response relationship exists, or whether smaller doses of thalidomide can be equally effective with fewer side-effects. There is also concern about peripheral neuropathy, particularly when the dose of thalidomide exceeds > 75 mg/day [21] and there is some correlation between cumulative dose of thalidomide and risk of peripheral neuropathy [22, 23]. The information regarding the neurotoxicity of thalidomide precluded the use of thalidomide beyond a total dose of 18 g when the trial was designed. Since thalidomide was planned to be given for a total duration of six months, we decided to use a dose of 100 mg/day as continuous therapy, with the total cumulative dose of 18 g. The combination of carboplatin and paclitaxel with thalidomide was not chosen in view of concern regarding the increased risk of peripheral neuropathy associated with the above combination. Increasing carboplatin doses above AUC 7 doses not necessarily improve the likelihood of response but does increase myelotoxicity [24]. Therefore we decided to use carboplatin at the dose of AUC 7 given every four weekly, based on EDTA clearance, which was standard dosing in our unit at the time the trial was designed.

Materials and Methods

The study was conducted after local ethical research committee approval and according to Helsinki’s declaration (1989). All newly diagnosed eligible ovarian cancer patients were recruited after written informed consent when they attended oncology outpatient clinics. To be eligible, each patient was required to meet the following criteria: (1) histologically confirmed diagnosis of epithelial ovarian cancer with Stage IC-IV; (2) WHO performance status 0, 1 or 2; (3) age over 18 years. To avoid potential teratogenicity from thalidomide all eligible ovarian cancer patients should be postmenopausal or if premenopausal, they must have had bilateral salpingo-oophorectomy and/or total abdominal hysterectomy. Patients who received previous chemotherapy for ovarian cancer were ineligible. Patients with diabetes mellitus, chronic neurological disease causing peripheral neuropathy and other concurrent invasive malignancies were excluded. All patients were given at least 24 hours before they could make the final decision about the study. This study was conducted in the Cancer Research UK Medical Oncology Unit, Churchill Hospital, Oxford and St. James’s University Hospital, Leeds.

Treatment plan

Patients were randomised without stratification to receive either (1) carboplatin alone at the dose of AUC 7 (7 x (creatinine clearance + 25)) using creatinine clearance calculated by EDTA clearance (uncorrected value) every four weeks up to six cycles; or carboplatin at the same dose and schedule, plus thalidomide 100-mg orally each day at bedtime. Thalidomide was taken continuously for a total of 24 weeks commencing on the first day of carboplatin therapy and stopped four weeks after the last dose of chemotherapy. All patients received standard premedications before the chemotherapy. Prior to each cycle of chemotherapy biochemistry and haematological tests were obtained. Serum CA-125 was measured before each cycle. All patients had a baseline CT scan of the abdomen and pelvis with or without chest and the scan was repeated after three cycles of chemotherapy and at the end of treatment to assess response.

Sensory nerve action potentials (SNAP) were performed in all patients prior to treatment and then at two, four and six months. Thalidomide was discontinued if there was a greater than 40% decrease in the sensory nerve action potential when compared with the baseline value. If the decrease was less than 40%, then the test was repeated after one month.

Toxicity and response evaluation

Toxicity was defined by Cancer and Leukaemia Group B (CALGB) expanded common toxicity criteria. Carboplatin was delayed by one week if absolute neutrophil count was less than 1.5 x 10^9/1 or platelets were less than 100 x 10^9/1. If the treatment was delayed by two or more weeks then the dose of carboplatin was reduced to the dose of AUC 5. If grade 3 or 4 myelosuppression occurred the dose of carboplatin was reduced to AUC 5. If the calculated creatinine clearance worsened by more than 25%, then EDTA clearance was repeated and the dose of carboplatin was recalculated. For those patients with evaluable or measurable disease, WHO criteria were used to assess response after completion of treatment.

Assessment of surrogate markers of angiogenesis

Prior to each cycle of treatment, two 10-ml blood samples for serum and plasma were obtained to analyse potential surrogate markers of angiogenesis. The blood samples were centrifuged at 2500 rpm for 10 min at 4°C and separated plasma and serum were stored at –20°C or below until analysis. Before analysis, samples were thawed slowly and mixed gently.

VCAM-1, E-Selectin, VEGF, bFGF and vWF were measured in the serum, in duplicates, following the protocols provided by the manufacturer. [Human VCAM-1 ELISA kit (R&D Systems, Abingdon, UK), human E-Selectin ELISA kit (R&D Systems Ltd.; Oxford, UK), human VEGF ELISA kit (R&D systems, Abingdon, UK), human bFGF ELISA kit (R&D Systems, Abingdon, UK), human vWF ELISA kit (Alpha Labs, Hants, UK)]. We chose the above markers as surrogate markers of angiogenesis based on previous phase II trials evaluating these markers with other anti-angiogenic drugs conducted in our unit [25-27].

Statistical methods

At the time of the design of the study the effects of thalidomide in combination with carboplatin chemotherapy were not known, particularly effects on the levels of plasma/serum surrogate markers of angiogenesis. Therefore, there was no data in the literature on which number of patients required could be decided. Forty patients were chosen to evaluate if there would be any effect on circulating angiogenic markers based on previous phase II trials evaluating these markers with other anti-angiogenic drugs conducted in our unit [25-27]. In all these studies effects on surrogate markers were observed with a similar number of patients. To clearly delineate the effects of thalidomide, we felt it was preferable to do a randomised study rather than a simple phase II trial. This would separate any effects of carboplatin on the measurement of surrogate markers. The paired t-test was used to analyse differences in surrogate markers of angiogenesis before and after treatment in both groups and linear regression analysis was used to evaluate the differences between the two groups.
Results

Patient data

From 1998 to 2002, a total of 40 patients were enrolled into the trial (20 patients to the carboplatin-alone group and 20 to the carboplatin and thalidomide group). Patient characteristics are summarised in Table 1. Apart from four patients with adverse histological features like clear cell and mucinous who were randomised by chance to the carboplatin and thalidomide group, patient characteristics were similar in both treatment groups. Ninety-five percent of patients (19 out of 20) in the carboplatin group and 70% of patients in the carboplatin and thalidomide group (14 out of 20) received six cycles of treatment. Six patients in the experimental arm received less than the intended six cycles of treatment. One patient died after completing five cycles and one patient died after completing two cycles of treatment. Two patients had progressive disease after three cycles and two patients discontinued treatment after three cycles due to toxicity (peripheral sensory neuropathy in one patient and fatigue in another patient).

Table 1. — Patient characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Carboplatin</th>
<th>Carboplatin/Thalidomide</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>69.5 (41 to 84)</td>
<td>68 (40 to 80)</td>
</tr>
<tr>
<td>WHO Performance status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stage at time of diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>III-IV</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Radical surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(BSO ± TAH + Omentectomy)</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Residual disease at the end of surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 cm</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>≥ 2 cm</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Histology of the tumour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Muscinous</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Clear cell</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Primary peritoneal carcinoma</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Grade of the tumour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Not known</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>No. of cycles of treatment received</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

BSO, bilateral salpingo-oophrectomy; TAH, total abdominal hysterectomy.

Table 2. — Incidence of treatment-related adverse events.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Carboplatin</th>
<th>Carboplatin/Thalidomide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemia</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Nausea/Vomiting</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Sensory neuropathy</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Constipation</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Tiredness</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

n = 20 patients. The figures represent number of patients.

Table 3A. — Mean value for serum markers in the carboplatin arm.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>E-selectin (ng/ml)</th>
<th>bFGF (pg/ml)</th>
<th>VEGF (pg/ml)</th>
<th>Urine VEGF (ng/g creatinine)</th>
<th>VCAM-1 (ng/ml)</th>
<th>vWF (IU/dl)</th>
<th>CA-125 (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>31</td>
<td>15</td>
<td>713.5</td>
<td>919.2</td>
<td>556.8</td>
<td>136.9</td>
<td>417.4</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>28.8</td>
<td>6.7</td>
<td>232.9</td>
<td>182.8</td>
<td>513.6</td>
<td>127.4</td>
<td>400.4</td>
</tr>
<tr>
<td>Cycle 3</td>
<td>23.2</td>
<td>5.5</td>
<td>273.7</td>
<td>121.8</td>
<td>450.3</td>
<td>134.6</td>
<td>223.9</td>
</tr>
<tr>
<td>Cycle 4</td>
<td>26.2</td>
<td>7.9</td>
<td>449.5</td>
<td>168.8</td>
<td>478.6</td>
<td>129.7</td>
<td>183.2</td>
</tr>
<tr>
<td>Cycle 5</td>
<td>24</td>
<td>3.9</td>
<td>360.6</td>
<td>145</td>
<td>486.6</td>
<td>131.5</td>
<td>164.4</td>
</tr>
<tr>
<td>Cycle 6</td>
<td>23</td>
<td>5</td>
<td>246.6</td>
<td>142.2</td>
<td>470.4</td>
<td>133</td>
<td>141.5</td>
</tr>
</tbody>
</table>

n = 19 patients for all markers and at least 3 cycles of chemotherapy to have been completed.

Table 3B. — Mean value for serum markers in the carboplatin/thalidomide arm.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>E-selectin (ng/ml)</th>
<th>bFGF (pg/ml)</th>
<th>VEGF (pg/ml)</th>
<th>Urine VEGF (ng/g creatinine)</th>
<th>VCAM-1 (ng/ml)</th>
<th>vWF (IU/dl)</th>
<th>CA-125 (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>49</td>
<td>14.5</td>
<td>671.3</td>
<td>415.7</td>
<td>568</td>
<td>139</td>
<td>417.4</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>41.5</td>
<td>10.8</td>
<td>369.4</td>
<td>227.5</td>
<td>513.6</td>
<td>148.5</td>
<td>404.4</td>
</tr>
<tr>
<td>Cycle 3</td>
<td>39.7</td>
<td>4.2</td>
<td>295</td>
<td>219.3</td>
<td>450.3</td>
<td>143.1</td>
<td>223.9</td>
</tr>
<tr>
<td>Cycle 4</td>
<td>38</td>
<td>4.4</td>
<td>376.7</td>
<td>208.6</td>
<td>478.6</td>
<td>142.2</td>
<td>183.2</td>
</tr>
<tr>
<td>Cycle 5</td>
<td>37.5</td>
<td>3.9</td>
<td>403.9</td>
<td>130.4</td>
<td>486.6</td>
<td>132.2</td>
<td>164.4</td>
</tr>
<tr>
<td>Cycle 6</td>
<td>38</td>
<td>5.9</td>
<td>429.2</td>
<td>142.2</td>
<td>470.4</td>
<td>132.9</td>
<td>141.5</td>
</tr>
</tbody>
</table>

n = 17 patients for all markers and at least 3 cycles of chemotherapy to have been completed.

Toxicity

All patients who received any treatment were evaluated for toxic effects. The observed toxicities are summarised in Table 2. Haematological toxicities were mild in both treatment groups. Of the non-haematological toxicities, the most common adverse events in both treatment groups were grade 1-2 nausea, tiredness, constipation and dizziness. Three patients in the carboplatin group experienced grade 3 nausea and one patient in the combination group developed grade 4 nausea. More patients in the carboplatin and thalidomide group developed constipation (5 vs 3), tiredness (16 vs 11) and symptoms suggestive of peripheral neuropathy (6 vs 3), when compared with the carboplatin group. No major abnormality was detected in baseline and follow-up SNAP tests in either treatment group except for one patient in the carboplatin and thalidomide arm who developed transient grade 3 peripheral neuropathy and SNAP test showed a mild axonal polyneuropathy.
Response to therapy

Patients who received three or more cycles of chemotherapy with or without thalidomide were included for analysis of efficacy. Nineteen out of 20 patients in each group were evaluable for response. In the carboplatin group 11 patients achieved complete response (CR) and seven patients achieved partial response (PR) (overall response rate 90%). Disease stabilised in one patient and none of the patients developed progressive disease. In contrast, in the carboplatin and thalidomide group eight patients achieved CR, and PR was seen in seven patients, and one patient achieved disease stabilisation (overall response rate 75%). However, three patients in this group had progressive disease on treatment. There was statistically no significant difference in overall response rate (CR+PR) (p = 0.41) and complete response (p = 0.34) between the two treatment groups.

Surrogate markers of angiogenesis

The results for the serum surrogate markers of angiogenesis and serum CA-125 were evaluated in 19 patients in the carboplatin group (Table 3A) and 17 patients in the combination group (Table 3B). There were wide inter and intra-individual differences in surrogate markers of angiogenesis in both treatment groups. There was a significant fall in CA-125 and E-selectin in the carboplatin group (p < 0.001) and in the carboplatin/thalidomide group (p = 0.001) after treatment. There was no significant change in the levels of E-Selectin between the two groups. C - Carboplatin (n = 19); C+T - carboplatin and thalidomide (n = 17).

Discussion

Targeting tumour angiogenesis as an anti-cancer strategy is well established in solid tumours. In addition, a combination of standard chemotherapy with angiogenesis inhibitor may enhance therapeutic efficacy as such an
approach allows the use of agents with complimentary mechanisms of action and potentially non-overlapping toxicities. We evaluated the role of thalidomide in combination with carboplatin in a phase II randomised trial in ovarian cancer. We also investigated the anti-angiogenic effects of thalidomide.

We have shown that the combination of carboplatin and thalidomide is well tolerated in most patients. As expected, the incidence of constipation, tiredness, dizziness and peripheral neuropathy was more common in the thalidomide and carboplatin arm. When compared with the most recent trials that used thalidomide at a higher dose (400 – 600 mg/day) in combination with chemotherapy, the relative lack of toxicity we observed in our study is not surprising [24, 25]. No significant neurotoxicity was observed either clinically or as assessed by SNAP test and this observation was similar to other published studies [28, 29].

We could not demonstrate the expected increased efficacy by the addition of thalidomide to carboplatin in chemotherapy naïve ovarian cancer patients. There was no significant difference between the carboplatin and carboplatin/thalidomide groups in terms of disease response (90% vs 75%, p = 0.41). In contrast to our study, increased response rate to a combination of thalidomide (median dose of 200 mg/day) and topotecan has been demonstrated in a prospective trial, which compared the above combination with topotecan alone in women with recurrent ovarian cancer (47% vs 21% p = 0.03) [30]. The lack of additional therapeutic effect in our study may be due to a number of factors such as the relatively small size of patient groups or to the relatively lower dose of thalidomide used in this study. In addition, there were more patients with relatively chemo-resistant clear cell and mucinous tumours (20%) in the carboplatin and thalidomide group compared to the carboplatin group and six patients in the combination group did not complete the intended six cycles of treatment either due to disease progression or toxicity.

We also investigated surrogate markers of angiogenesis by serial blood sampling in patients. There was a significant decrease in CA-125 and serum E-selectin in both groups and VCAM-1 in the carboplatin group alone. None of the analysed markers showed significant differences between the groups. We propose that the reductions seen in levels of the above markers may suggest a tumour response to treatment rather than a specific anti-angiogenic action of thalidomide. In addition, it is possible that chemotherapy alone could have effects on the markers of angiogenesis. Similar to our study, no significant changes in the angiogenic markers like VEGF and bFGF were seen in prostate cancer patients treated with thalidomide [31]. However, this contrasts with studies reported in haematological disorders where significant reductions in the bone marrow microvesSEL densities and circulating levels of bFGF and VEGF had been observed in patients who responding to escalating doses of thalidomide (100 to 600 mg/day) [32, 33]. Our inability to demonstrate anti-angiogenic activity of thalidomide may be due to the dose and schedule of thalidomide used in our study and may also be related to the relatively small number of patients recruited into this study. Given the general lack of consensus among investigators with regards to optimal surrogate markers of angiogenesis to be used in clinical trials, it has recently been suggested that circulating endothelial precursor cells (CEPs), which are genetically predetermined and regulated by angiogenic factors may be a reliable marker [34]. In colorectal cancer patients treated with an anti-VEGF antibody, there was lowering of the levels of CEPs in whom the antibody was active in slowing tumour growth [35]. Thus measurement of CEPs in peripheral blood by flow cytometry may help in the future to optimise the dose of angiogenesis inhibitors and also to monitor their efficacy [36].

We conclude that the addition of thalidomide at the dose used in our study in combination with carboplatin as a first-line treatment in patients with ovarian cancer is safe and well tolerated. Failure to demonstrate increased efficacy of the above combination over carboplatin alone may be due to many factors as discussed above. Thalidomide and its analogues may have a role in recurrent ovarian cancer either alone or in combination with chemotherapy [17, 18, 30]. The GOG 198 trial explored the role of thalidomide in a completely different clinical scenario. Relapsed ovarian cancer patients with biochemical recurrence (rising CA-125) only with no evidence of macroscopic relapse are randomised to receive thalidomide (200 mg/day with gradual increment to maximum of 400 mg/day) or tamoxifen (40 mg/day) for the period of one year. After the interim analysis of 139 patients, the trial was closed as thalidomide did not reduce the recurrence rate relative to tamoxifen and it was more toxic [37]. We believe the role of thalidomide and its analogues in ovarian cancer is yet to be defined.

Acknowledgements

We are grateful to patients who kindly participated in the study. Cancer Research UK supported the study. We acknowledge the support of the Department of Neurophysiology for SNAP tests. We thank Pamela White for her support in preparing the manuscript.

References


Nodal metastasis in endometrial cancer

G. Willis, J.E. Misas, W. Byrne, E. Podczaski
Women’s Cancer Center of Central Pennsylvania, Harrisburg (PA) USA

Summary

Purpose: Besides hysterectomy and bilateral salpingo-oophorectomy, the goal of surgery in early endometrial cancer is to identify extraperitoneal disease. The purpose of this study was to evaluate disease characteristics and survival of patients found to have nodal metastasis at staging for endometrial cancer. Methods: All patients presenting to our practice from January 1993 to July 2009 underwent pelvic and paraaortic lymph node sampling at the time of surgery as permitted by the body mass index. Patient and disease characteristics of patients with nodal metastasis were abstracted by retrospective chart review. Factors contributing to disease-free and overall corrected survival were evaluated. Results: Forty-three patients with an early endometrial cancer were found to have pelvic and/or paraaortic nodal metastasis. Thirty-three percent of patients with nodal metastasis had papillary serous or clear cell cancers. Such tumors were often superficially invasive, yet were more likely to demonstrate lymphovascular space involvement as compared to endometrioid cancers. Furthermore, in a global model of disease-free and overall corrected survival, only tumor histology (endometrioid vs non-endometrioid) was a significant prognostic factor. Excluding clear cell and papillary serous tumors, only tumor grade was a significant prognostic factor in disease-free survival and overall corrected survival in patients with endometrioid adenocarcinomas and nodal involvement. Following adjuvant treatment after surgery, the recurrences were nearly evenly divided between pelvic, paraaortic nodal and distant sites. Only four of 33 (12%) patients treated with adjuvant pelvic radiation experienced a failure in the irradiated field. Furthermore, none of the patients experiencing a paraaortic nodal recurrence received adjuvant radiation to this site. Conclusions: The data suggest a benefit to the use of adjuvant radiation for local control of disease. Furthermore, the use of paclitaxel and carboplatinum chemotherapy also appears a promising adjunct in patients with endometrioid histologies and nodal spread. Papillary serous and clear cell cancers contributed disproportionately to the incidence of nodal metastasis and an adverse prognosis following further adjuvant therapy of patients with nodal disease. Despite taxol/carboplatinum chemotherapy, over half of the patients with non-endometrioid cancers recurred, as opposed to one of 19 endometrioid cancers so treated. The ideal form of adjuvant treatment for such patients remains problematic.

Key words: Endometrial cancer; Nodal metastasis; Non-endometrioid endometrial cancers.

Introduction

Endometrial cancer is the most frequent malignancy arising in the female genital tract, accounting for approximately 42,000 cases on an annual basis [1]. Fortunately, 75% of endometrial cancers appear to be confined to the uterus at diagnosis and represent clinical Stage I disease. However, despite the use of adjuvant therapy, about 20% of patients with “early disease” will develop a recurrence [2].

The early studies of the Gynecologic Oncology Group identified prognostic factors contributing to adverse outcomes as determined by systematic surgical staging. In a study of 621 patients with clinical Stage I disease and comprehensive staging, malignant cells were identified in the peritoneal cytologies in 12% of the patients [3]. Five percent had adnexal metastases, and 11% of the patients had either pelvic or paraaortic nodal metastasis. Tumor grade and depth of myometrial invasion closely correlated with nodal metastasis. Only 3% of patients with well-differentiated cancers had nodal metastasis, as opposed to 18% of those with grade 3 disease. Furthermore, the frequency of pelvic lymph node metastasis increased from 5% in patients with superficial invasion, to 25% in those with deep myometrial invasion.

Data from early studies of the Gynecologic Oncology Group provided much of the foundation for the current FIGO surgical staging of endometrial cancer. Comprehensive surgical staging defines the extent of disease and offers a rational basis for any further treatment. Lymphadenectomy contributes prognostic information and provides opportunities for tailored adjuvant therapy in patients with nodal metastasis.

The purpose of this study was to analyze disease characteristics and survival of patients with nodal metastasis at surgery for endometrial cancer. Disease features identified at surgery were evaluated as independent prognostic factors influencing disease-free and overall corrected survival in patients with either pelvic or paraaortic nodal disease. Finally, patterns of recurrence were analyzed according to the nature of the adjuvant therapy administered after surgery.

Materials and Methods

This retrospective study was approved by the Institutional Review Board of the Pinnacle Health Hospitals. All patients felt to be surgical candidates presenting to the practice with a new diagnosis of endometrial cancer from January 1993 to July 2009 underwent pelvic and paraaortic lymph node sampling (in addition to hysterectomy and salpingo-oophorectomy) as permitted by the body mass index (BMI). Patients with pelvic and/or paraaortic lymph node metastasis were identified by review of the office electronic medical record or through the Tumor Registry Service of the Pinnacle Health System. All patients had clinical Stage I or II (occult) endometrial adenocar-
cinomas and underwent hysterectomy, bilateral salpingo-oophorectomy and staging without preoperative radiotherapy. Definitive surgery was performed, on average, within 32 days following the pathologic diagnosis of an endometrial carcinoma. Surgery was performed as an open procedure in 32 patients (74%). Eleven patients had minimal access surgery with a robotic approach in four, LAVH in five and total laparoscopic hysterectomy in two patients. All but one of the patients underwent removal of pelvic lymph nodes and 35 of the 43 also underwent paraaortic lymph node sampling. Given adequate visualization, pelvic lymphadenectomy was performed with skeletonization of the obturator nerve, external iliac vein and external iliac artery, as opposed to selective node sampling. Enlarged, bulky lymph nodes were removed in an effort to reduce residual tumor burden. Both left and right paraaortic lymph nodes were obtained below the level of the inferior mesenteric artery given adequate exposure.

Charts of all identified individuals were abstracted for patient characteristics, surgical procedures and pathologic findings. Pathology reports were specifically reviewed as to tumor histology, tumor grade, depth of myometrial invasion, lymphovascular space involvement, cervical disease, adnexal metastasis, and peritoneal cytology. Lymph nodes excised were scored as to total number removed, number of positive nodes and location (pelvic vs paraaortic).

Following surgery, patients were encouraged to undergo both radiotherapy and paclitaxel/carboplatinum chemotherapy. Patients without paraaortic disease were treated with only pelvic radiotherapy. The pelvis was treated with approximately 45 Gy by AP/PA or four-field technique. In patients with paraaortic nodal disease, the treatment field was extended up to T12. The chemotherapy administered consisted of paclitaxel/platinum-based regimens using dosages and schedules identical to those used for ovarian epithelial cancers.

Three patients declined further treatment after surgery. Thirty-three patients received pelvic radiotherapy (with or without a paraaortic field). Twenty-four of the patients also received taxol/carboplatinum chemotherapy and the remaining nine received only radiation. Eight of the 33 patients were also given extended field radiation to the paraaortic area. Thirty-one patients received paclitaxel and carboplatinum chemotherapy; seven of the 31 were treated without radiotherapy.

Statistical analyses were performed using SYSTAT 11 (Chicago, IL) or Medcalc. Associations between categorical variables were evaluated by the Fisher exact test or chi-square. The Kaplan-Meier method was used to generate life-table survival curves and calculate disease-free and overall corrected survival. The Cox proportional hazards model was used to identify independent prognostic factors in disease-free and overall corrected survival. Variables entered into the stepwise Cox proportional hazards model were retained if the respective p value was less than 0.05, and eliminated if the p value exceeded 0.10. Differences in survival for patient subgroups were compared by the log-rank test.

Results

In patients undergoing primary surgery and staging for clinical Stage I and Stage II occult endometrial adenocarcinoma from January 1, 1993 to July 1, 2009, 43 patients were found to have pelvic and/or paraaortic nodal metastasis. Thirty-eight patients presented with abnormal or postmenopausal bleeding. The remaining five had smears showing endometrial cells or atypical glandular cells. The diagnosis of endometrial cancer was made by outpatient sampling of the uterine cavity in 17 patients (40%). Twenty-five individuals required curettage to establish a diagnosis. One patient was found to have an endometrial cancer at intraoperative evaluation of the hysterectomy specimen.

Of the 43 patients with nodal metastasis, ages ranged from 38.7 to 83.8, with an average of 63.8 years at the time of diagnosis. Two of the patients were African American and the remaining individuals were Caucasian. Hypertension was the most frequent medical problem with a prevalence rate of 49%. Seven of the 43 patients (16%) had a diagnosis of diabetes mellitus. BMI ranged from 19.7 to 60.9 with an average of 32.5. Seven patients (16%) had a BMI of < 25. Seven individuals had a BMI > 40 with three of the patients having a BMI in excess of 50.

Fourteen of the 43 patients (33%) had non-endometrioid (3 clear cell and 11 papillary serous) cancers with the remaining individuals having tumors of endometrioid histology. Patient age at diagnosis and BMI were not statistically different for the two tumor histologies. Furthermore, the frequency of cervical involvement, ovarian metastasis and positive cytologies was not statistically greater in patients with clear cell and papillary serous cancers as compared to endometrioid cancers. However, patients with non-endometrioid cancers were statistically more likely to show either no or superficial myometrial invasion ($p = 0.02$) and lymphovascular space involvement ($p < 0.01$) as compared to endometrioid adenocarcinomas giving rise to lymph node metastasis. Eight of 14 non-endometrioid cancers had either no invasion or superficial, inner third invasion, of the myometrium as compared to only two of 29 endometrioid cancers (Figure 1). All 14 hysterectomy specimens with clear cell or pap-

![Figure 1. Depth of myometrial invasion for all 43 patients with nodal metastasis, and those with endometrioid vs non-endometrioid tumor histology.](image-url)
illary serous cancers demonstrated lymphovascular space involvement as compared to 52% of endometrioid cancers.

Forty-two of the 43 patients (98%) underwent pelvic lymph node sampling with documented nodal metastasis in 36 individuals. The number of lymph nodes removed ranged from one to 25 with an average of 8.6 nodes per patient. A total of 363 pelvic lymph nodes were removed, of which 76 (21%) contained metastasis. Thirty-five of the 43 patients (81%) underwent sampling of the lower paraaortic chain, removing from one to 18 (average of 3.9) lymph nodes. Thirty-three percent of the paraaortic lymph nodes removed (45/135) were positive for metastasis. Twelve patients had both positive pelvic and positive paraaortic lymph nodes at the time of staging. The distribution of regional lymph node metastasis is summarized in Table 1. Correlates of lymph node metastasis included six patients with positive peritoneal cytologies, seven with ovarian tumors, and 21 individuals with cervical involvement. Lymphovascular space involvement was demonstrated in 29 of the 43 hysterectomy specimens (67%). Depth of tumor invasion in the 43 hysterectomy specimens is shown in Figure 1.

With an average follow up of 3.3 years after surgery, the two- and five-year actuarial disease-free survivals for all patients were 81% and 48%, respectively. The type of nodal metastasis (pelvic vs paraaortic), total number of positive lymph nodes, tumor histology (endometrioid vs non-endometrioid), pelvic cytology, ovarian involvement, presence of peritoneal disease (e.g., positive cytology, ovarian involvement, or peritoneal tumor), cervical disease, depth of myometrial invasion and lymphovascular space involvement were used as covariates in a global model of disease-free survival and overall corrected survival. In a stepwise Cox proportional hazards regression of both disease-free and overall corrected survivals, the only variable retained in these models using a $p < 0.05$ was tumor histology. Two- and five-year actuarial disease-free survivals for patients with clear cell or papillary serous cancers were 51% and 15%, respectively, as compared to 89% and 65% for individuals with endometrioid histology. The Kaplan-Meier life-table for disease-free survival in patients with endometrioid and non-endometrioid cancers is shown in Figure 2. The subgroup of patients with endometrioid histology was also evaluated in terms of disease-free survival and overall corrected survival using similar covariates by the use of the log-rank test. Only tumor grade was a significant predictor of outcome in terms of disease-free ($p = 0.02$) and overall corrected survival ($p < 0.01$). The Kaplan-Meier life table plots for disease-free survival in patients with grade 3 and grade 1 to 2 tumors is shown in Figure 3.

There were 16 treatment failures contributed by eight of 14 non-endometrioid and eight of 29 endometrioid cancers. The recurrences were nearly evenly divided between pelvic, paraaortic nodal, and distant disease failures. Despite further therapy after documentation of recurrent disease, 14 of the 16 patients have succumbed to disease from 0.1 to 11 years after documentation of disease recurrence. Table 2 summarizes the location of the recurrent disease, tumor histology and the nature of the adjuvant treatment administered. Eight of the 16 recurrences were detected by CA 125 follow-up.

A total of 33 patients received pelvic radiotherapy (with or without a paraaortic field). Only four of the 33 patients (12%) treated with pelvic radiotherapy demonstrated a recurrence within the irradiated field. Furthermore, none of the five patients with a paraaortic recurrence received radiation to this area. Thirty-one patients received paclitaxel and carboplatinum chemotherapy with or without radiotherapy, accounting for eight recurrences. There were two pelvic treatment failures, three recurrences in the paraaortic chain, and three distant failures (one with local disease). Twelve of the 31 patients (39%) receiving chemotherapy had tumors of papillary serous or clear cell histology with recurrences in seven of
Radiation appears to be a useful adjuvant following surgery in endometrial cancer patients with nodal metastasis. Only four of 33 patients treated with pelvic radiation developed recurrences in the irradiated field. Furthermore, none of the five patients with paraaortic recurrences received radiation to the paraaortic chain. These observations are consistent with recent reports in the literature. Klopp and co-workers noted a high rate of locoregional recurrences in endometrial cancers with nodal involvement without the use of tailored radiotherapy [10]. Five-year relapse-free survival and overall survival were significantly better with the use of regional radiotherapy. Furthermore, in node-positive patients treated without regional radiotherapy, the most frequent site of relapse was the pelvis. Similarly, data from other institutions also suggest that adjuvant radiotherapy is associated with a significant survival benefit in women with node-positive endometrial cancers [11]. Adjuvant radiotherapy improved the survival from 54% to 74% in patients with a single positive node and from 52% to 60% in those with two to five positive nodes.

The role of chemotherapy, especially taxane and platinum-based regimens remains to be defined in patients with nodal metastasis. A randomized, prospective, Gynecologic Oncology Group study showed that doxorubicin and cisplatin chemotherapy in patients with advanced (Stage III and IV) endometrial cancer and residual disease ≤ 2 cm resulted in improved survival as compared to those patients treated with whole abdominal radiotherapy [12]. In the present study, despite taxol and carboplatinum chemotherapy in 31 patients, there were two pelvic recurrences, three failures in the paraaortic chain and three distant recurrences (with local disease in one patient). The benefit of chemotherapy is unclear given the heterogeneity of tumor histologies and grades. However, any benefit appears to favor those patients with endometrioid histology. Twelve of the 31 patients (nearly 40%) receiving chemotherapy had tumors of papillary serous or clear cell histology accounting for seven recurrences. However, only one of 19 endometrioid adenocarcinomas recurred after adjuvant paclitaxel and carboplatinum.

**Discussion**

Although two recent studies have questioned the therapeutic benefit of lymphadenectomy in “early” endometrial cancer [4, 5], the information obtained clearly provides prognostic information. The literature and data from the present study demonstrate that patients with nodal metastasis have an adverse prognosis. Hirahatake and colleagues observed cumulative five-year survival rates of 94% in patients without nodal metastasis, 75% in those with metastasis limited to the pelvic nodes, and 38% in patients with both pelvic and paraaortic nodal disease [6]. At a median follow-up of 37 months, McMeekin et al. observed a three-year survival estimate of 70% for patients with paraaortic nodal disease and 87% for those with isolated pelvic node metastasis [7]. More recent data has shown that patients with retroperitoneal lymph node metastasis had a five-year overall survival of 55% and a progression-free survival of 48% [8]. In the present series, two- and five-year disease-free actuarial survivals were 81% and 48%, respectively, observations consistent with those previously reported in the literature. The present data also suggest that tumor histology is a significant factor in determining outcome for those patients with nodal disease. However, further stratification of prognostic factors, such as positive cytologies or adnexal metastasis was not observed as previously described by the Mayo group [9].

### Table 1. — Incidence of positive pelvic and paraaortic metastasis for the 43 patients with nodal metastasis.

<table>
<thead>
<tr>
<th>Pelvic lymph nodes</th>
<th>Paraaortic lymph nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>12</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
</tr>
</tbody>
</table>

### Table 2. — Location of disease recurrence, tumor histology, and nature of preceding adjuvant therapy for the 16 patients with recurrent disease.

<table>
<thead>
<tr>
<th>Location of recurrence</th>
<th>Histology</th>
<th>Nature of adjuvant therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvis</td>
<td>Non-endometrioid: 2</td>
<td>Chemotherapy in 2 with radiation (pelvic &amp; paraaortic fields) in 1 patient</td>
</tr>
<tr>
<td></td>
<td>Endometrioid: 3</td>
<td>Pelvic radiation in 3 patients</td>
</tr>
<tr>
<td>Paraaortic area</td>
<td>Non-endometrioid: 3</td>
<td>Chemotherapy in 3 with RT in 1 patient</td>
</tr>
<tr>
<td></td>
<td>Endometrioid: 2</td>
<td>Pelvic radiotherapy in 2 patients</td>
</tr>
<tr>
<td>Local and distant disease</td>
<td>Non-endometrioid: 1</td>
<td>Chemotherapy in 1</td>
</tr>
<tr>
<td>Distant disease</td>
<td>Non-endometrioid: 2</td>
<td>Pelvic radiation in 1 &amp; chemotherapy in 1</td>
</tr>
<tr>
<td></td>
<td>Endometrioid: 3</td>
<td>Pelvic radiation in 3 patients with chemotherapy in 1</td>
</tr>
</tbody>
</table>

The 12 patients. However, only one of 19 endometrioid adenocarcinomas recurred after adjuvant paclitaxel and carboplatinum.
logic behavior of non-endometrioid histology is also demonstrated in a population of patients limited to those with nodal metastasis. Despite the use of paclitaxel and carboplatinum chemotherapy with or without additional radiation, seven of the 12 patients eventually developed recurrent disease. The ideal form of adjuvant therapy in patients with nodal metastasis as a result of non-endometrioid cancers remains problematic and will require randomized studies in patients with these uncommon, yet biologically aggressive cancers.

Conclusions

Although lymphadenectomy may not provide a therapeutic benefit, it does identify prognostic information that provides for subsequent tailored adjuvant treatment of patients with nodal metastasis. The data suggest a benefit to the use of adjuvant radiation for local control of disease. The use of paclitaxel and carboplatinum chemotherapy also appears promising in patients with endometrioid histologies. Papillary serous and clear cell cancers contributed disproportionately to the incidence of nodal metastasis and an adverse prognosis in a population with nodal metastasis, despite subsequent adjuvant treatment. The optimal treatment of such patients with nodal involvement remains problematic.

References


Address reprint requests to:
G. WILLIS, D.O.
Women’s Cancer Center of Central Pennsylvania
3901 North Front Street
Harrisburg, PA 17110 (USA)
e-mail: gwillis@pinnaclehealth.org
Metastasis gene expression analyses of choriocarcinoma and the effect of silencing metastasis-associated genes on metastatic ability of choriocarcinoma cells

L. Huining¹, C. Jingting², H. Keren³

¹Department of Obstetrics and Gynecology, Xiangya Hospital, Central South University, Changsha, Hunan
²Department of Gynecological Oncology, Hunan Tumor Hospital
³Shenzhen Maternity and Child Healthcare Hospital, Shenzhen, Guangzhou (People’s Republic of China)

Summary

Objective: Obtaining choriocarcinoma metastasis-associated genes and identifying the role and mechanism of VEGF-B in the progression of human choriocarcinoma. Study Design: (1) cDNA microarray technique was used to compare the transcriptional profiles between highly metastatic JEG-3 cells and lowly metastatic JAR cells; (2) An inhibitory effect of VEGF-B shRNA was demonstrated by RT-PCR; (3) The effect of VEGF-B shRNA on invasion of JEG-3 cells in vitro was detected by Matrigel invasion assay. Results: (1) In upregulated genes, 51 genes were correlated with the cell metastasis ability, and FN, MMP-2, uPA, CA V-1 and VEGF-B were the first five genes; (2) Afterwards transfected VEGF-B shRNA, VEGF-B mRNA expression decreased obviously; (3) VEGF-B shRNA transfection significantly downregulated invasion level of JEG-3 cells in vitro (p < 0.05). Conclusion: VEGF-B plays an important role in the metastatic capability of human choriocarcinoma. Reducing the expression of VEGF-B can help weaken the invasion ability of human choriocarcinoma.

Key words: cDNA microarray; RNAi; VEGF-B; Choriocarcinoma; Invasion; Metastasis.

Introduction

Choriocarcinoma develops from reproductive tissue cells which are very active. When these cells undergo cancerous changes, they grow and multiply very rapidly. A tumor forms and sheds cancer cells into the bloodstream at an early stage. The cancer cells in the bloodstream develop new cancers in other parts of the body, a process known as metastasis. If choriocarcinoma is not treated successfully, these tumors throughout the body can result in damage, and that can quickly lead to death. So how to weaken the invasive ability of choriocarcinoma cells is very crucial.

In a previous study [1] it was pointed out that JEG-3 was more metastatic than JAR. To gain insight into the alterations in metastasis-related gene expression that governs metastasis of choriocarcinoma, we first used a cDNA microarray technique to compare the transcriptional profiles between human choriocarcinoma JEG-3 cell lines (highly metastatic) and JAR cell lines (lowly metastatic). Afterwards vascular endothelial growth factor-B (VEGF-B) gene specific short hairpin RNA (shRNA) expressing plasmid was constructed and transfected into JEG-3 cells. Finally we investigated the effects of shRNA transfection on VEGF-B expression and the ability of invasion and metastasis in human choriocarcinoma cell lines JEG-3 (highly metastatic) in vitro.

Material and Methods

Cells

JEG-3 and JAR human choriocarcinoma cell lines were acquired from American Type Culture Collection (ATCC, Manassas, VA).

cDNA microarray analysis

Briefly, first the total RNA was reverse transcribed into complementary DNA (cDNA) using T7-promotor primer and MMLV reverse transcriptase. The cDNA was transcribed into complimentary RNA (cRNA), during which it was fluorescently labelled by incorporation of cyanine Cy5-CTP (JAR cell line) or Cy3-CTP (JEG-3 cell line). After purification, using the RNeasy mini kit (Qiagen), cRNA yield and Cy incorporation efficiency (specific activity) into the cRNA were determined using a NanoDrop Spectrophotometer (NanoDrop Technologies), cRNAs showing a yield > 825 ng and a specific activity of 8-20 pmol/μg cRNA were selected for further processing. Equal amounts of the exposed and negative control sample were competitively hybridized onto Agilent whole (14K) human oligonucleotide arrays in a hybridization oven at 60°C for 17 h. Slides were washed according to the manufacturer’s instructions with washing buffers and finally dipped in stabilization and drying solution (Agilent Technologies) to protect them from environmental ozone. The arrays were scanned on an Agilent scanner (G2565BA) and further processed using Agilent Feature Extraction Software (Version 9.5.1). The software automatically finds and places microarray grids, rejects outlier pixels, accurately determines feature intensities and ratios, flags outlier pixels, and calculates statistical confidences. For the two-color microarrays, gProcessedSignal values from Agilent’s Feature Extraction software were used as input into experimental analyses and includes additional preprocessing to adjust for possible dye bias within a microarray. Data used in

Revised manuscript accepted for publication August 26, 2010

Eur. J. Gynaec. Oncol. - ISSN: 0392-2936
XXXII, n. 3, 2011
the two-color analyses was either the red and green ProcessedSignal or LogRatio values. Dye normalization included both linear scaling and Lowess normalization to a rank invariant set of microarray features. Further details on the data processing steps used to generate the Agilent two-color output can be found in the Agilent protocol GE2-v5_95_Feb07.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA were reverse transcribed in 30 ml of a solution (Fermentas), and then PCR was performed to detect the expression of VEGF-B mRNA in JEG-3 and JAR cells. Based on published DNA sequences of human VEGF-B and GAPDH gene, primers for VEGF-B (sense, 5'- GAG ATG TCC CTA GAA GAA CAC AG -3', antisense, 5'- AAA GCC ATG TGT CAC CTT CGC AG -3') and GAPDH (sense, 5'-GCT GCC GCT GAG TAC GTC GT-3', antisense, 5'-TGG GTG TCG CTG TGT AAG TC-3') were obtained. PCR was performed using a MJR PCR System (MJ Research Corp.). Aliquots (10 μl) of the amplification products were resolved by 1.5% agarose gel (Promega Corp.) electrophoresis and visualized by ethidium bromide staining, and the fragment size and signal intensity were analyzed by Genescan Analysis software and Genescan Genotyper software (Applied Biosystems).

Short hairpin RNA

The sequence targeted to 5'- AAA GGA CAG TGC TGT GAA GCC AGA C -3' in VEGF-B messenger RNA (mRNA) were designed without off-target effects. The sense and antisense strands of shRNAs were: 5'- AAA GGA CAG UCG UGU GAA GCC AGA CAA -3' (sense), 5'- AAU UUC CUG UCA CGA CAC UUC GGU CUG -3' (antisense). The negative-control mismatch sequence was: 5'- GAC TTC ATA AGG CGC GCA CAC UUC GGU CUG -3' (antisense). The negative-control mismatch sequence expressing plasmid is transfected into JEG-3 cells by lipofectamineTM 2000.

negative control group: negative-control mismatch sequence expressing plasmid is transfected into JEG-3 cells by lipofectamineTM 2000.

blank group: JEG-3 cells are treated by mixture of lipofectamineTM 2000 and free RPMI medium.

Briefly, JEG-3 cells were seeded in 60 mm dishes and plasmid-lipofectamine compounds were disposed. The plasmid-lipofectamine compounds were added into JEG-3 cells, and the final concentration of the shRNA-VEGF-B-plasmid was 200 μg/l. After six hours, the compounds were abandoned. The JEG-3 cells were incubated for an additional 48 h, selected with G418, and then lysed.

Matrigel invasion assay

Diluted 1:2 Matrigel (1.75 μg/l) (BD Biosciences, Beit-Ha’Emek, Israel) in serum free cell culture media was added to the upper chamber of a 24-well transwell plate, and incubated at 37°C 3-4 h for gelling. All cells were harvested from tissue culture flasks by Trypsin/EDTA, washed and resuspended in 0.1% FCS in DMEM medium and added to upper wells at a density of 10^5 cells/well in 100 μl medium, while 600 μl medium was added to the lower well. Plates were incubated at 37°C for 24 h, and then, the cells remaining on the upper surface of the membrane were removed with a cotton swab and the filters were fixed by 95% ethanol for 30 min. Cells that had invaded the lower surface of the filter were counted under an inverted microscope; 10 fields per well were counted. All experiments were performed in duplicate and the results from five separate sets of experiments were averaged.

Statistical analysis

Results are shown as mean ± SEM. Statistical significance of differences in mean values was assessed by using Student’s t-test with SAS software (SAS Institute, Cary, NC). Differences among means were considered significant at p values of < 0.05.

Results

Gene expression profiles of the cell lines by cDNA microarray analysis

As shown in Figure 1, green spots represent downregulated genes of JEG-3 vs JAR, red spots represent upregulated genes, while indistinctive genes are represented as yellow spots. The results demonstrated that there were 216 genes and 105 expressed sequence tag (EST) which ratio (JEG-3(Cy3)/JAR(Cy5)) was > 2 and there were 334 genes and 88 EST with a ratio < 0.5. In all differential genes, there were 128 genes belonging to oncogenes, 51 genes belonging to metastatic genes, 44 genes belonging to energy metabolism-related genes, 39 genes belonging to angiogenesis-related genes, 25 genes belonging to protein synthesis-related genes, eight genes belonging to cell-cycle-related genes, eight genes belonging to cell proliferation-related genes, four genes belonging to apoptosis-related genes, and three genes belonging to cytoskeleton-related genes. Fibronectin (FN), matrix metalloproteinase-2 (MMP-2), urokinase-type plasminogen activator (uPA), Caveolin-1 (CAV-1) and VEGF-B show markedly increased expression of metastatic genes when compared to JEG-3 with JAR.

RT-PCR analysis of selected genes

As shown in Table 1 and Figure 2, the result of RT-PCR analysis for VEGF-B was in agreement with the microarray data, although the change in the expression level was not exactly the same.

<table>
<thead>
<tr>
<th>Group</th>
<th>Times</th>
<th>VEGF-B mRNA</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>JEG-3</td>
<td>5</td>
<td>1.862 ± 0.11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>JAR</td>
<td>5</td>
<td>0.479 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

Effect of VEGF-B shRNA on JEG-3 cells

Effect of VEGF-B shRNA on VEGF-B gene expression in JEG-3 cells.

As shown in Figure 3, there was an almost 4.3 fold decrease in VEGF-B mRNA in JEG-3 cells when these cells were treated with VEGF-B shRNA; the difference was significant (p < 0.01). While comparing the blank group with negative control group, we found that the difference was meaningless.
Effect of VEGF-B shRNA on invasion ability of JEG-3 cells.

As illustrated in Figure 5, transfection with VEGF-B shRNA induced an almost 2.1 fold decrease in JEG-3 cells migrated through Matrigel-coated filters, indicating that VEGF-B shRNA restrained choriocarcinoma cell invasion. At the same time, we also found that there were no differences between the blank group and negative control group.

Discussion

It is generally accepted that genetic alternations in tumor cells may endow a special subpopulation with the ability needed for invasion through the basement membranes and metastatic colony formation in distant organs; thus a comparative analysis of genetic alterations between highly metastatic cell lines and lowly metastatic cell lines and metastases would be helpful to unravel the
mechanisms of metastasis. Abundant studies have indicated that a variety of genes, which encode those proteins involved in multiple processes of metastasis, such as heparanase [2], insulin growth factor-II [3] and interleukin-17 [4], may play crucial roles in invasion and metastasis in choriocarcinoma.

By cDNA microarray technique, we found that there were 51 known human metastasis-related genes when compared JEG-3 cells with JAR cells and the alteration in the expression of the first five genes, FN, MMP-2, uPA, CAV-1 and VEGF-B, is obvious.

Many publications now clearly link the expression of FN [5], MMP-2 [6, 7], uPA [8] and CAV-1 [9] to the invasive and metastatic properties of choriocarcinoma, but there is a lack of research to prove that these and VEGF-B are relevant to the metastasis of choriocarcinoma. In this study, we found that after transfected with VEGF-B shRNA, not only was the expression of VEGF-B mRNA in JEG-3 cells reduced, but also the invasion ability of JEG-3 cells was depressed, indicating that the expression of VEGF-B plays an important role in the metastatic capability of human choriocarcinoma, and inhibiting the expression of VEGF-B may contribute to weakening the invasive and metastatic ability of choriocarcinoma cells.

Gunningham [10] measured the level of VEGF-B by ribonuclease protection assay and immunohistochemistry in 13 normal breast samples and 68 invasive breast cancers. He found that there was a significant association between VEGF-B and node status and the number of involved nodes. In a study of pancreatic carcinoma, Wey et al. [11] found out that VEGFR-1 appears to be expressed ubiquitously in pancreatic carcinoma cell lines, in which it induces signaling and promotes migration and invasion. Overexpression of VEGF-B in tumors may activate tumor cells bearing VEGFR-1 via an autocrine pathway. Agents targeting VEGF-B or its receptors may have a dual inhibitory effect on tumor growth by suppressing both angiogenesis and tumor cell function. All of these results are consistent with our study.

In Olofsson et al.’s. research [12], they pointed out that the binding of VEGF-B to its receptor on endothelial cells leads to increased expression and activity of urokinase type plasminogen activator (u-PA). Cancer cell invasion, both locally in primary tumors and in metastatic sites, involves extensive tissue remodeling that is performed by matrix-degrading proteinases. u-PA is a serine proteinase that can digest a broad spectrum of extracellular matrix (ECM) substrates including fibrin, fibronectin and laminin [13]. In addition, u-PA can activate other matrix-degrading proteinases including MMPs that can digest collagens and a variety of other matrix proteins [14]. This hints that VEGF-B can promote the invasion ability of choriocarcinoma cells just because it enhances the uPA pathway (an invasion and metastasis implicated pathway).

Choriocarcinoma is a kind of disease that has strong invasive ability, so how to depress the invasive ability of choriocarcinoma cells is a question that draws attention. The results of our study suggest that VEGF-B is the element that enhances the invasion of choriocarcinoma cells; therefore, controlling the expression of VEGF-B could result in depressing the invasive ability of choriocarcinoma cells.

In our study, we found that shRNA-mediated silencing of VEGF-B not only significantly suppresses the invasion ability of JEG-3 cells, but also can inhibit the growth of hypodermal transplant tumor and the number of carmine nodes in lungs of nude mice. These results emphasize the potential clinical applications of VEGF-B inhibitors in choriocarcinoma.

Acknowledgments

The Department of Obstetrics and Gynecology and the State Key Lab of Medical Genetics of China. The study was supported by the technological foundation of Hunan.

References


Address reprint requests to:
C. JINGTING, M.D.
Xiangya Hospital
Xiangya Road #87
ChangSha, HuNan (China)
e-mail: cjingting114@yahoo.com.cn
Safety and efficacy of a splenectomy during debulking surgery for Müllerian carcinoma


Gynecologic Oncology Division, National Cancer Center Hospital, Tokyo (Japan)

Summary

Purpose: This study was designed to assess the safety and efficacy of a splenectomy and to analyze the prognostic factors of Müllerian carcinoma with spleen metastasis. Methods: We reviewed the medical records of 11 patients with Müllerian carcinoma who underwent a splenectomy between 1997 and 2007. The treatment outcome of these patients was examined and the possible prognostic factors were investigated by univariate analysis. Results: Four and seven patients underwent a splenectomy for primary and recurrent disease, respectively. A complete resection was achieved in eight patients. A blood transfusion was not required and only two mild postoperative complications were observed. The median and five-year survivals of all patients following treatment were 39 months and 39%, respectively. Older patients (> 60 years old) and patients with a poor performance status (PS2) had a poorer prognosis by univariate analysis. Conclusions: A splenectomy can be performed safely and effectively during debulking surgery for appropriately selected patients with primary or recurrent Müllerian carcinoma.

Key words: Debulking surgery; Müllerian carcinoma; Prognosis; Spleen metastasis; Splenectomy.

Introduction

More than two-thirds of ovarian cancers are diagnosed in the advanced stages of disease. Even if patients successfully achieve a complete remission, cancer will recur in more than half of these patients. The prognostic importance of residual disease following primary debulking surgery (PDS) for primary disease is now widely accepted since multiple studies show an inverse correlation between the size of the residual tumor mass and the patient outcome [1]. A similar correlation in the results of secondary debulking surgery (SDS) for recurrent disease has been shown in a smaller number of studies conducted with highly selected patients [2]. The spleen is often involved in either primary or recurrent ovarian cancer. In both PDS and SDS, a splenectomy can be performed safely with an acceptable morbidity [3-10]. We have also performed a splenectomy as a part of debulking surgery for selected patients with primary or recurrent Müllerian carcinomas. This study assessed the safety and the efficacy of a splenectomy and analyzed the factors which influence survival after treatment which includes a splenectomy.

Patients and Methods

Patients

We performed retrospective reviews of the surgical records and pathological reports of patients with Müllerian carcinomas including ovarian, tubal, and peritoneal carcinomas at the National Cancer Center Hospital between January 1997 and December 2007. We found 11 patients with advanced or recurrent Müllerian carcinomas who had undergone a splenectomy for the purpose of debulking during the study period. The detailed medical records of these patients were obtained and the patients were selected for this study. According to the Japanese ethical guidelines for an epidemiologic study, this study was approved by the Institutional Review Board of the National Cancer Center.

In our institution, patients with advanced Müllerian carcinomas generally undergo combined surgery and chemotherapy as the primary treatment. The standard surgical procedures include a total abdominal hysterectomy (TAH), bilateral salpingo-oophorectomy (BSO), omentectomy (OM) and maximal tumor debulking. A pelvic lymph node biopsy (PLB) and/or paraaortic lymph node biopsy (PALB) are performed if swollen nodes are present. A systematic pelvic lymphadenectomy (PLA) and a paraaortic lymphadenectomy (PALA) are performed when intraperitoneal optimal debulking surgery (maximum residual tumors less than 1cm in diameter) is done and biopsy proven lymph node metastases are present. For patients with spleen metastasis detected by either preoperative or intraoperative findings, a splenectomy is performed when the procedure is expected to effectively reduce the residual tumor mass.

Chemotherapy is usually administered following surgery. For the patients with apparently unresectable tumors or for patients with poor performance status (PS) because of advanced disease or other factors, chemotherapy may precede the debulking surgery. If chemotherapy precedes debulking surgery, then the surgery is performed during the chemotherapy as interval debulking surgery or following the completion of chemotherapy. The timing of surgical debulking depends on the patient’s response to chemotherapy and the improvement of PS. Until 1997, the CAP (cyclophosphamide, doxorubicin and cisplatin) was the standard chemotherapeutic regimen for Müllerian carcinoma in our institution. Paclitaxel and docetaxel were introduced into use for ovarian cancer in Japan in 1997 and 2000, respectively. Combination chemotherapy using taxane (paclitaxel or docetaxel) and platinum (cisplatin or carboplatin) have been used as the standard chemotherapy regimen at our institution since the taxane agents were introduced.

Surgery is the treatment of choice for recurrent Müllerian car-
cinoma only when the disease is not persistent and when the recurrent tumors seem resectable based on preoperative evaluations by CT scan and/or MRI plus physical examination. Chemotherapy is usually provided following surgery irrespective of the presence or the absence of residual tumors. Although there is no standard chemotherapy regimen, platinum- and taxane-based regimens are commonly used postoperatively.

We obtained informed consent of the patients for each treatment.

**Statistical methods**

Survival was measured from the first day of treatment for primary disease and for recurrent disease. The survival curves were determined by the Kaplan-Meier product limit method. Factors influencing survival were analyzed using the log-rank test (univariate analysis); $p < 0.05$ was considered to indicate statistical significance. All analyses were performed using the JMP software program (SAS Institute Inc., USA).

**Results**

**Patients characteristics**

The relevant characteristics of the 11 patients who underwent a splenectomy are shown in Table 1. Eight patients had ovarian cancer, one patient had tubal cancer and two patients had peritoneal cancer. Histologic types of all 11 patients were serous adenocarcinoma. Five patients had a parenchymal metastasis and six patients had only capsular involvement of the spleen. The performance status at diagnosis of primary or recurrent disease was PS 0 in two patients, PS 1 in six patients and PS 2 in three patients. The median age of the patients was 52 years (range, 27 to 73 years). The median follow-up duration after treatment, including a splenectomy, was 31 months (range, 13 to 83 months), excluding the patients who died.

Four patients underwent a splenectomy during primary treatment. Three patients underwent a splenectomy as interval debulking surgery after three to four cycles of neoadjuvant chemotherapy and one patient underwent debulking surgery after completion of six cycles of primary chemotherapy. The debulking procedure was the first debulking intent surgery for all four patients. Two patients were FIGO Stage IIIc and two patients were FIGO Stage IV.

Seven patients underwent a splenectomy for recurrent disease, six patients for first recurrence and one patient for a second recurrence. The surgery, including a splenectomy, was a second debulking surgery for five patients and a third debulking surgery in two patients. The median interval from initial treatment to recurrence was 33 months (range, 23 to 174 months). The median treatment-length for a second recurrence was 33 months (range, 23 to 174 months). The median treatment-duration for a third recurrence was 33 months (range, 23 to 174 months). The median treatment-

---

### Table 1. — The characteristics of patients who underwent a splenectomy due to metastatic Müllerian cancer.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>PS</th>
<th>Primary site</th>
<th>FIGO stage</th>
<th>SPL (preop-postop)</th>
<th>Procedures</th>
<th>Chemotherapy</th>
<th>Additional procedures</th>
<th>Residual disease</th>
<th>Postop morbidity</th>
<th>Operative time</th>
<th>Blood loss</th>
<th>Hospital stay</th>
<th>Outcome*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>2</td>
<td>Ovary</td>
<td>IV</td>
<td>Recurrence</td>
<td>None/None</td>
<td>Yes/None</td>
<td>TAH+BSO+OM+</td>
<td>None</td>
<td>None</td>
<td>90 min</td>
<td>280 ml</td>
<td>18 days</td>
<td>9M DOD</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>1</td>
<td>Ovary</td>
<td>IIIc</td>
<td>None/None</td>
<td>None/Yes</td>
<td>Yes/Yes</td>
<td>TAH+BSO+OM+</td>
<td>None</td>
<td>None</td>
<td>197 min</td>
<td>192 ml</td>
<td>19 days</td>
<td>38M DOD</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>2</td>
<td>Ovary</td>
<td>IIIc</td>
<td>None/None</td>
<td>None/Yes</td>
<td>Yes</td>
<td>Pancreas tail and InG Tumor Res</td>
<td>Yes</td>
<td>None</td>
<td>113 min</td>
<td>85 ml</td>
<td>20 days</td>
<td>39M DOD</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>1</td>
<td>Ovary</td>
<td>IV</td>
<td>Recurrence</td>
<td>Yes/None</td>
<td>Yes/Yes</td>
<td>TAH+BSO+OM+</td>
<td>Yes</td>
<td>None</td>
<td>156 min</td>
<td>142 ml</td>
<td>13 days</td>
<td>83M AWD</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>1</td>
<td>Tube</td>
<td>IV</td>
<td>Primary</td>
<td>Yes/Yes</td>
<td>Yes</td>
<td>pHPT+PLB</td>
<td>Yes</td>
<td>None</td>
<td>270 min</td>
<td>342 ml</td>
<td>13 days</td>
<td>81M NED</td>
</tr>
<tr>
<td>6</td>
<td>63</td>
<td>1</td>
<td>Peritoneum</td>
<td>IV</td>
<td>Recurrence</td>
<td>None/Yes</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>153 min</td>
<td>224 ml</td>
<td>14 days</td>
<td>29M DOD</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>1</td>
<td>Ovary</td>
<td>IIIc</td>
<td>None/None</td>
<td>None/Yes</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>85 min</td>
<td>130 ml</td>
<td>14 days</td>
<td>31M NED</td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>1</td>
<td>Ovary</td>
<td>IIa</td>
<td>Recurrence</td>
<td>None/Yes</td>
<td>None</td>
<td>TAH+BSO+OM+</td>
<td>Yes</td>
<td>None</td>
<td>275 min</td>
<td>322 ml</td>
<td>13 days</td>
<td>31M AWD</td>
</tr>
<tr>
<td>9</td>
<td>52</td>
<td>1</td>
<td>Peritoneum</td>
<td>IV</td>
<td>Primary</td>
<td>Yes/Yes</td>
<td>Yes</td>
<td>TAH+BSO+OM+</td>
<td>No</td>
<td>None</td>
<td>255 min</td>
<td>132 ml</td>
<td>14 days</td>
<td>26M AWD</td>
</tr>
<tr>
<td>10</td>
<td>61</td>
<td>0</td>
<td>Ovary</td>
<td>IV</td>
<td>Primary</td>
<td>Yes/Yes</td>
<td>Yes</td>
<td>PALB</td>
<td>None</td>
<td>None</td>
<td>430 min</td>
<td>703 ml</td>
<td>15 days</td>
<td>17M NED</td>
</tr>
<tr>
<td>11</td>
<td>73</td>
<td>0</td>
<td>Ovary</td>
<td>IV</td>
<td>Recurrence</td>
<td>None/None</td>
<td>None</td>
<td>PALB</td>
<td>None</td>
<td>None</td>
<td>109 min</td>
<td>107 ml</td>
<td>7 days</td>
<td>13M NED</td>
</tr>
</tbody>
</table>


### Table 2. — Univariate analysis for possible prognostic factors after splenectomy.

<table>
<thead>
<tr>
<th>Possible prognostic factors</th>
<th>Number of patients</th>
<th>Median survival</th>
<th>Five-year survival</th>
<th>$p$ value (Log-rank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60</td>
<td>7</td>
<td>39M</td>
<td>50%</td>
<td>0.012</td>
</tr>
<tr>
<td>≥ 60</td>
<td>4</td>
<td>29M</td>
<td>0%</td>
<td>0.05</td>
</tr>
<tr>
<td>PS (0, 1 vs 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0, 1</td>
<td>8</td>
<td>NR</td>
<td>80%</td>
<td>0.007</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>38M</td>
<td>0%</td>
<td>0.006</td>
</tr>
<tr>
<td>Disease origin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ovariube/ peritoneum</td>
<td>9</td>
<td>39M</td>
<td>44%</td>
<td>0.195</td>
</tr>
<tr>
<td>History of distant metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>absent</td>
<td>7</td>
<td>39M</td>
<td>29%</td>
<td>0.02</td>
</tr>
<tr>
<td>present</td>
<td>4</td>
<td>29M</td>
<td>50%</td>
<td>0.08</td>
</tr>
<tr>
<td>Disease status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>primary</td>
<td>4</td>
<td>38M</td>
<td>50%</td>
<td>0.12</td>
</tr>
<tr>
<td>recurrent</td>
<td>7</td>
<td>39M</td>
<td>34%</td>
<td>0.14</td>
</tr>
<tr>
<td>Preoperative chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>6</td>
<td>39M</td>
<td>0%</td>
<td>0.006</td>
</tr>
<tr>
<td>any</td>
<td>5</td>
<td>NR</td>
<td>67%</td>
<td>0.17</td>
</tr>
<tr>
<td>Postoperative chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>4</td>
<td>38M</td>
<td>38%</td>
<td>0.05</td>
</tr>
<tr>
<td>any</td>
<td>7</td>
<td>39M</td>
<td>40%</td>
<td>0.006</td>
</tr>
<tr>
<td>Residual disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>absent</td>
<td>8</td>
<td>NR</td>
<td>53%</td>
<td>0.34</td>
</tr>
<tr>
<td>present</td>
<td>3</td>
<td>39M</td>
<td>0%</td>
<td>0.34</td>
</tr>
</tbody>
</table>

PS: performance status, NR: not reached.
free interval from any previous treatment was 21 months (range, 11 to 135 months). At primary treatment, one patient was FIGO Stage Ib, one patient was FIGO Stage IIc and two patients were FIGO Stage IIIa, three patients were FIGO Stage IIIc and two patients were FIGO Stage IV.

Safety of the splenectomy

For patients with primary disease, a splenectomy was performed following standard procedures such as TAH, BSO and OM. One patient had already undergone TAH during treatment for myoma earlier. One patient underwent PALB, and one patient underwent a partial hysterectomy and PLB. The median operative time was 263 minutes (range, 197 to 430 minutes) and median blood loss was 267 ml (range, 132 to 703 ml). A blood transfusion was not required for these four patients. There were no postoperative complications reported in any of the patients with primary disease. The median postoperative hospital stay was 15 days (range, 13 to 20 days).

Among the patients with recurrent disease, four patients had only inspection of the peritoneal cavity plus splenectomy. One patient had a pancreas tail and inguinal tumor resection, one patient had a peritoneal tumor resection and one patient had a superficial inguinal tumor resection. The median operative time for patients with recurrent disease was 113 minutes (range, 85 to 275 minutes) and the median blood loss was 142 ml (range, 85 to 322 ml). There were no blood transfusions needed for these seven patients. Two patients exhibited mild postoperative morbidity, one had a fever and one had bowel obstruction. The median postoperative hospital stay was 13 days (range, 7 to 18 days). Although postoperative complications were observed in patients who underwent a third debulking surgery, there were no correlations between the number of debulking surgeries and the operative time, blood loss or postoperative hospital stay.

Outcome of the treatment including splenectomy

A complete resection of all visible tumors was accomplished in all four patients with primary disease. One patient died 38 months after initial treatment, one patient remained alive with disease at 26 months, and two patients remained alive with no evidence of disease at 17 months and 81 months. The median survival of patients with primary disease was 38 months and five-year survival rate was 50%.

Among the patients with recurrent disease, three patients had residual tumor less than 1 cm in diameter and four patients had complete resection of all visible tumors. Among the four patients without residual disease after a splenectomy, one patient died 29 months after treatment of recurrent disease, one patient remained alive with disease at 83 months and two patients remained alive with no evidence of disease at 13 months and 31 months. Among the three patients with residual tumor after a splenectomy, two patients died at nine months and 39 months and one patient remained alive with disease at 31 months. While there were no five-year survivors among this group of patients, the median survivals of the patients without residual tumor and with minimal residual tumor were not reached and 39 months, respectively. The median survival of all patients with recurrent Mullerian cancer was 39 months.

The median survival for all patients was 39 months and the five-year survival rate was 39% (Figure 1).

Factors influencing survival

Possible prognostic factors after splenectomy were analyzed by univariate analysis. Table 2 shows the results of the analyses. Age (< 60 or ≥ 60), PS (0-1 or 2), disease origin (ovary/tube or peritoneum), history of distant metastasis (absent or present), disease status (primary or recurrent), preoperative chemotherapy (none or any), postoperative chemotherapy (none or any), residual dis-

---

**Table 3. — Review of the literature regarding prognosis and complications of splenectomy in the treatment of ovarian and Mullerian cancer.**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of patients</th>
<th>Survival Median</th>
<th>Surgery</th>
<th>Complications Rate</th>
<th>Operative</th>
<th>Time</th>
<th>Postoperative Complications</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uehara et al.</td>
<td>11 [4/7]</td>
<td>39M [31M/32M] 59% [50%/54%] (5Y)</td>
<td>NoRT</td>
<td>73%</td>
<td>100%</td>
<td>0%</td>
<td>156 min</td>
<td>18%</td>
</tr>
<tr>
<td>Matigibay et al. [9]</td>
<td>112 [66/46]</td>
<td>NA</td>
<td>Transfusion</td>
<td>22%</td>
<td>76%</td>
<td>4 units</td>
<td>NA</td>
<td>25%</td>
</tr>
<tr>
<td>Eisenkop et al. [8]</td>
<td>49 [490]</td>
<td>[65M/22M] 44% [46%/42%] (2Y)</td>
<td>Operative</td>
<td>100%</td>
<td>100%</td>
<td>5 units</td>
<td>*245 min</td>
<td>41%</td>
</tr>
<tr>
<td>Manci et al. [10]</td>
<td>24 [0/24]</td>
<td>54M [48%/56%] (5Y)</td>
<td>Complications</td>
<td>67%</td>
<td>100%</td>
<td>21%</td>
<td>155 min</td>
<td>13%</td>
</tr>
<tr>
<td>Bilgin et al. [7]</td>
<td>13 [7/6]</td>
<td>6M [91%] (3Y) a</td>
<td>Postoperative</td>
<td>NA</td>
<td>77%</td>
<td>NA</td>
<td>0%</td>
<td>8%</td>
</tr>
<tr>
<td>Ayhan et al. [6]</td>
<td>34 [340]</td>
<td>[18M/18M] 37% [57%/NA] (5Y)</td>
<td>Hospital stay</td>
<td>NA</td>
<td>100%</td>
<td>NA</td>
<td>NA</td>
<td>0%</td>
</tr>
<tr>
<td>Chen et al. [5]</td>
<td>35 [13/22]</td>
<td>37M [57M/NA]** 37% [37%/NA] (5Y)</td>
<td>Hospital stay</td>
<td>NA</td>
<td>100%</td>
<td>NA</td>
<td>NA</td>
<td>0%</td>
</tr>
<tr>
<td>Scarabelli et al. [4]</td>
<td>34 [12/22]</td>
<td>[NR/41M] [NA/NA] (NA/NA)</td>
<td>Hospital stay</td>
<td>NA</td>
<td>100%</td>
<td>NA</td>
<td>NA</td>
<td>0%</td>
</tr>
<tr>
<td>Nicklin et al. [3]</td>
<td>18 [11/7]</td>
<td>[37M/27M] a [83%/78%] (2Y) a</td>
<td>Hospital stay</td>
<td>NA</td>
<td>100%</td>
<td>NA</td>
<td>NA</td>
<td>0%</td>
</tr>
</tbody>
</table>


*: median, **: mean, a: survival for the patients who underwent complete resection, b: including patients who had splenectomy for iatrogenic injury.

c: excluding a patient who died postoperatively, d: including patients who had splenectomy for iatrogenic injury.
ease (absent or present) and metastatic status of spleen (parenchymal or capsular) were all assessed. Age ≥ 60 years old emerged as a significantly poor prognostic factor ($p = 0.012$) and PS 2 was revealed to be marginally significant ($p = 0.086$).

**Discussion**

In the present study, we evaluated the safety and efficacy of splenectomy in a group of selected patients with Müllerian cancer and evaluated the prognostic factors influencing survival after treatment.

Several studies have reported the safety of splenectomy in the treatment of ovarian cancer. We reviewed previous studies of splenectomy for patients with ovarian cancer which included more than ten patients (Table 3) [3-10]. Magtibay et al. reported the largest study of patients with ovarian cancer who underwent a splenectomy [9]. Of 112 patients included in their study, 66 patients had primary disease and 46 patients had recurrent disease. The authors reported a total of 26 complications (23%), an overall perioperative mortality rate of 5% and a median of four units of transfused packed red blood cells (PRBCs). The next largest study, by Eisenkop et al., reported the results of treatment for 49 patients with primary ovarian cancer requiring a splenectomy [8]. They reported a total of 20 complications (41%), a median total blood loss of 1500 ml and a median five units of transfused PRBCs. The studies with smaller numbers of patients reported a higher incidence of complications (44%-165%) [3-6] and postoperative mortality rates (8%-9%) [6, 7]. From the results of these studies, it may be concluded that splenectomy is an invasive procedure which frequently requires a blood transfusion. Magtibay et al. stated that splenectomy as part of debulking surgery is associated with modest morbidity and mortality [9]. Complications reported to be associated with splenectomy are injury of the pancreas tail or stomach, infection, thrombocytosis, thromboembolism, atelectasis, pneumonia and so on [6, 9, 11]. However, the parameters of surgical invasiveness depend on the extent of disease and the procedures performed in addition to a splenectomy. Many authors [3, 4, 7, 8, 10] concluded that a splenectomy could be performed safely during debulking surgery for either primary or recurrent ovarian cancer. Although the number of patients is small and the patients were only selected patients, our experience with 11 splenectomies showed this procedure to be associated with a low incidence of morbidity and blood transfusion. Our data suggest that splenectomy as part of the debulking procedure for primary or recurrent Müllerian cancer can be performed safely.

The median survivals of the patients with primary or recurrent disease in our study were 38 months and 39 months, respectively. Magtibay et al. reported a median survival in patients with primary disease of 22 months and a two-year survival of 46%. Patients with recurrent disease had a median survival of 20 months and a two-year survival of 42% [9]. Our results may therefore be better. For patients with primary Stage IIIc ovarian cancer, Eisenkop et al. [8] reported a median survival of 56 months and a five-year survival of 48%. For surgical patients with recurrent disease, Manci et al. reported a median survival of 56 months [10]. One of the reasons these results vary so widely may be differences in patient selection. Eisenkop et al. included only patients who underwent complete resection of all visible tumors [8] while Magtibay et al. included more than 50% of patients who had gross residual disease (residual tumor larger than 1 cm in diameter) or unknown residual tumor size at the time of debulking surgery [9]. In addition, the studies by Magtibay et al., Manci et al. and our group included patients with primarily Stage IV disease [9, 10], while the study by Eisenkop et al. was limited to patients with Stage IIIc disease [8]. Although the patient selection criteria were not the same, the results of Eisenkop et al. and Manci et al. [8, 10] are comparable with the results of the Gynecologic Oncology Group (GOG) study for optimally debulked Stage III ovarian cancer, including patients without spleen metastasis [12], and the results of several studies of SDS including patients without spleen metastasis [13, 14]. These favorable results are not consistent with poor outcome of patients with liver metastases. The reasons for the difference is not obvious, the differences of both vital importance and possibility of total organ resection between liver and spleen may be related. Nonetheless, we conclude that patients with spleen metastasis from either primary disease or recurrent disease may show an improved outcome following adequate debulking surgery that includes splenectomy, based on the results of these studies and our research. We also conclude that the improved outcome is comparable to outcomes in patients without spleen metastasis.

In the previous studies, some factors, such as splenic parenchymal involvement [3], residual tumor [10], histologic type of tumor [6] and PS [6], have been reported to correlate with patient prognosis. We also investigated the prognostic factors that influence survival by a univariate analysis. Histologic type was not included in the analysis.
because all 11 patients in our study had serous adenocarcinoma. We did not detect any prognostic impact of disease status \( p = 0.612 \), residual disease \( p = 0.341 \) or metastatic status of spleen \( p = 0.347 \), possibly due to the small sample size in this study. However, we determined that an age \( \geq 60 \) years old was a significantly poor prognostic factor \( p = 0.012 \) and PS 2 was a poor prognostic factor with marginal significance \( p = 0.086 \). These two factors are both common prognostic factors of ovarian cancer. Thus, poor prognosis of older patients or patients with poor PS may not be specific to the procedure of splenectomy. Possibly, these factors affect survival through affecting surgical completeness, time to initiation of postoperative chemotherapy, interval of chemotherapy, dose of chemotherapy and so on. We can suggest that for older patients or patients with poor PS, the risks and benefits of surgery should be taken into consideration before performing a splenectomy.

Our study has several important limitations, such as small number of patients, data from a single institution, retrospective nature of the study, long study period allowing change of chemotherapeutic regimen, diverse disease origin or disease status and so on. However, we can say that splenectomy can be performed safely during debulking surgery for primary or recurrent Müllerian cancer and that the prognosis of patients with spleen metastasis can be improved when debulking surgery, that includes splenectomy, is performed on appropriately selected patients. Age and PS of the patients should be one of the important factors in the selection of patients.

Conclusion

A splenectomy can be performed safely during debulking surgery for primary or recurrent Müllerian cancer, and the prognosis of patients with spleen metastasis can be improved when debulking surgery including a splenectomy, is performed on appropriately selected patients. Age and PS of the patients should be one of the important factors in the selection of patients.

References


Address reprint requests to:
T. UEHARA, M.D.
Department of Obstetrics and Gynecology
Chiba University Hospital
Chiba University School of Medicine, 1-8-1 Inohana, Chuo-ku
Chiba 260-8677 (Japan)
e-mail: tak-uehara@hospital.chiba-u.jp
**XRCC1 Arg399Gln polymorphism and risk for cervical cancer development in Argentine women**


*IGEVET (Institute of Veterinary Genetics “Ingeniero Fernando Noel Dulout”)*
Faculty of Veterinary Science, National University of La Plata, Buenos Aires (Argentina)

**Summary**

**Background:** XRCC1 (X-ray repair cross-complementing group 1) plays a central role in the DNA base excision repair mechanism. Single nucleotide polymorphisms (SNPs) in the XRCC1 gene are thought to modulate DNA repair capacity and have been linked to cancer risk in several studies. **Materials and Methods:** We conducted a case-control study comprising 217 cervical samples, including 103 cervical carcinomas and 114 normal tissue samples. Cervical samples were genotyped for two XRCC1 SNPs (Arg194Trp and Arg399Gln) by PCR-RFLPs. **Results:** Subjects carrying heterozygous Arg399Gln or the combined Gln399Gln + Arg399Gln variant genotypes had a significantly reduced risk for cervical cancer development. In addition, the J94Arg-399Gln haplotype was also found to be associated with a decreased risk for cervical carcinoma. **Conclusion:** Our findings suggest that XRCC1 genotypes and haplotypes contribute in reducing the risk for cervical cancer development. Furthermore, genetic susceptibility conferred by Arg399Gln polymorphism operates independently of human papillomavirus infection of cervical tissue.

**Key words:** Cervical Cancer; HPV; Single Nucleotide Polymorphisms; XRCC1.

**Introduction**

Cervical cancer is the second most common cancer among women worldwide, with an estimated global incidence of 493,000 new cases and 274,000 deaths in the year 2002. In developing countries, where widespread screening is still unavailable, cervical cancer accounts for 15% of female cancers. The highest incidence rates are observed in sub-Saharan Africa, Melanesia, the Caribbean, South central and Southeast Asia and Latin America [1]. In Argentina, where disparate distribution of cervical cancer prevalence is remarkable, mortality rates associated with cervical cancer are higher in provinces with a lower socioeconomic level [2, 3].

Infection with high-risk HPV has been considered to be the major etiological factor in the development of cervical cancer. However, most of HPV infections are transient and only 1% of women infected with high-risk HPV will develop cervical carcinoma. This indicates that HPV infection is a necessary event but not sufficient for cervical carcinogenesis. Therefore, other factors, including environmental agents and host genetic background, may play crucial roles in the development of cervical cancer [4].

Exposure to different endogenous and exogenous mutagens and carcinogens can result in various types of DNA damage. These alterations, if not repaired, can cause genetic instability, mutagenesis and cancer [5]. The damage is fixed by multiple DNA repair pathways including base excision repair, nucleotide excision repair, mismatch repair, and double-strand break repair. A defect or reduced efficiency in any of these DNA repair mechanisms plays a critical role in the development of various age-related diseases, including cancer [5].

Among the five main DNA maintenance mechanisms operating in humans, base excision repair (BER) is the primary adopted by cells against reactive oxygen species, methylation, deamination, and hydroxylation [5]. The XRCCI gene (X-ray repair crosscomplementing group 1) is located on chromosome 19q13.2 and encodes a scaffold protein involved in DNA BER. This protein interacts with a complex of DNA repair proteins, including poly (ADP-ribose) polymerase, DNA ligase 3, and DNA polymerase-β, promoting efficiency of the BER pathway [6, 7]. Shen et al. have identified three coding polymorphisms in the XRCCI gene at codons 194 (Arg to Trp), 280 (Arg to His) and 399 (Arg to Gln). Arg194Trp polymorphism is located in a linker region connecting the domains that interact with poly (ADP-ribose) polymerase and DNA polymerase-β, while Arg399Gln polymorphism is located in the functionally poly (ADP-ribose) polymerase binding region, within an identified BRCA1 COOH terminus domain [8]. These polymorphisms, involving an amino acid change at evolutionarily conserved regions, can cause subtle structural alterations in the repair protein and consequently change the host susceptibility to cancer.

To date, there are controversial results related to the contribution of XRCCI SNPs to cancer susceptibility. Several molecular epidemiologic studies revealed that XRCCI polymorphisms were significantly associated with increased risk of cervical cancer [9, 10], as well as breast cancer [11, 12], lung cancer [13, 14], bladder cancer [15] and melanoma [16]. However, a null association with XRCCI polymorphisms has been reported in other studies [17-19].

Revised manuscript accepted for publication August 26, 2010
The XRCC1 protein has no enzymatic activity and during repair interacts with DNA polyb, PARP and DNA ligase III [20]. Moreover, the XRCC1 protein may also regulate the activity of many other DNA repair enzymes [21] and genetic polymorphisms in the same and other DNA repair genes may influence cancer risk [22]. Because of these reasons, we hypothesized that variations in the XRCC1 gene may contribute to cervical carcinoma susceptibility. Consequently, we carried out a population-based case-control study considering XRCC1 as a candidate susceptibility gene for cervical cancer. We assessed the association of two XRCC1 SNPs, Arg194Trp and Arg399Gln, with the risk for cervical cancer development and their interaction with HPV infection.

Materials and Methods

A total of 217 cervical samples were obtained from an anonymous cervical specimen database in La Plata, Argentina. The specimens comprise 114 normal cytologies (collected from women who were attending screening), and 103 squamous cervical cancers. The mean age of women comprising the control group was ~40 years old (± 10.26 SD), and ~44 years old (± 9.87 SD) for those in the case group. Cervical specimens comprised exfoliated cells from the ecto-endocervix, collected using a cytobrush or spatula and kept frozen at −80°C. Cervical-biopsy specimens were formalin-fixed and paraffin-embedded or freshly frozen.

DNA extraction

Paraffin-embedded samples were washed twice with xylol and finally with 100% ethanol, re-suspended in 350 μl of proteinase K digestion buffer (250 mg/ml), and incubated for two hours at 56°C. Cervical exfoliated cell pellets and frozen biopsies were suspended and washed twice with 1 ml of PBS, and incubated for 24 hours at 56°C in 400 μl of digestion buffer (50 mM Tris-Cl pH 8.5; 1 mM EDTA; 1% Triton X-100 and 0.5% Tween 20) containing 250 μg/ml of proteinase K (Genbiotech, Buenos Aires, Argentina). After proteinase digestion, the samples were redigested for genotype confirmation. Homozygous Arg399Gln genotype is determined by the presence of 100 bp band. The heterozygous Arg194Trp genotype is determined by the presence of 100, 80, 55, and 20 bp bands.

Statistical analysis

Descriptive statistics were analyzed using the SPSS™ (Statistical Package for Social Sciences) software version 15.0. Pearson’s Chi-square test was applied to examine the Hardy-Weinberg equilibrium and 5’-acc cac gag tct agg tct caa cc-3’ (X194F) and 5’-acc cac gag tct agg tct caa cc-3’ (X194R) for forward and reverse primers. Primers defined a region of 150 base pairs. The reaction mixture was performed in a final volume of 25 μl: 5 μl of genomic DNA; 0.85 pmol/μl of each primer; 20 mM of each deoxynucleoside triphosphate; PCR buffer 1X (50 mM KCl and 10 mM Tris-HCl-pH 8.3), and 0.1% Triton X-100; 1.2 mM of MgCl₂, and 1.5 units of Taq DNA polymerase (Invitrogen). The amplification reaction was carried out under the following conditions: an initial melting step of 92°C for 3 min followed by 35 cycles of 30'' at 92°C, 50° at 60°C and 40'' at 72°C; with a final elongation step of 72°C for 5 min.

The primers for Arg399Gln XRCC1 amplification were: 5’-gca tgc gta agg gtt-3’ (X399F) and 5’-cag gag cag cgt cgg ctt-3’ (X399R) for forward and reverse primers. These primers define a 100 base pairs fragment. The PCR reaction mixture (25 l) consisted of 5 l of genomic DNA, 20 mM of deoxynucleotide triphosphate, 1X PCR buffer (50 mM KCl and 10 mM Tris-HCl; pH 8.3), 1.2 mM MgCl₂, and 1.5 units of Taq polymerase. The conditions for the amplification reaction included an initial melting step of 92°C for 3 min followed by 35 cycles of 30'' at 92°C, 40'' at 90°C and 40'' at 72°C; and a final extension step of 72°C for 5 min.

The PCR products for both polymorphisms were checked on 2% agarose gels, stained with Safer Green and visualized with Safe Imager (Invitrogen, USA).

Genotyping of both polymorphisms was assessed by restriction enzyme digestion. PCR products were digested overnight at 37°C with five units of MspI (Genbiotech, Argentina) per sample. The obtained fragments were resolved on 10% polyacrylamide gels, stained with Sybr Safe and visualized with blue light. The homozygous XRCC1 codon 194 Arg allele yields bands of 80, 55, and 20 bp while the 194Trp allele yields only two bands of 100 and 55 bp. The heterozygous Arg194Trp genotype is determined by the presence of 100, 80, 55, and 20 bp bands, although 20 bp is too small to be clearly appreciated in the gel. The 55 bp band, generated by a constant restriction site, was used as an internal control for complete digestion. On the other hand, XRCC1 codon 399 digestion yields 65 and 35 bp bands for the homozygous Arg allele, while the homozygous Arg399Gln allele generates a unique 100 bp band. The heterozygous Arg399Gln genotype is determined by the presence of three bands at 100, 65, and 35 bp. To avoid misclassification, a randomly selected subset of 20% of the samples was re-tested for genotype confirmation.
Results

Population data

XRCC1 polymorphisms were successfully genotyped in all samples. The specimens were also tested for the presence of human papillomavirus (HPV) DNA. Among the 217 samples included in this study, the global age ranged from 26 to 69 years, with a median age of 39.6 years (SD ± 10.26) for the control group, and 43.9 years (SD ± 9.87) for the case group. The prevalence of HPV ranged from 31.6% among the control group to 85.3% among squamous cell carcinomas (OR = 12.57; CIs = 6.39-24.59; X² = 63.35; \( p = 0.01 \)).

XRCC1 allele frequencies

Gene and genotype frequencies for XRCC1 codon 194 and 399 polymorphisms in controls and cases are shown in Table 1. While there was no significant differences between the XRCC1 Arg194Trp alleles between controls and carcinomas (91.2% vs 86.4%, respectively), the XRCC1 Arg399Gln polymorphism showed a higher prevalence of 399Arg in controls (58.4% for Arg399 and 41.6% for 399Gln) than cases (67.5% for Arg399 and 32.5% for 399Gln), this difference being statistically significant (OR = 0.67; CI = 0.45-0.99; \( p = 0.049 \)). These findings suggest an inverse association between the Gln399 allele and cervical cancer risk.

XRCC1 genotypes frequencies and risk for cervical cancer development

The distribution of genotypes for both polymorphisms fit well as expected by the Hardy-Weinberg model in the control group. Results for the XRCC1 codon 399 showed a statistically significant difference in genotypes distribution (X² = 11.03; \( p = 0.004 \)). Age-adjusted logistic regression analysis revealed that the heterozygous condition Arg399Gln was inversely related with cervical cancer risk (OR = 0.45; CIs = 0.24-0.85; \( p = 0.014 \)) compared with women homozygous for the Arg399 allele. Besides, when we combined the Gln399Gln and Arg399Gln genotypes they also presented the same relationship with respect to cervical carcinoma (OR = 0.47; CIs = 0.25-0.89; \( p = 0.02 \)).

On the other hand, we did not find significant differences in Arg194Trp genotype distribution. Conversely, when the analysis was carried out with the combination of Arg194Trp+Trp194Trp genotypes, they provided a modest risk of borderline statistical significance, with a crude OR of 1.86. However, no association was found when adjusting for age. Risk estimations for cervical cancer and statistical tests are presented in Table 1.

Haplotype analysis

For the analyses of haplotypes, all potential models were taken into consideration. We found that subjects carrying the Arg194-Gln399 combination have a protective effect for cervical cancer development (\( \beta = -0.92 \); Wald = −2.04; \( p = 0.040 \); Akaike AIC = 682.20), and this haplotype reached the strongest association under the dominant model.
Table 2. — Analysis of association between XRCC1 polymorphisms and risk for HPV infection in control group.

<table>
<thead>
<tr>
<th>XRCC1 Genotypes</th>
<th>HPV Positive N=36</th>
<th>HPV Negative N=78</th>
<th>Adjusted OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codon 194</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg (CC)</td>
<td>31 (86.1%)</td>
<td>67 (85.9%)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>Arg/Trp (CT)</td>
<td>4 (11.1%)</td>
<td>8 (10.3%)</td>
<td>1.09 (CI = 0.30-3.96)</td>
<td>0.89</td>
</tr>
<tr>
<td>Trp/Trp (TT)</td>
<td>1 (2.8%)</td>
<td>3 (3.8%)</td>
<td>0.67 (CI = 0.065-6.78)</td>
<td>0.73</td>
</tr>
<tr>
<td>Arg/Trp +Trp/Trp (CT+TT)</td>
<td>5 (13.8%)</td>
<td>11 (14.1%)</td>
<td>0.97 (CI = 0.31-3.07)</td>
<td>0.96</td>
</tr>
<tr>
<td>Allele frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg (C)</td>
<td>66 (91.7%)</td>
<td>142 (91.1%)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>Trp (T)</td>
<td>6 (8.3%)</td>
<td>14 (8.9%)</td>
<td>0.92 (CI = 0.33-2.50)</td>
<td>0.87</td>
</tr>
<tr>
<td>Codon 399</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg (GG)</td>
<td>14 (38.9%)</td>
<td>23 (29.5%)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>Gln/Arg (AG)</td>
<td>15 (41.7%)</td>
<td>44 (56.4%)</td>
<td>0.56 (CI = 0.22-1.37)</td>
<td>0.21</td>
</tr>
<tr>
<td>Gln/Gln (AA)</td>
<td>7 (19.4%)</td>
<td>11 (14.1%)</td>
<td>0.74 (CI = 0.31-1.78)</td>
<td>0.51</td>
</tr>
<tr>
<td>Gln/Gln+Gln/Arg (AA+AG)</td>
<td>22 (61.1%)</td>
<td>55 (70.5%)</td>
<td>0.65 (CI = 0.28-1.51)</td>
<td>0.32</td>
</tr>
<tr>
<td>Allele frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg (G)</td>
<td>43 (59.7%)</td>
<td>90 (57.7%)</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>Gln (A)</td>
<td>29 (40.3%)</td>
<td>66 (42.3%)</td>
<td>0.92 (CI = 0.52-1.62)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Discussion

Recent studies have focused the attention on identifying the effect of single nucleotide polymorphisms (SNPs) in several DNA repair genes, suggesting that these polymorphisms could affect individual’s susceptibility to cancer. The XRCC1 protein plays an important role in the base-pair excision repair (BER) pathway. Shen et al. reported three coding nonsynonymous SNPs, Arg280His, Arg280His, and Arg399Gln, in the XRCC1 gene. Polymorphism in codon 399 of this gene has been extensively investigated in many epidemiological studies in relation to various types of cancer. The variant Gln allele was thought to reduce DNA repair activity and hence lead to increased DNA damage [26]. Moreover, this genetic polymorphism has been linked to an increased risk of lung cancer [14, 27], stomach cancer [28], and head and neck cancer [29]. On the other hand, this allele was also reported to be associated with a reduced risk of bladder cancer [30], esophageal cancer [31], non-melanoma skin cancer [32] and head and neck cancer [33].

Our population-based case-control study of 103 cases and 114 controls, revealed a significant association between Arg399Gln polymorphism and cervical cancer risk. Our findings are consistent with those from previous studies that reported a reduced risk of cancer for this polymorphism [30-33]. A study by Nelson et al. reported a decreased risk of non-melanoma skin cancer for those individuals who carried the Gln399Gln genotype. In a hospital-based case-control study conducted by Olshan et al. [33], both the Gln399Gln and the combined Arg399Gln/Gln399Gln genotypes were found to be associated with a decreased risk of head and neck cancer among patients in North Carolina. Stern et al. [30] also reported a slight decrease in risk of bladder cancer for subjects who carried the homozygous Gln399Gln genotype compared with those homozygous for the Arg399 allele, although this difference was not statistically significant. Accordingly to these works, we found that both Arg399Gln and the combined Gln399Gln/Arg399Gln variant genotypes provided a protective effect against cervical cancer development among women in La Plata, Argentina. Other epidemiological investigations reported contrary findings. Two population-based case-control studies conducted by Huang et al. [9] among Chinese women and Niwa et al. [34] among Japanese subjects revealed that individuals with the homozygous Gln399Gln genotype are at higher risk of developing cervical carcinoma.

It is worth noting that, in our study, genotypes frequencies among women are remarkably different to those observed among subjects in Chinese and Japanese studies. These significant differences in frequency distribution between Caucasian and Asian populations have already been reported [35]. Comparison of the obtained allele frequencies for XRCC1 codon 194 and codon 399 with those published for Caucasian, African and Asian populations [14] revealed that the Argentine population is highly similar to Caucasians and quite different from Asian populations. This situation is very important at the moment of searching associations and interpreting results, since different findings in different populations could be attributed to genetic and ethnic disparities.

Whether the Arg399Gln polymorphism is associated with increased or reduced cancer risk may be a function of type and location of tumor. Although the Gln allele has been shown to diminish XRCC1 protein efficiency, our results showed that Arg399Gln and Gln399Gln/Arg399Gln genotypes still provide a decreased risk for cervical cancer. An Interesting model was proposed by Stern et al. [30] who hypothesized that cells with excessive oxidative damage that carry such variants would have a decreased ability to repair DNA damage and might be more likely to undergo apoptosis or senescence. Such decreased efficiency could be an “advantage” if it prevents the transmission and clonal expansion of mutations that could arise during BER.
Controversial data was also found for the Arg194Trp polymorphism. For instance, Trp194Trp genotype was reported to be associated with increased risk of certain carcinomas, including colorectal carcinoma and esophageal SCC [36, 37]. Conversely, this genotype was regarded in other investigations as a protective factor against bladder [38] and gastric carcinoma [39]. Our findings revealed that the combined Arg194Trp/Trp194Trp variant genotype provided a modest risk of borderline statistical significance, with crude OR of 1.86. However, no association was found when adjusting for age.

Although XRCC1 Arg194Trp polymorphism occurs at an evolutionary conserved site involving an amino acid substitution, we hypothesize that it would be less likely to cause a significant change in protein repair function, and affect cancer susceptibility, since it resides in the linker region of XRCC1 N-terminal domain.

It has been widely demonstrated that HPV infection is a crucial factor in cervical cancer development. Consequently, we further investigated the association between the two SNPs of XRCC1 gene and the risk for HPV infection. For the 217 women analyzed in this study the prevalence of HPV ranged from 32.3% among the control group to 84.7% among the squamous cell carcinomas. The prevalence of HPV infection was similar to that previously reported by our group among control subjects but lower among women with SCC [40]. No significant differences were observed in genotype and allele frequencies of both SNPs between HPV positive and HPV negative women. Furthermore, none of all variant genotypes were found to confer higher risk of viral infection. We suggest that genetic susceptibility found for Arg399Gln polymorphism operates independently of the infection status of cervical tissue.

In addition to single polymorphism analyses, we have examined the effect of haplotype combinations for both XRCCI loci. All potential models were taken into consideration. Interestingly, we found that subjects carrying the Arg194-Gln399 haplotype have a decreased risk for cervical cancer development. We also observed that the protective haplotype reached the strongest association under a dominant model, denoting that a single copy is enough to show the inverse association between the Gln399 allele and cervical cancer risk.

In summary, our data supply evidence for the association between the XRCC1 Arg399Gln polymorphism and the risk of cervical cancer. Our findings revealed that both Arg399Gln and the combined Gln399Gln/Arg399Gln variant genotypes provided a reduced risk for cervical cancer development among women in La Plata, Argentina. Furthermore, haplotypes analysis confirmed and strengthened the association of the XRCC1 gene with disease susceptibility. In this way, haplotype Arg194-Gln399, acting with a dominant effect, was found to decrease risk of cervical cancer. On the other hand, no association between XRCCI Arg194Trp polymorphism and disease outcome has been demonstrated. Both studied SNPs and haplotypes did not confer more risk for HPV infection.

Our results allow for only preliminary conclusions due to the small sample size, and thus larger studies are needed to further test the effects of XRCCI genetic polymorphisms on the risk of cervical cancer.

References

XRCC1 Arg399Gln polymorphism and risk for cervical cancer development in Argentine women

Address reprint requests to:
G. BARBISAN, M.D.
IGEVET (Instituto de Genética Veterinaria “Ingeniero Fernando Noel Dulout”) Facultad de Ciencias Veterinarias Universidad Nacional de La Plata calle 60 y 118 s/n, (CP1900) La Plata, Buenos Aires (Argentina)
e-mail: gbarbisanla@yahoo.com
Compliance to adjuvant therapy in breast cancer patients

C. Dittmer1, K. Roeder1, F. Hoellen1, D. Salehin2, M. Thill1, D. Fischer1

1Department of Obstetrics and Gynaecology, University hospital Schleswig-Holstein, Campus Luebeck, Luebeck
2Helios Hospital Krefeld, Krefeld (Germany)

Summary

During recent years a continuous reduction of mortality from breast cancer has taken place in the Western countries. We wanted to verify whether the actual therapy for our own cases deviates from our recommendations, although the surgeon, radiotherapist and gynaecological oncologist are on the same premises. We sent out questionnaires to all newly diagnosed breast cancer patients in the last seven years regarding their adjuvant therapy. Comparing these answers to our own recommendation showed a very good compliance regarding chemotherapy and radiation therapy. Adjuvant endocrine therapy showed a very poor compliance with an adherence of 77%. Overall we can conclude that endocrine therapy causes many side-effects that seem to burden the patients. In combination with the duration of the therapy this causes a severe reduction in compliance and length of the therapy.

Key words: Compliance; Breast cancer; Adjuvant therapy; Endocrine therapy.

Introduction

With 55,000 new occurrences per year, breast cancer is the most common cancer of women in Germany; each year breast cancer is responsible for 17,780 deaths, making this the most common cause of death by a malign entity [1]. In Europe, breast cancer is the most common cancer in females and two-thirds occur in postmenopausal women aged 55 years and older [2]. An interesting fact is shown by epidemiologic data evaluated in the EURO-CARE-2: the quality of care in Germany could be better – a survival rate of 72% in the first five years after the diagnosis places us in the European midfield [3].

During recent years a continuous reduction in breast cancer mortality has taken place in Western countries. This is on one hand due to the improvement of early diagnosis and on the other hand to the constant progress of diverse targeted therapies [4]. A large number of patients with chronic diseases do not show a good compliance with the prescribed medication. A study of the University of Marburg has shown that a lot of patients discontinue their antihormonal medication within the first year of therapy [5, 6]. Data provided by German pharmacies was evaluated via patient self-reports with a detailed questionnaire. A study of the University of Marburg has shown that a lot of patients discontinue their antihormonal medication within the first year of therapy [5, 6]. Data provided by German pharmacies showed that three months after the start of the therapy only 66% use their follow-up prescription, 18 months later 50% are remaining [5, 6]. The effects of this lack of compliance in the long run and ways to improve the adherence are currently being evaluated by different studies. They are looking at postmenopausal women who are taking an aromatase inhibitor to see whether more information on the medication and the disease can improve the compliance.

It has been shown that therapy according to the guidelines improves survival rate [4, 7, 8]. Apart from the correct therapy compliance plays an essential role.

Using our own cases in the last seven years we wanted to verify whether the actual therapy deviates from our recommendations, although the surgeon, radiotherapist and gynaecological oncologist are on the same premises.

Materials and Methods

In the context of a retrospective study we evaluated the data of all patients with the first diagnosis of breast cancer between 2000 and 2007 in our clinic, excluding the deceased. Adherence was evaluated via patient self-reports with a detailed questionnaire. Data collected from medical records included, histology, tumour staging and grading, HR status, and primary oncological treatment (surgery, radiation therapy, chemotherapy). We sent questionnaires to 1,621 patients and received 663 back. We did not pursue any other means of getting information since we wanted to rely on the testimony of the women. The answers were compared with our last recommendations for adjuvant therapy.

Our recommendations were fixed when we finished surgery, received the histological results, and the staging results. These recommendations were set up as a letter and sent to the gynaecologist, general practitioner, radiotherapist and gynaecological oncologist if applicable.

The questionnaires contained the following:

- Did you receive chemotherapy/radiotherapy/endocrine therapy/antibodies?
  - If yes, do you know which?
  - How long did you receive the medication?
  - Did it come to an early stop of the medication?
  - In case of yes, do you know why?
  - Did you receive alternative medicine?

We divided the adjuvant therapy in chemotherapy, radiotherapy and endocrine therapy. Altogether we received feedback from 616 patients; 276 patients were pT1, 183 pT2, 24 pT3 and 14 pT4. Sixteen patients were suffering from a ductal carcinoma in situ (DCIS) and 22 received neoadjuvant chemotherapy. Another ten patients showed bilateral breast cancer. The rest of the patients showed incomplete data and could not be included in the analysis. One hundred and seventy-nine patients were node positive and 353 node negative.

Revised manuscript accepted for publication August 26, 2010

Eur. J. Gynaec. Oncol. - ISSN: 0392-2936
XXXII, n. 3, 2011
Compliance to adjuvant therapy in breast cancer patients

Results

Chemotherapy

Of 318 patients, to whom we recommended chemotherapy, 277 completed the treatment. This corresponds to a compliance of 87%. Of those who did not follow the recommendations 11 refused chemotherapy, three patients discontinued the therapy due to febrile neutropenia, two patients showed cardiac problems and another three had side-effects. Twenty-two patients did not give reasons for the discontinuation.

Radiotherapy

The greatest compliance was in the field of adjuvant radiotherapy. Five hundred and seventeen patients were advised to undergo radiation; 511 completed the treatment and six refused the treatment. This corresponds to a compliance of 98.8%.

Endocrine therapy

The greatest difference between recommended and received therapy was found regarding endocrine therapy. We divided the patients into four groups: group 1 was recommended five years of tamoxifen (n = 205), group 2 – two years of tamoxifen followed by three years of an aromatase inhibitor (AI) (n = 112), group 3 - five years of AI and group 4 was recommended five years of tamoxifen and two years of GnRH-Analog (n = 28) according to the premenopausal status.

Treatment with tamoxifen

Five years of tamoxifen were completed by 83 of 205 patients, 121 discontinued the therapy early and one patient refused endocrine therapy. Of the 121 patients, 75 were switched to an AI, the time span here was inbetween six weeks and four years. Twenty-six patients discontinued the therapy without giving a reason and 17 patients stopped taking tamoxifen due to side-effects. Ten of these were switched over to an AI. Because of progressive disease the medication was stopped for three patients.

The discontinuation of tamoxifen therapy occurred mainly due to side-effects. Seven patients reported hyperplastic endometrium, one patient postmenopausal bleeding and one patient suffered from endometrial cancer. One patient developed a thrombosis and one a stroke. These patients were switched to an AI. Five patients claimed to have perimenopausal side-effects as a reason for the discontinuation. One patient stopped taking tamoxifen when she developed a benign ovarian tumor.

Group 2: Five years of AI

The upfront therapy with an AI was completed by 70 of the 93 patients; 23 patients discontinued the therapy. The main reasons for discontinuation were rheumatic complaints and arthritis. There were also cases of hair loss and elevation of liver enzymes.

Group 3: Sequential therapy

Of 112 patients 69 completed the recommended therapy and 43 discontinued the therapy or were switched over to a different regime. Five patients received an AI upfront. For three patients tamoxifen therapy was discontinued due to side-effects. One patient was switched over to an AI; she claimed to have had no side-effects. One other patient was operated three months after starting tamoxifen. After the operation the tamoxifen treatment was discontinued and not replaced. One patient was switched over to five years of tamoxifen.

Group 4: Tamoxifen and GnRH-Analog

The combination of tamoxifen and GnRH-Analog was tolerated by 12 of the 28 patients and 16 discontinued therapy. Two patients claimed peripheral edema and menopausal flushing to be the reason for stopping the goserelin. These patients received tamoxifen mono.

Discussion

The data evaluation in this group of patients shows a representative collection regarding compliance with the adjuvant setting of breast cancer patients.

The greatest compliance is shown in the field of radiotherapy. The reason for this could be the relatively short duration and the minor side-effects of the therapy, for example compared to chemotherapy. A positive factor in our setting is that an appointment for the radiotherapy is determined at the dismissal of the patient (at the latest) so that continuous guidance is given.

In second place is chemotherapy, with a compliance of 87%. More than 90% of the women received the chemotherapy in the attached chemotherapy unit of the women’s hospital. The first appointment is like the one for the radiotherapy, already set at time of release.

A very poor compliance is shown in the field of endocrine therapy with 77%; 56% actually received the recommended therapy, but those who discontinued due to medical decisions were not taken into account regarding compliance. The highest rate of discontinuation was shown by those women who were supposed to receive five years of tamoxifen with a compliance of 40%. Side-effects like perimenopausal problems were the reason given most often. It should be kept in mind that this group also consisted of those patients that were switched to an AI upfront due to the new findings that were discovered. Five years of AI were completed by 75% of the patients. The main reasons for discontinuation were muscle and bone pain.

The sequential therapy was completed by 62%. Four patients were switched to an AI upfront; three patients were switched over due to the side-effects of the tamoxifen.
Conclusion

Overall we can conclude that endocrine therapy caused many side-effects that seemed to burden patients. In combination with the duration of the therapy this causes a severe reduction in compliance and length of therapy [9].

A recently published study by McCowan et al. showed that tamoxifen treatment taken incompletely increases the risk of a disease-associated death [10].

How do we improve compliance? This question has been subject to discussions for several years now. In 2004 Fink et al. published a study that showed that patients who did not believe in the effect of endocrine therapy tend to discontinue the medication very soon [11]. This was confirmed by Hadji et al. in a publication on improving compliance to adjuvant hormonal therapy in 2010 [12]. Another recently published study concerning compliance to endocrine treatment in the postmenopausal patient showed that compliance can be increased by a close connection to an oncological unit [13].

The limitation of this study is first of all the fact that it is a retrospective study and second that there is no gold standard in measuring adherence. In order to receive a more detailed overview the prescription data has to be collected.

Patient awareness and an understanding of their disease could influence adherence [14, 15]. Studies evaluating influential factors on compliance are the PACT study and the evaluate study. In this setting the collection of data for the management of therapy and compliance in the treatment of postmenopausal primary breast cancer with letrozol is the main goal.

We must not forget that the patients usually don’t understand the endocrine therapy to be one of their main oncologic therapies. After completing radiation and/or chemotherapy they perceive themselves as subjectively healthy. The effectiveness and the importance with regard to prevention are not understood by many patients. Due to this a close connection to an oncologic unit also in the field of the endocrine therapy is of great importance.

Aknowledgement

This work was sponsored by Novartis.

References


Discrepancy of pre- and postoperative grades of patients with endometrial carcinoma

A. Karateke, N. Tug, C. Cam, S. Selcuk, M.R. Asoglu, S. Cakir

Department of Obstetrics and Gynecology, Zeynep Kamil Hospital, Istanbul (Turkey)

Summary

Purpose: To investigate the diagnostic accuracy of endometrial curettage in patients with endometrial carcinoma. In this retrospective study, pre- and postoperative histopathologic findings of patients with endometrial cancer were investigated. Methods: 168 patients with the final diagnosis of endometrial cancer were enrolled in the study. Pre- and postoperative histopathologic diagnoses and grades (according to the 1988 FIGO classification) of the patients were compared retrospectively. Results: 22 patients were diagnosed as having endometrial hyperplasia and the remaining 136 patients had endometrial carcinoma preoperatively. Overall discrepancy rate of grades was 39% (31% upgrade, 8% downgrade; p < 0.05). There was also 9% discrepancy between the pre- and postoperative histopathological types. Conclusion: It has been suggested that since endometrial cancer patients with low grades according to the preoperative pathologic diagnosis have a potential to upgrade, the management of these patients if myometrial invasion is less than one-half thickness, simply by hysterectomy plus bilateral salpingo-oophorectomy (without lymph node sampling), might actually miss some patients who actually deserve surgical staging. Further studies are needed to draw a sufficient conclusion.

Key words: Endometrial carcinoma; Endometrial curettage; Grade; Discrepancy.

Introduction

Endometrial cancer is one of the most common female genital tract malignancies. Nearly 90% of patients with endometrial carcinoma present with abnormal vaginal bleeding, usually during menopause [1]. Several preoperative endometrial sampling tools have been used for the diagnosis of endometrial disorders in patients with abnormal uterine bleeding but dilatation and curettage is the most commonly used sampling technique with a high predictive power for tumor grade [2].

The clinical staging of endometrial cancer includes a thorough pelvic examination, imaging techniques, and endometrial sampling. However, preoperative evaluation could not provide sufficient information for histopathologic type, grade, myometrial invasion and lymphovascular involvement, so the final staging has to rely on the postoperative pathologic examination of the hysterectomy material of the patients with endometrial cancer. Many studies conducted on patients subject to adequate surgical evaluation demonstrated significant rates of clinical understaging [3, 4]. For this reason, in 1988, the International Federation of Obstetrics and Gynecology (FIGO) switched the staging system of endometrial cancer from clinical to surgical [5].

In this retrospective study, pre- and postoperative histopathologic findings of patients with endometrial cancer were investigated.

Materials and Methods

A total of 172 patients operated in the Gynecologic Oncology Clinic of Zeynep Kamil Hospital between January 2000 and December 2009 with the final diagnosis of endometrial cancer were analyzed retrospectively. All patients underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy with or without pelvic-paraaortic lymphadenectomy. The period of time between endometrial sampling and surgery was less than one week. None of the patients received any chemotherapy or other anti-cancer treatments before surgery. The study group was composed of 158 patients for whom the method of endometrial sampling was curettage. Histopathologic evaluation of both the samples and final hysterectomy specimens were performed by the same team according to 1988 FIGO classification [5]. Tumor grades and histological types of endometrial samples and final hysterectomy specimens of all patients were compared.

Statistical analyses were performed by the Pearson chi-square, Kruskal Wallis, Mann Whitney U, two-sample Kolmogorov Smirnov and Wilcoxon signed rank tests as appropriate, using SPSS version 11.5. Data are given as percent or mean ± standard deviation (SD). A level of 0.05 was chosen to indicate statistical significance. All reported p values are two-tailed.

Results

Among the analyzed 158 postoperative endometrial cancer specimens, preoperative diagnosis of 22 patients (14%) was atypical endometrial hyperplasia and the remaining 136 curettage samples were diagnosed as endometrial carcinoma. Mean age, gravidity and parity of these 136 patients were 4.1 ± 2.9, 3.0 ± 2.2 and 59.5 ± 9.8, respectively. Surgical stages of the patients according to FIGO were as follows: 16 IA, 58 IB, 32 IC, one IIa, five IIb, three IIIa, two IIIb, 17 IIIC, two IVA and none IVb. Twenty-two patients (16%) were premenopausal (12 grade 1, seven grade 2, three grade 3) and 112 patients
(84%) were postmenopausal (58 grade 1, 32 grade 2, 22 grade 3). No difference was found by means of menopausal state of the preoperative grade groups (Pearson chi-square test).

Preoperative grades of the samples diagnosed as endometrial carcinoma were: 72 (53%) grade 1, 39 (29%) grade 2 and 25 (18%) grade 3. However, in the final hysterectomy specimens, 49 (37%) patients were diagnosed as grade 1, 46 (34%) as grade 2, 41 (29%) as grade 3. The pre- and postoperative grades of the patients with endometrial cancer differed significantly (n = 136, Wilcoxon signed rank test, p = 0.000; Table 1).

The concordance rates of the pre- and postoperative diagnoses of the preoperative grade 1-3 patients were 58%, 51% and 84%, respectively (n = 136, Kruskal Wallis test, X² = 7.271, p = 0.026; Table 1). The concordance rates of preoperative grade 1-2 patients did not differ from each other (Mann Whitney U test, Z = -0.711, p = 0.477). However, the grade 3 group differed both from grade 1 (Mann Whitney U test, Z = -2.305, p = 0.021) and grade 2 groups (Mann Whitney U test, Z = -2.641, p = 0.008) significantly.

According to the final histopathologic examination, 11 patients were down graded (seven grade 2 patients, four grade 3 patients) and 42 patients (30 grade 1 and 12 grade 2 patients) were upgraded (Table 2). Rate of upgrade differed between grade 1 and 2 patients significantly (n = 111, two-sample Kolmogorov Smirnov Z = 5.030, p = 0.000).

The comparison of tumor histological type between D&C and hysterectomy specimen revealed that for 22 patients with a preoperative diagnosis of atypical endometrial hyperplasia, postoperative diagnosis was endometrioid adenocarcinoma. Among 136 preoperative endometrial cancer samples, 118 were endometrioid adenocarcinoma, nine were papillary serous, two were clear cell, four were mixed type and one was undifferentiated carcinoma. However, histo-pathological types of 12 (9%) patients changed postoperatively. Pre- and postoperative histopathological types did not differ significantly (Wilcoxon signed rank test, p = 0.139; Table 2).

### Discussion

In this retrospective study, 136 patients with a diagnosis of endometrial carcinoma were analyzed. The method of the preoperative sampling was endometrial curettage for all patients and it was found that grades of the disease differed significantly between pre- and postoperative diagnoses with an overall discrepancy rate of 39% (Table 1). There was also a 9% discrepancy between the pre- and postoperative histopathological types of the disease, but it was not statistically significant (Table 2).

Tumor grade has a strong correlation with the risk of spread outside the uterus. In a report conducted by Gynecologic Oncology Study Group, the risk of pelvic node metastases were prospected as 2.8%, 8.7%, and 18.3%, respectively, and the risks for aortic node metastases were 1.6%, 4.9%, and 11.1%, respectively in clinical Stage I grade 1-3 endometrial carcinoma. Consequently clinical

---

### Table 1. — Cross-tabulation of pre- and postoperative grades of the 136 patients with endometrial cancer. Pre- and postoperative grades differed significantly (Wilcoxon signed rank test, p = 0.001; Discrepancy rate of grade 3 also differed both from those of grade 1 and 2; Mann Whitney U test, p = 0.021).

<table>
<thead>
<tr>
<th>Preoperative Grade (n)</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Discrepancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 (72)</td>
<td>42</td>
<td>22</td>
<td>8</td>
<td>30 (42%)†</td>
</tr>
<tr>
<td>Grade 2 (39)</td>
<td>7</td>
<td>20</td>
<td>12</td>
<td>12 (31%)†</td>
</tr>
<tr>
<td>Grade 3 (25)</td>
<td>0</td>
<td>4</td>
<td>21</td>
<td>–</td>
</tr>
<tr>
<td>Total (136)</td>
<td>49</td>
<td>46</td>
<td>41</td>
<td>42 (31%)‡</td>
</tr>
</tbody>
</table>

*Mann Whitney U test, p < 0.05.
†Kolmogorov-Smirnov test, p < 0.05.

### Table 2. — Cross-tabulation of pre- and postoperative histological types of 136 patients with endometrial cancer (Wilcoxon signed rank test, p = 0.139).

<table>
<thead>
<tr>
<th>Preoperative Histology</th>
<th>Endometrioid</th>
<th>Serous</th>
<th>Mucinous</th>
<th>Clear cell</th>
<th>Mixed</th>
<th>Undifferentiated</th>
<th>Total</th>
<th>Discrepancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid</td>
<td>109</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>117</td>
<td>7</td>
</tr>
<tr>
<td>Serous</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>Clear cell</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Mixed</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>136</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>136</td>
<td>9</td>
</tr>
</tbody>
</table>
staging is strongly recommended for patients higher than clinical Stage I, grade 1-2 [6].

Endometrial sampling by hysteroscopy, pipelle, lavage and Vabra are the commonly used techniques with high sensitivity and specificity rates but D&C of the whole cavity has been accepted as a reference together with hysterectomy specimens in the evaluation of the efficacy of these techniques in the literature [3, 7-9]. There have been many studies comparing the pre- and postoperative diagnoses in patients with endometrial cancer, with concordance rates of grades ranging from 50% to 85% with higher concordance rates for grade 3 and up to 93% for histological types if the method of preoperative sampling was curettage. The accuracy of frozen sections was even lower than the endometrial curettage [3]. In the present study, the overall concordance of pre- and postoperative grades was 61% and of histopathological types 91% (Tables 1 and 2).

The diagnostic accuracy of endometrial sampling has been found to be even lower in grade 1 and 2 compared to grade 3 [7-13] as was the case in the present study. The concordance of grades in the present study was 58, 51 and 84% for grade 1-3 patients, respectively, and the upgrade rates for grade 1 and 2 were 42% and 31%, respectively (p < 0.05; Table 1).

Together with the findings of this study and the review of the literature, as a considerable rate of patients with preoperative diagnoses of Stage I, grade 1-2 endometrial cancer would upgrade to higher levels according to the final histopathological diagnosis, and the grading according to frozen sections is also not entirely accurate so, it could be suggested that the management of patients with preoperative diagnoses of Stage I, grade 1-2 with hysterectomy plus salpingo-oophorectomy (without lymph node sampling), might miss some patients who actually deserve surgical staging.

In conclusion, according to the results of this retrospective study, for patients with endometrial carcinoma, preoperative sampling with endometrial curettage has a diagnostic accuracy of 91% for histopathologic types and 61% for grades of the tumors. Further studies are needed to draw a sufficient conclusion.

References


Address reprint requests to:
N. TUG, M.D.
Rasimpasa M., Rihtim C., Uzunhafiz S.
No. 34/3, Yeldegirmani, Kadikoy
PK: 34716, Istanbul (Turkey)
e-mail: niyazitug@hotmail.com
Diagnostic test for ovarian cancer composed of ovarian cancer symptom index, menopausal status and ovarian cancer antigen CA125

R. Macuks¹, I. Baidekalna¹, S. Donina²,³

¹Riga Stradins University; ²Latvian Oncology Center of Riga Eastern Clinical University Hospital; ³Riga Stradins University, A. Kirhenstein’s Microbiology and Virology Institute (Latvia)

Summary

The objective of the study was to evaluate accuracy of the diagnostic test composed of the ovarian cancer symptom index, ovarian cancer antigen CA125 and menopausal status. Methods: A case-control study consisting of 75 women - 24 patients with ovarian cancer, 20 patients with benign ovarian diseases, and 31 age-matched healthy controls. Results: Sensitivity and specificity for the ovarian cancer symptom index alone was 83.3% and 48.3%, respectively. Specificity improved up to 70.9% when menopausal status was added. When CA125 (at cut-off level of 21 U/ml) was added to the ovarian cancer symptom index, the highest sensitivity and specificity was achieved resulting in 79.1% and 100.0%, respectively. Conclusions: The ovarian cancer symptom index could be used as a first-step screening tool in combination with serum biomarkers followed by TVS examination with an acceptable sensitivity and specificity. However, further prospective studies with larger sample size are needed to reach clear conclusions.

Key words: Ovarian cancer; Symptoms; Screening.

Introduction

Due to lack of specific symptoms and effective screening, a majority of patients with ovarian cancer are diagnosed at advanced stages. However, recently attention has turned again to the development of the ovarian cancer symptom index which was first described by Goff et al. in the USA in 2007 [1]. A correlation was found between ovarian cancer and eight symptoms with defined duration and frequency. Then, in 2007, a consensus statement from the American Cancer Society, the Gynecologic Cancer Foundation, and the Society of Gynecologic Oncologists recommended that women discuss the following symptoms with a physician: bloating, pelvic or abdominal pain, difficulty eating or feeling full quickly, and urinary symptoms (urgency or frequency). Although these symptoms can be caused by conditions other than ovarian cancer, women who experience these symptoms almost daily for more than a few weeks are encouraged to see their physicians, preferably a gynecologist [2].

Previous studies showed a sensitivity and specificity of the ovarian cancer symptom index comparable to ovarian cancer antigen CA125 alone – 64% and 88%, respectively [3]. The main role of the ovarian cancer symptom index is still to select patients for referral to gynecological consultation and further investigations.

Material and Methods

Ethical approval was given for this study by the Ethics Committee of Riga Stradins University. A case-control study consisting of 75 women - 24 patients with ovarian cancer in Group A, 20 patients with benign ovarian diseases in Group B, and 31 age-matched healthy controls in Group C. Patients were divided into the two study groups after surgery according to final histological diagnosis. Group B consisted of patients thought to have had ovarian cancer before the operation. Patients with severe co-morbidities, previous or other coexisting malignancies were not included in the study. In both study groups tumors arising only from epithelial origin were included. In study Group A most of the patients had serous type ovarian adenocarcinomas, in addition also one patient with mucinous and one with an endometroid adenocarcinoma subtype were included. In study Group B, the majority of patients similarly had serous type cystadenomas, but also three endometroid and five mucinous benign ovarian cystadenomas were included.

For the control group serum samples were taken after transvaginal ultrasonographic (TVS) examination to ensure there was no gynecological pathology. For the control group women were asked about the frequency and duration of eight symptoms (pelvic pain, abdominal pain, increased abdominal size, abdominal bloating, difficulty in eating, feeling full quickly, urinary urgency and urinary frequency). Symptoms were considered positive, if any of them were present for < 1 year and had occurred > 12 days per month. All questions were asked by the doctor ensuring that all patients had understood the asked questions. In this questionnaire patients were not asked about symptom severity.

In the control group women were chosen who attended gynecologists in an outpatient clinic. Tumor marker CA125 was detected in patient’s serum by standard enzyme-labeled chemiluminescent immunometric assay ADVIA Centaur CA125 II™, Multi-Diluent 1, Bayer, using Siemens analyzer Immulite-2000 [4, 5]. Sensitivity, specificity and positive predictive value (PPV) for the ovarian cancer symptom index together with women’s menopausal status and ovarian cancer associated antigen CA125 among study and control groups were calculated using the Vassarstat statistical program [6].
Sensitivity and specificity of the ovarian cancer symptom index combined with serum antigen CA125 was calculated at cut-off levels of 21 U/ml, 35 U/ml and 65 U/ml. Individually sensitivity and specificity of the ovarian cancer symptom index was calculated after addition of menopausal status as an independent factor and after that in combination with CA125 at different cut-off levels. A statistically significant correlation or difference between variables and groups were accepted at the level of 0.05.

Results

Sensitivity and specificity for ovarian cancer antigen CA125 alone was higher than for the ovarian cancer symptom index alone or in combination with other variables – 95.8% and 100.0%, respectively (Table 1).

In Group A and B there were 15 menopausal women in each group and 22 menopausal women in Group C. Addition of menopausal status to the ovarian cancer symptom index alone improved specificity of the diagnostic test. The highest rates of sensitivity and specificity were observed when the ovarian cancer symptom index was used in combination with ovarian cancer biomarker CA125 elevated above 21 U/ml without addition of menopausal status. Sensitivity and specificity of the ovarian cancer symptom index remained low when applied for discrimination of patients with benign ovarian tumors from the control group women. The highest sensitivity and specificity for ovarian cancer patient isolation from patients with benign ovarian tumors was observed when ovarian cancer antigen CA125 was added to the ovarian cancer symptom index at the cut-off level of 21 U/ml. Specificity improved by 5% for each factor when menopausal status and higher cut-off level for ovarian cancer antigen CA125 was applied with remarkable decrease in sensitivity (Table 1).

Table 1. — Comparison of sensitivity and specificity of the ovarian cancer symptom index in combination with menopausal status and ovarian cancer antigen CA125 at different cut-off levels.

<table>
<thead>
<tr>
<th>Ovarian cancer symptom index alone</th>
<th>Group A/Group C (%)</th>
<th>Group B/Group C (%)</th>
<th>Ovarian cancer symptom index and CA125 (&gt; 21 U/ml) alone</th>
<th>Group A/Group B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menopausal status alone</td>
<td>83.3/48.3%</td>
<td>55.0/48.3%</td>
<td>83.3/45.0%</td>
<td>50.0/45.0%</td>
</tr>
<tr>
<td>CA125 (&gt; 21 U/ml) combined with CA125 (&gt; 21 U/ml)</td>
<td>79.1/100.0%</td>
<td>25.0/100.0%</td>
<td>79.1/75.0%</td>
<td>50.0/75.0%</td>
</tr>
<tr>
<td>(&gt; 35 U/ml) combined with CA125 (&gt; 35 U/ml)</td>
<td>70.8/100.0%</td>
<td>20.0/100.0%</td>
<td>70.8/80.0%</td>
<td>50.0/80.0%</td>
</tr>
<tr>
<td>(&gt; 65 U/ml) combined with menopausal status and CA125 (&gt; 65 U/ml)</td>
<td>70.8/100.0%</td>
<td>20.0/100.0%</td>
<td>70.8/80.0%</td>
<td>50.0/80.0%</td>
</tr>
<tr>
<td>All three factors combined (&gt; 21 U/ml)</td>
<td>50.0/100.0%</td>
<td>20.0/100.0%</td>
<td>50.0/80.0%</td>
<td>50.0/80.0%</td>
</tr>
<tr>
<td>(&gt; 35 U/ml) combined with menopausal status and CA125 (&gt; 35 U/ml)</td>
<td>45.8/100.0%</td>
<td>15.9/100.0%</td>
<td>45.8/85.0%</td>
<td>50.0/85.0%</td>
</tr>
<tr>
<td>(&gt; 65 U/ml) combined with menopausal status and CA125 (&gt; 65 U/ml)</td>
<td>45.8/100.0%</td>
<td>15.9/100.0%</td>
<td>45.8/85.0%</td>
<td>50.0/85.0%</td>
</tr>
</tbody>
</table>

PPV for the ovarian cancer symptom index alone was 0.06% at a sensitivity and specificity of 83.3% and 48.3%, respectively, but when combined with serum CA125 elevated above 21 U/ml, it was 3.06% at test sensitivity and specificity of 79.1% and 100% with an estimated average disease prevalence of 0.04%.

Discussion

Previously it was thought that ovarian cancer has no specific symptoms, especially for early-stage detection. Recently, researchers from the USA observed that symptoms, in combination with their frequency and duration, had a sensitivity of 56.7% for identifying early-stage disease and 79.5% for identifying advanced-stage disease with specificities ranging from 86% to 90%. In that study the symptom index performed similarly to CA125 for detecting any stage of the disease [7]. In similar studies the ovarian cancer symptom index revealed sensitivity and specificity ranging from 64.0%–68.0% and 84.7%–95.0%, respectively, among all stages [8–10].

At first it was observed by Goff et al., in a particular study where more pronounced symptom expression between ovarian cancer patients compared to patients with benign ovarian diseases; control group patients were also found [1]. A correlation between the ovarian cancer symptom index and stage among ovarian cancer patients was not statistically significant, but in other studies a positive symptom index prevalence of 44.8–56.7% for patients with Stage I/II disease and in 72.9–79.5% for patients with Stage III/IV disease was found [1, 7]. Moreover no statistically significant correlation was observed between the ovarian cancer symptom index and ovarian cancer antigen CA125. The reason for this might be a quite frequent expression of symptoms among control group women. Particular control groups do not reflect the average symptom distribution in the whole population. Average distribution of a positive ovarian cancer symptom index in the population was reported to be about 3% [11]. Regardless of control group selection bias, a positive ovarian cancer symptom index was observed up to 51.6% of control group women without finding any ovarian cancer. Even more - six women from the control group had three and more frequently repeating symptoms that had appeared during the previous 12 months.

Despite attempts to eliminate distribution bias of ovarian cancer antigen CA125 among ovarian cancer patients, they were normally distributed before and after exclusion of patients with ovarian cancer antigen CA125 exceeding 1000 U/ml, but a correlation between the ovarian cancer symptom index was still not achieved. Irrespectively of a high prevalence of the positive ovarian cancer symptom index in the control group, a strong correlation between the ovarian cancer symptom index and study groups was observed.

Overall, the sensitivity and specificity of the symptom index alone was 83.3 and 48.3, which is not similar to the data reported before. In a case control study by Mi-Kyung et al. consisting of 116 women with epithelial ovarian
cancer and 209 control group women sensitivity and specificity were 65.5% and 84.7%, respectively [8]. Specificity of the diagnostic test improved with addition of menopausal status and ovarian cancer antigen CA125, because none of the control group women had elevated CA125 and specificity reached 100% with slightly decreasing sensitivity. The highest sensitivity/specificity of the ovarian cancer symptom index according to our study data was achieved after addition of only one parameter – CA125 at a cut-off level of 21 U/ml which corresponds to other studies. Andersen et al. reported even higher diagnostic values for the combined symptom index with CA125 - sensitivity and specificity of 80.6% and 83.5% for early-stage ovarian cancer and 95.1% and 83.5% for late-stage cancers, respectively [8]. The addition of menopausal status to the ovarian cancer symptom index with simultaneously elevated serum ovarian cancer antigen decreased test sensitivity because there were a lot of ovarian cancer patients at premenopausal age which were lost with such approach. In the same study the symptom index identified cancer in 50% of the affected women who did not have elevated CA125 levels and 11.8% of the high-risk women without cancer also received a positive symptom index score [8].

According to our data, PPV for the ovarian cancer symptom index alone was lower than previously published, but when applied in combination with serum concentration of CA125, it was even higher than reported before. The estimated positive predictive value of the symptom index or symptoms meeting the consensus criteria was 0.6%-1.1% overall and less than 0.5% for early-stage disease in the study of 812 case patients and 1,313 population-based control subjects [12].

It is estimated that there is only one ovarian cancer patient found among 100 patients with the ovarian cancer symptom index. Historically the goal of a screening test has been to achieve a PPV greater than 10% to be considered cost effective and have an acceptable risk for the population being screened. Results from one of the largest trials on ovarian cancer symptom research suggest that there are a lot of women with false-positives with the ovarian cancer symptom index and that the test could be improved with addition of some other biomarkers. In the same study most case patients had a positive ovarian cancer symptom index result within five months before diagnosis [12]. That means that despite a rather short period between symptom appearance and diagnosis, it still remains a significant period in context of optimal debulking surgery.

Conclusions

The ovarian cancer symptom index could be used as first-step screening tool in combination with serum bio-markers followed by TVS examination with an acceptable sensitivity and specificity. However, further prospective studies with a larger sample size are needed to reach clear conclusions.

Acknowledgements

The study was done within the framework of Latvian University project (no. 2009/0220/1DP/1.1.2.0/09/APIA/VIAA/016) and P. Stradins University project (no. 2009/0147/1DP/1.1.2.1.2/09/IPIA/VIAA/009).

References


Address reprint requests to: R. MACUKS, M.D.
Mazciema Street 23/1-55
Riga, LV1079 (Latvia)
e-mail: r.macuks@gmail.com
Ovarian germ cell tumors in children: a 20-year retrospective study in a single institution

Chao Yang, Shan Wang, Chang-Chun Li, Jun Zhang, Xiang-ru Kong, Jun Ouyang

Department of Pediatric Surgical Oncology, Children’s Hospital of Chongqing Medical University, Chongqing (China)

Summary

Purpose: Ovarian germ cell tumors are rare in childhood. The goal of the study is to provide information that may help guide the evaluation and surgical management of future children with ovarian tumors. Methods: A retrospective review of patients with ovarian germ cell tumors between January 1990 and January 2010 was performed. Results: 137 patients were included with a median age of 9.5 years. Teratomas were found most frequently (mature: 78, immature: 6), followed by yolk sac tumors (n = 51), dysgerminoma (n = 1) and embryonal carcinoma (n = 1). Abdominal pain (81.8%) and abdominal distension (58.4%) were the most common symptoms. Twenty-six infants were found prenatally. Twenty-one patients presented torsion of the ovary. Alpha-feto-protein levels were elevated in all pure yolk sac tumors, two immature teratomas and one embryonal carcinoma. Most patients (84) were Stage I, 16 were Stage II, 23 Stage III, and four Stage IV. All patients with mature and immature teratomas (grade 1) underwent surgery alone. Surgery + chemotherapy were conducted in 55 other patients. The surgical procedures consisted of salpingo-oophorectomy (n = 68), oophorectomy (n = 21) and ovarian-sparing tumorectomy (n = 48). Sixteen patients gave up the treatment and died. Excluding this subset, 5-year relapse-free survival and overall survival was 93.4% and 98.3%, respectively. No recurrences were observed in any patients. Conclusion: Ovarian germ cell tumors have an excellent prognosis. With accurate staging, complete resection, and adjuvant chemotherapy, patients should be expected to have excellent survival rates. Preservation of ovarian tissue should be considered whenever safe and feasible, however, this needs to be confirmed by studies on larger numbers of patients.

Key words: Germ cell tumor; Ovary; Teratoma; Ovary; Yolk sac tumor; Dysgerminoma; Pediatric.

Introduction

Ovarian tumors, whether cystic, solid, or both, have been considered rare in the pediatric population. The incidence is estimated to be approximately 2.6 cases per 100,000 girls per year, with malignant ovarian neoplasms making up about 1% of all pediatric cancers [1]. Germ cell tumors are the most common type of ovarian tumors in children and adolescents, comprising 3% to 4% of all pediatric patients [2, 3]. About one-third of all childhood ovarian neoplasms are reported to be malignant, therefore the possibility of malignancy should be considered in all cases. Pediatric ovarian germ cell tumors (GCTs) are highly chemosensitive with a high curability rate. Surgery with adjuvant chemotherapy is the mainstay of treatment. The aim of this study was to review the clinical presentation, management and outcome in a series from a single institution over a 20-year-period. The goal of this study is to provide information that may help guide the evaluation and surgical management of future children with ovarian tumors.

Patients and Methods

With approval from the hospital Human Research Ethics Committee, the medical records of 172 consecutive girls with ovarian problems treated surgically at Children’s Hospital of Chongqing Medical University, Chongqing, China between January 1990 and January 2010 were studied. Our hospital provides secondary and tertiary pediatric care in Chongqing City and is also a major pediatrics referral center for southwest China. Every patient was diagnosed by surgery or puncture biopsy and pathology; ovarian cysts, follicular cysts of the ovary and granular cell tumors were excluded. At last, 137 records were included in the study. We recorded symptoms that led to further investigation and to diagnosis, age at diagnosis, the radiological and biochemical methods employed in the diagnosis, methods of treatment, and complications. Clinical staging was according to the Children’s Oncology Group staging, a modification of the FIGO (International Federation of Gynecology and Obstetrics staging for ovarian tumors) classification [4]. This classification defines Stage I as a tumor limited to the ovary, Stage II as a tumor with pelvic extension, microscopic residual or positive lymph nodes < 2 cm, Stage III as a tumor with gross residual or biopsy only, contiguous visceral involvement, lymph nodes with malignant metastatic nodules > 2 cm, and Stage IV as a tumor with distant metastases, including the liver. Histological typing of tumors followed the WHO classification. Immature teratomas were graded according to the Norris classification.

Results

Patient characteristics

Median age at presentation was 9.5 years (range from neonatal to 15 years). Malignant tumors were diagnosed in girls at a median age of 8.5 years (range from 4 months to 14 years). Past medical histories and family histories were unremarkable. Ninety percent of the patients were from rural areas with most of them having upper lower or lower socioeconomic status. The parents of one-third of the patients were illiterate.
Presenting signs and symptoms

The vast majority of patients presented with a combination of symptoms and signs; however the most frequent primary symptom was abdominal pain, either acute, chronic, or both and was reported for 112 patients (81.8%). The second most frequent symptom, major abdominal distension by the abdominal or pelvic mass, was noted in 80 patients (58.4%), associated with pain in 45 and painless in 35; however it was the primary sign in only seven. Other symptoms and signs included nausea and vomiting, constipation, urinary symptoms and fever. For 26 infants, the tumor was found prenatally, and surgery was performed within two months after their birth. Twenty-one patients presented torsion of the ovary, 20 of which were mature teratomas, and the rest were dysgerminoma. For two patients the ovarian tumor was an incidental finding during investigations for an unrelated pathology (both of them were consulted for pneumonia). The duration of symptoms ranged from six hours to two years.

Imaging

Various imaging studies were used for these patients over the years, except in those with an acute abdomen. Abdominal ultrasound was the most common investigation, performed in all patients. Plain abdominal films were used for 65 patients. Computed tomography was performed in 127 patients. Calcifications were observed in 40 patients. The space-occupying lesion was reported to be purely cystic in six, purely solid in 65, and mixed cystic-solid in 66 patients. Tumor dimensions ranged from 2.5×1.5×1.5 cm till 21 cm in diameter.

Tumor markers

Alpha-feto-protein (AFP) levels were elevated in 54 patients, 51 with pure yolk sac tumors, two with immature teratomas (Gr 2 and 3), one with an embryonal carcinoma, and were normal in all others. Beta human chorionic gonadotropin (β-HCG) and AFP levels were both elevated in the patients with embryonal carcinoma.

Staging

According to postsurgical staging all 84 patients with mature and immature teratomas and ten patients with a malignant tumor were Stage I. Sixteen patients with malignant tumors had Stage II, 23 had Stage III and four patients with lung metastases (2), liver metastasis (1), peritoneum metastasis (1) had Stage IV disease (Table 1).

Histology

The histologic diagnosis of the 137 tumors is summarized in Table 2. Mature teratomas were the commonest tumors (n = 78), followed by yolk sac tumors (n = 51), immature teratomas (n = 6), dysgerminoma (n = 1) and embryonal carcinoma (n = 1). The distribution of benign or malignant tumor by age groups is shown in Table 2.

### Table 1. — Distribution of benign/malignant GCTs by age groups.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Total GCTs</th>
<th>Benign</th>
<th>Malignant (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>57</td>
<td>16</td>
<td>41 (71.9%)</td>
</tr>
<tr>
<td>4-6</td>
<td>24</td>
<td>22</td>
<td>2 (9.1%)</td>
</tr>
<tr>
<td>7-10</td>
<td>26</td>
<td>21</td>
<td>5 (19.2%)</td>
</tr>
<tr>
<td>11-15</td>
<td>30</td>
<td>19</td>
<td>11 (36.7%)</td>
</tr>
</tbody>
</table>

### Table 2. — Histopathologic diagnosis in 137 patients with ovarian GCTs.

<table>
<thead>
<tr>
<th>Histology</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teratoma</td>
<td>84</td>
</tr>
<tr>
<td>Mature</td>
<td>78</td>
</tr>
<tr>
<td>Immature</td>
<td>6</td>
</tr>
<tr>
<td>Grade 1</td>
<td>4</td>
</tr>
<tr>
<td>Grade 2</td>
<td>1</td>
</tr>
<tr>
<td>Grade 3</td>
<td>1</td>
</tr>
<tr>
<td>Yolk sac tumor</td>
<td>51</td>
</tr>
<tr>
<td>Dysgerminoma</td>
<td>1</td>
</tr>
<tr>
<td>Embryonal carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>137</td>
</tr>
</tbody>
</table>

### Table 3. — Stage and outcome.

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>DFS (%)</th>
<th>OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>94</td>
<td>95.7%</td>
<td>96.8%</td>
</tr>
<tr>
<td>II</td>
<td>16</td>
<td>62.5%</td>
<td>75%</td>
</tr>
<tr>
<td>III</td>
<td>23</td>
<td>52.2%</td>
<td>78.2%</td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>25%</td>
<td>50%</td>
</tr>
</tbody>
</table>

DFS indicates disease free survival; OS, overall survival.

### Treatment

For all patients with mature and immature teratomas (grade 1) treatment consisted of surgery alone. Surgery + chemotherapy were done in 55 other patients; no patient received radiotherapy.

Twenty-two patients with acute symptomatology had undergone emergency surgery: 21 for torsion of the tumor (16 right-sided) and one for apparent tumor rupture. The others had been operated upon selectively. Stage III and Stage IV patients had biopsy first, either puncture or surgery, when the diagnosis was confirmed. Neoadjuvant chemotherapy was given for three to six courses, then followed by surgery, and chemotherapy was also given after surgery. Sixteen patients with malignant tumors gave up treatment because of the poor economic conditions (including 3 Stage I, 4 Stage II, 7 Stage III and 2 Stage IV). The tumors were right-sided in 76 patients and left-sided in 61 patients. Tumor dimensions ranged from 2.5×1.5×1.5 cm to 21×18×15 cm. No bilateral tumors were found in surgery. The procedures consisted of salpingo-oophorectomy (n = 68), oophorectomy (n = 21) and ovarian-sparing tumorectomy (n = 48). The contralateral ovary was always inspected and palpated very carefully in every patient. Five patients were suspected of having malignant tumors and biopsy of the contralateral ovary was made; meanwhile, no one had tumor. The tumors themselves were not subjected to frozen section.
histology. Ascites was sent for cytologic analysis when present, and revealed malignant cells in one patient with peritoneum metastasis.

Postoperatively, six patients developed a wound infection and 12 mechanical small intestinal obstructions by adhesions. In eight patients the obstruction subsided with conservative measures, while in the four others surgical adhesiolysis was necessary to relieve the obstruction.

Based on the disease stage, patients underwent protocol treatment with surgery and cisplatin-based chemotherapy (PEB). The chemotherapy regimen used was PEB which was administered once every three weeks for six to eight cycles. Bleomycin was given at a dose of 30 units D2, etoposide 120 mg/m²/d D1-3, and cisplatin at a dose of 100 mg/m²/in divided doses D1-3. All malignant patients received chemotherapy except with Stage I immature teratoma grade 1.

Outcome

There were no recurrences in patients with malignant tumors. The median duration of follow-up was 7.3 years (3 months to 15 years). Sixteen patients refused chemotherapy and died because of widely metastasized tumor. Excluding the 16 patients who gave up treatment, the 5-year relapse-free survival and overall survival was 93.4% and 98.3%, respectively. The 5-year disease-free survival and overall survival was 100% for patients with dysgerminoma. Patients with early-stage disease had an excellent survival (Table 3).

There were no recurrences in patients with malignant tumors. All patients are on long-term follow-up with monitoring of endocrine function and fertility. Seventy out of 78 patients in puberty had regular menstrual cycles. Six patients had menostaxis and two patients required psychological input during the follow-up period. None of these patients experienced pregnancy because none have married yet.

Discussion

GCTs are rare in childhood. In our hospital, which performs more than 8,000 general pediatric surgeries per year, there have been only 137 GCTs in the last 20 years. Over a 20-year-period 137 GCTs do not really constitute a large series, yet it is the largest of its kind reported in the past decade. The age distribution for malignant tumors varies in the literature. Malignant tumors have been reported to occur more frequently at lower ages [5], although it has also been reported that the risk of malignancy increases with age, especially around puberty [6]. The age distribution of our study showed that tumors were diagnosed at every age with the peak age 10-15 years and below three years. The proportion of malignant tumors was even higher in infants than teenagers. From our study, we might conclude that younger infants and teenagers are at higher risk for malignancy than other children are. We also noticed that most parents of the patients were illiterate (1/3 of the patients) and from rural areas (> 90%) with a low socioeconomic status. Late stage of presentation may be owing to late presentation to the doctor or delayed referral to the tertiary care center.

Patients with ovarian tumors seek medical attention in a variety of ways. Most patients present with acute abdominal pain and signs of peritonitis that can be difficult to distinguish from acute appendicitis. Patients may present with a large pelvic or abdominal mass. Some patients may refer with precocious puberty or other signs of endocrine disorder. Bowel obstruction, ureteral compression and hydronephrosis may be present in some patients with a mass effect from an enlarged ovary. Distinguishing patients with ovarian torsion, acute appendicitis or other surgical lesions may be challenging for a pediatric surgeon. In our experience, the presence of nausea, anorexia, and vomiting may favor appendicitis over ovarian pathology, however, these symptoms are not specific or reliable. Although ultrasound can be helpful in confirming a preoperative diagnosis of ovarian torsion and in differentiating nonoperative ovarian pathology from appendicitis and other acute surgical conditions, delays in obtaining the examination and false-negative results reinforce the role that operation (and perhaps laparoscopy) plays in evaluating these patients [7].

Tumor markers are a significant factor for management, prognosis and follow-up of germ cell tumors. AFP is the commonest one used in pediatric population. AFP may be elevated in patients with teratomas and it is invariably elevated in yolk sac tumors [8]. Germinoma may present with either positive β-HCG, a marker associated with choriocarcinoma, or CA-125, which is associated with epithelial tumors. In our study, all patients with malignant germ cell tumors had significantly elevated levels of AFP or AFP and β-HCG. Two patients with immature teratoma (grade 2/3) presented with elevated AFP, and all other patients had tumor markers within the normal range. The AFP levels were all within the normal range after treatment at follow-up.

Surgical excision has a central role in the management of GCTs, apart from being the only treatment required in many cases, surgery provides valuable information for staging. Because GCTs often occur in girls and young woman, preservation of ovarian function and fertility is an important goal of treatment. More recently, investigations have recommended detorsion and preservation of all ovaries (either open or laparoscopic), even of those that appear frankly necrotic [9]. Radical ovarian excision was performed if the mass proved to be malignant. However, for benign tumors, unilateral salpingo-oophorectomy or ovarian-sparing tumorectomy is often sufficient. Bilateral ovarian involvement is rare in children, and in our study none were found to have bilateral involvement. In our opinion, if the contralateral ovary appears normal, biopsy is not necessary and may contribute to adhesion formation. Although it is not clear to what extent oophorectomy affects the fertility of these patients, it is known that there is a dramatic increase in the incidence of women with a single ovary compared with the general population in infertility clinics. However, pregnancy rates appear to be the same as that of general population when patients
undergo follow-up longitudinally [10]; the fate of remaining with a single ovary does not imply a reduced fertility potential to conceive. Therefore, considering the risk of asynchronous torsion or other contralateral ovarian disorders, we suggest preserving ovarian tissue whenever safe and feasible. However, more work is needed to shed more light on the fertility potential of patients with GCTs after surgery.

The rise of minimally invasive surgical procedures, such as the laparoscopic technique for ovarian tumors was developed in the past decades. The effectiveness of laparoscopic or open cystectomy for mature teratomas is well documented [11]. Skills in pediatric laparoscopic surgery have increased as well. However, GCTs in childhood are often very large, rendering laparoscopic removal less advantageous when compared with adults. Moreover, the reported spillage rates in laparoscopic excision of ovarian tumors range considerably, from 13% to 52% [12, 13]. In our opinion, laparoscopic excision should be considered when the tumor is small and more likely to be benign. If tumors are large and malignance cannot be excluded, open exploration should be a better approach. However, a laparoscopic procedure is helpful in tumor staging with minimal invasion.

Chemotherapy plays an important role in malignant tumor treatment and outcome. Immature teratomas, although not truly malignant, have been treated as malignant neoplasms in our department because of their potential to recur as malignant tumors. Chemotherapy based on cisplatin was given to patients with grade 2 or 3 immature teratomas. There has been no recurrence of immature teratomas in our study, and the overall survival rate was 100%. All patients are alive, aged 6-20 years now, and are between five months and 12 years post diagnosis.

Overall survival for GCTs was 86.9% in our study, which is lower than the literature reports ranging between 97-100% [6, 7]. Lower overall survival rate may be owing to 16 patients with malignant tumors who gave up chemotherapy. When excluding this subset, the 5-year relapse-free survival and overall survival was 93.4% and 98.3%, respectively. The application of platinum-based agents in pediatric chemotherapy regimens has improved survival significantly in children with malignant GCTs. The 6-year overall survival rate with the PEB protocol has been 95.1% for Stage I, 93.8% for Stage II, 97.35% for Stage III and 93.9% for Stage IV disease [14]. In our series, excluding the 16 patients who gave up therapy, the 5-year overall survival rate with the PEB protocol was 100% for Stage I, 100% for Stage II, 87.5% for Stage III and 100% for Stage IV disease.

Conclusion

Ovarian germ cell tumors are uncommon in children. Both benign and malignant tumors have an excellent prognosis. For benign ovarian neoplasms operation should be designed to optimize future fertility. Malignant tumors are highly chemosensitive with a high curability rate. With accurate staging, complete resection, and adjuvant chemotherapy, patients should be expected to have excellent survival rates. Preservation of ovarian tissue should be considered whenever safe and feasible; however, this needs to be confirmed by studies on larger numbers of patients in the future.

References

Specific downregulation of death-associated protein kinase enhances Fas-mediated apoptosis in the human differentiated endometrial adenocarcinoma cell line, HHUA

T. Tanaka¹, T. Bai¹, K. Yukawa²

¹Department of Obstetrics and Gynecology, Wakayama Medical University, Wakayama
²Department of Physiology, Faculty of Pharmacy, Meijo University, Nagoya (Japan)

Introduction

Death-associated protein kinase (DAPK) is a 160-kDa Ca²⁺/calmodulin-dependent serine/threonine kinase that functions as a positive mediator of apoptosis triggered by interferon-γ, tumor necrosis factor-α, anti-Fas antibodies, transforming growth factor-β, c-myc and E2F oncoproteins, ceramide and by detachment from the extracellular matrix [1-8]. Moreover, loss of DAPK expression has been implicated in tumorigenesis and metastasis [9, 10] thus suggesting a crucial role for DAPK in the apoptotic process under pathological conditions. On the other hand, several lines of evidence have indicated that DAPK may have an anti-apoptotic function. Inhibition of DAPK expression in HeLa cells, 3T3 fibroblasts and primary human vascular smooth muscle cells using an antisense DAPK was found to increase apoptosis [11, 12]. In our previous studies, we detected higher DAPK protein expression levels in differentiated endometrial adenocarcinoma cells than in normal primary endometrial cells or in several ovarian and uterine carcinoma cells including HeLa cells [13]. These results suggest that DAPK regulates cell survival or apoptosis of human endometrial adenocarcinoma cells.

Constitutive expression of apoptosis-inducible cytokine receptors, such as type 1 receptor of tumor necrosis factor (TNFR1) and Fas antigen (CD95) have been reported in normal human endometrial tissues [14-16] and in differentiated endometrial adenocarcinoma cells [17]. Some of these endometrial adenocarcinoma cells express functional Fas antigens that mediate Fas-mediated apoptotic signals [17]. The DAPK protein is also expressed in endometrial adenocarcinoma cells [13]. DAPK was first reported to positively regulate tumor necrosis factor-α-- and Fas-induced apoptosis via the death domain of the DAPK molecule [3]. Co-immunoprecipitation studies in brief seizure-induced neuronal death revealed binding of DAPK to the cytoplasmic domain of TNFR1 and to the Fas-associated death domain protein, suggesting that DAPK directly and positively regulates apoptosis mediated by the TNFR-family [18]. We recently reported that Fas-mediated apoptosis in chemically damaged ovarian granulosa cells is strongly suppressed in mutant murine ovaries in which the kinase domain of DAPK is deleted [19]. Epigenetic downregulation of DAPK gene expression by hypermethylation of its promoter region has been reported in many cancer cells including T-cell lymphomas [20], B-cell lymphomas [20], non-small cell lung carcinomas [21], head and neck cancers [22], gastric and colorectal carcinomas [23, 24], ovarian carcinomas [13] and uterine carcinomas [25]. However, Matsumoto et al. reported that the status of DAPK protein expression closely correlated with Fas expression but not with methylation of the promoter region [26]. Our previous studies also showed that DAPK protein expression levels correlated with Fas expression but not with methylation of the promoter region [26].

Summary

Purpose of investigation: Death-associated protein kinase (DAPK) is a serine/threonine kinase that is well-known as a positive mediator of Fas-mediated apoptosis. Previous reports have shown that DAPK and Fas are expressed in human endometrial adenocarcinoma cells. In this study, we examined the effects of specific downregulation of DAPK expression on Fas-mediated apoptosis in the human endometrial adenocarcinoma cell line, HHUA.

Methods and results: Transfection of DAPK small-interfering RNAs (siRNAs) into the HHUA cells reduced DAPK protein expression, and enhanced Fas-mediated apoptosis, in a dose-dependent manner.

Conclusions: These results indicate that, in contrast to cases with other malignant tumor cells, DAPK negatively regulates Fas-mediated apoptosis in these human differentiated endometrial adenocarcinoma cells.

Key words: Death-associated protein kinase; Fas; Endometrial adenocarcinoma; siRNA.
Materials and Methods

Cell line and culture

The HHUA cell line [27] was obtained from the Riken Cell Bank (Tsukuba, Japan). The cells were cultured in OPTI-MEM (Invitrogen, Corp., Carlsbad, CA) supplemented with 5% fetal bovine serum (FBS) (Equitech Bio, Inc., Ingram, TX), penicillin (100 U/ml), streptomycin (100 U/ml) and Fungizone (0.25 μg/ml; Invitrogen, Corp.) under 5% CO2 and 95% air at 37°C.

Transfection of DAPK siRNAs

Two DAPK siRNA duplexes were designed and synthesized by iGENE Therapeutics, Inc. (Tsukuba, Japan). The siRNA sequences are shown in Table I. A negative control siRNA was purchased from Ambion, Inc. (Austin, TX). Lipofectamine 2000 (Invitrogen, Corp.) was used as the transfection reagent according to the manufacturer’s instructions. For experiments, cells were seeded in 6-well plates (2.5 x 10⁶ cells/well) or 10-cm dishes (2 x 10⁶ cells/dish), cultured for 24 h and then transfected with the DAPK siRNAs or control siRNA. Subsequently, the cells were cultured for 48-72 h for protein assays before being harvested as indicated.

Western blotting

For Western blotting analysis, the cells were collected 48-72 h after transfection with the DAPK siRNAs or control siRNA and lysed in phosphate-buffered saline containing 1% NP-40, 0.1% sodium dodecyl sulfate, complete protease inhibitor cocktail (Roche Diagnostics,Corp.,Indianapolis, IN) and 1 mM phenylmethyl sulfonil fluoride. The protein concentrations of the cell lysates were quantified by Coomassie Plus Protein assays (Pierce Biotechnology, Inc., Rockford, IL). Equal amounts of the total proteins were separated by SDS-PAGE using a 7.5% gel and then transferred to a polyvinylidene fluoride membrane (Atto, Corp., Tokyo, Japan). After sequential incubations with primary and secondary antibodies, the immunocomplexes on the membranes were detected using enhanced chemiluminescence (ECL) or ECL plus kits (Amersham Pharmacia Biotech, Uppsala, Sweden). The antibodies used were purchased from the following sources: mouse monoclonal anti-DAPK antibody (Sigma, St. Louis, MO); rabbit polyclonal anti-poly (ADP-ribose) polymerase (PARP) and anti-cleaved PARP antibodies (Cell Signaling Technology, Beverly, MA). The membranes were stripped and reprobed with an anti-β-actin antibody (Sigma).

Cell viability assay

A Cell Counting kit (Dojindo Chemical Laboratory Co., Ltd., Tokyo, Japan) was used to evaluate the abilities of the DAPK siRNAs to enhance the cytotoxic effects of an agonistic anti-Fas IgM antibody (Medical & Biological Laboratory Co., Ltd., Nagoya, Japan). Cells were plated in quadruplicate in 96-well plates at 5 x 10⁴ cells/well and cotransfected with Lipofectamine 2000 and 25-50 nM DAPK siRNAs or with 50 nM control siRNA. At 24 h after transfection, cells were incubated with various concentrations of anti-Fas IgM for a further 48-72 h. At the end of the treatments, viable cell numbers were determined using the Cell Counting kit according to the manufacturer’s instructions. Absorbance at 450 nm was measured using a microplate reader. Cell viability of 100% was defined as absorbance obtained for cells without anti-Fas IgM. The data are expressed as means ± SD. Comparisons between experimental groups were performed by analysis of variance (ANOVA). The level of statistical significance was set at p < 0.05.

Results

Specific downregulation of endogenous DAPK expression by DAPK siRNA transfection induces cleavage of PARP in HHUA cells

To investigate the role of endogenous DAPK protein in HHUA cells, we first assayed the effect of specific down-regulation of DAPK expression in HHUA cells following transfection with double-stranded siRNAs (DAPK siRNA-1 or siRNA-2) (Table 1) targeted against human DAPK mRNA. Western blot analyses revealed dose-dependent suppression of DAPK protein expression in DAPK siRNA-transfected HHUA cells at 48 h after transfection (Figure 1). Concurrently, specific downregulation of endogenous DAPK expression by DAPK siRNA transfection induced cleavage of PARP, an apoptosis marker, in the cells. No nonspecific inhibitory effects of DAPK siRNA were detected on β-actin protein expression, used as an internal control.

Table 1. — DAPK siRNA sequences.

<table>
<thead>
<tr>
<th>DAPK siRNA</th>
<th>siRNA sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>siRNA-1</td>
<td>5'-CAACAUCAUGCAAAGUGAAACAGUU-AG-3'</td>
</tr>
<tr>
<td>siRNA-2</td>
<td>5'-AGCCAAGAUAAGGCAGAACGAUU-AG-3'</td>
</tr>
</tbody>
</table>

Reduced endogenous DAPK expression increases the sensitivity of HHUA cells to anti-Fas IgM treatment

Since HHUA cells express functional Fas antigen [17], we directly examined the effects of DAPK knock down on Fas-mediated apoptosis in HHUA cells. In these experiments, the dose-response curve of the DAPK siRNA-transfected HHUA cells in response to anti-Fas IgM antibody treatment was significantly shifted to the
Specific downregulation of death-associated protein kinase enhances Fas-mediated apoptosis in the human differentiated etc.

Discussion

This is the first report to show a relationship between Fas-mediated apoptosis and DAPK expression in human endometrial adenocarcinoma cells. Specific downregulation of DAPK significantly and dose-dependently enhanced Fas-mediated apoptosis in HHUA cells, indicating that endogenous DAPK expression negatively regulates Fas-mediated apoptosis. Moreover, suppression of DAPK expression may itself have induced apoptotic signals in the cells since DAPK siRNA transfection induced the cleavage of PARP, an apoptosis marker, in HHUA cells (Figure 1). It has been widely believed that DAPK positively and directly mediates apoptosis via the TNFR family members [3, 18, 28-30]. However, this study has shown that DAPK can negatively regulate Fas-mediated apoptosis in certain cancer cells. Our results also suggest that DAPK might have potential as a target of molecular targeting anticancer therapy.

HHUA cells express high levels of functional Fas antigen [17] as well as functional estrogen and progesterone receptors [27], similar to that in normal human endometrial epithelium. HHUA cells also form glandular luminal structures in collagen gel cultures, similar to the structures formed by normal glandular epithelial cells [31]. Analysis of 20 HHUA cells indicated that all cells had a normal 46XX karyotype [32]. Based on these characteristics, HHUA cells are considered to retain many of the intracellular signaling pathways found in normal endometrial epithelial cells. The present experimental data indicate that endogenous DAPK expression inhibits Fas-mediated apoptotic signaling in HHUA cells. There are several reports that DAPK plays cytoprotective roles in healthy cells under normal growth conditions [11, 12]. Therefore, our results may suggest the possibility that menstrual cycle-dependent changes in DAPK expression in the endometrium can also regulate normal endometrial epithelial cell survival or human endometrial remodeling. Since DAPK expression is often regulated by the methylation status of the DAPK promoter region in various cells [13, 20-25], some human endometrial remodeling may also be regulated epigenetically.

References


Immunological evaluation of vaginal secretion in patients with high-grade cervical intraepithelial neoplasia treated with intraligual interferon α-2b

M.C. Mardegan¹, M.C. Ramos², S.J. Adad³, M.A. Michelin⁴, D. Shimba⁵, E.F.C. Murta⁶

¹Gynecology and Obstetrics, UFTM ²General Pathology, Oncology Research Institute (Instituto de Pesquisa em Oncologia-IPON), Federal University of the Triângulo Mineiro (UFTM), ³Discipline of Special Pathology, UFTM, ⁴Oncology Research Institute (IPON)/Discipline of Immunology, UFTM, ⁵UFTM, ⁶Oncology Research Institute (IPON)/Discipline of Gynecology and Obstetrics, UFTM, Uberaba, Minas Gerais (Brazil)

Summary

Introduction: Conservative treatment with intraligual interferon (IFN) is a therapeutic option for cervical intraepithelial neoplasia (CIN) patients of childbearing age. Materials and Methods: The study group was made up of patients diagnosed with a high-grade lesion and treated with intraligual human recombinant IFNα-2b. Vaginal secretion was collected during IFNα-2b treatment for analysis of cytokines and viral load. Results: The initial histology diagnostic was 62.5% (n = 5) with CIN 2 and 37.5% (n = 3) with CIN 3. In terms of clinical evaluation and anatomopathology, 6.5% (n = 5) had a good clinical response, while 37.5% (n = 3) had therapeutic failure. All the patients with therapeutic failure were smokers. Interleukin 6 and tumor necrosis factor-α concentrations were raised at the sixth application for the patient group who failed to respond to therapy compared to the responsive group (p = 0.0357). Patients with a good response exhibited a reduction in human papillomavirus viral load (p = 0.03). Conclusions: Patients that had a good response had lower concentrations of inflammatory cytokines than did non-responders.

Key words: Human papillomavirus virus; Cervical intraepithelial neoplasia; Treatment; Interferon α-2b; Cytokines.

Introduction

Cervical cancer is a world health problem. Globally, it is the second most common tumor among women [1]. It is preceded by pre-malignant lesions, called cervical intraepithelial neoplasias (CINs), which can take years to evolve [2]. The development of these lesions and, consequently, of cervical cancer is intimately associated with the human papillomavirus (HPV) infection [3].

Cervical HPV infection is temporary for the majority (70-90%) of women infected, with the virus being eliminated 12-24 months after initial diagnosis. However, persistent HPV, principally in its oncogenic forms, is closely associated with the development of high-grade cervical lesions [4]. The virus’s persistence is closely linked to mechanisms that evade the host’s immune response [5, 6].

Cell mediated immune response, at systemic and local levels, is important to the course of HPV infection [7]. Evidence shows that the interaction of cytokines liberated during cellular and humoral immune responses is responsible for the remission, persistence, and progression of HPV-related lesions, although the mechanisms involved in these responses are not yet well understood [8].

Currently, treatment of high-grade lesions by ablative (laser) or excisional (cold conization, laser conization, loop diathermy) methods is recommended, with the former only being advocated following a satisfactory colposcopy [9]. However, various studies have noted an increase in obstetric complications in women who had previously received these procedures, with the most common of these being pre-term delivery, low birth weight, and premature membrane rupture [10-13].

Given the rising number of people infected with HPV and the appearance of CIN in young people [14], alongside the fact that women are having children later and later in life, the need to develop therapies that do not interfere with patients’ future reproductive capability has become pressing. Conservative treatment with intraligual interferon (IFN) could be a therapeutic option for women of childbearing age since it does not alter the cervix’s anatomy, which is a major factor in the development of complications during pregnancy.

Since the 1980s, various studies have used IFN for treating gynecological cancers with varying results [15]. With IFN treatment, studies have found remission of CINs in 30-80% of the cases [16-18]. With respect to invasive neoplasia, there are reports of curing invasive vaginal carcinoma using intraligual IFNα-2b [19].

Knowledge of the immunological changes that occur with interferon therapy is of fundamental importance in the development of strategies to treat cancer and its precursor lesions. To date, no study has evaluated the immunological changes in vaginal secretion caused by the intraligual use of IFN and the relationship these changes have with clinical response to treatment. The aim of this study was to analyze the concentration of cytokines in vaginal secretion, before and after treatment with intraligual IFNα-2b.
Materials and Methods

Patients

A prospective study was performed at the Oncology Research Institute (Instituto de Pesquisa em Oncologia, IPON) at the Federal University of the Triângulo Mineiro. The study group consisted of patients between 18 and 50 years of age with a diagnosis of CIN grades 2 and 3 who had not received prior treatment. Information about age, habits, and lifestyle (smoking, drug use, number of partners), and contraceptive methods used was gathered. Patients were advised to use condoms during their entire course of treatment. Patient identification was by number, with the first patient to participate in the study being labeled “1”, the second “2”, and so on.

The exclusion criteria were: immunosuppressant illness, serious cardiopathies or change in liver or kidney function; pregnancy; use of anti-inflammatory or immunosuppressants that could not be suspended during IFN treatment; reported intolerance of interferon; absence of a lesion visible by colposcopy or a very small lesion (diameter < 1 cm²).

The research ethics committee of the Federal University of the Triângulo Mineiro approved the study. All patients or their family members signed a free and clear consent form in writing.

Colposcopy and biopsies

The selected patients already had a biopsy that was positive for a high-grade CIN. Before the first and last treatment application, patients received a colposcopic examination; the images were photographed using a videocolposcope (Video Diagnose “Software” Program).

During the final application of interferon, a biopsy and a triple screen pap smear were performed. The biopsy was sent for colposcopy with the aid of a 24 cm, 2 mm Thomas Gaylor forceps. The fragment was embedded in a formaldehyde solution for anatomopathological study to confirm response to treatment.

IFN application

This study used 3,000,000 U of human recombinant IFN -2b (Blauferon B Blausiegel). The applications were made using a 1.0 ml syringe and a 13 x 0.45 needle three times a week on alternate days (Mondays, Wednesdays, and Fridays) for six consecutive weeks, making a total of 18 applications. Each application administered a dose of 3,000,000 U.

The cervix was exposed using a vaginal speculum, and antisepsis of the cervix and vaginal walls involved gauze soaked in topical povidone, using a Cherron forceps. The medicine was then applied. In the case of multiple lesions or those occupying more than one quadrant of the cervix, alternate applications were made on each lesion (in the case of isolated lesions) or in each quadrant (in the case of continuous lesions). During treatment, lactate dehydrogenase, hemoglobin, leukocytes, plaques, alkaline phosphatase, prothrombin time, and activated partial thromboplastin time, urea, creatine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured, since IFN can influence these factors.

Collection of vaginal secretion for cytokine dosage

Vaginal secretion was collected by introducing a cytology brush. After being introduced, the brush was rotated once at the bottom of the vaginal pocket. This was then placed in a 0.5 ml Eppendorf tube containing 0.3 ml of saline solution. After breaking the stem, the seal was closed, and the tube was shaken on a vortex for 1 min, turned upside down, and a hole was made in the lower part of the tube. The contents were carefully transferred to a 1.5 ml Eppendorf tube and subjected to centrifugation for 500 min at 300 g. After centrifugation, the first tube was thrown out, and the material was finally stored at −20°C for later measurement. Vaginal secretion was collected after the first, sixth, tenth, and eighteenth (final) application.

Flow cytometry

Detection of the IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, tumor necrosis factor (TNF), INF-γ molecules was by flux cytometry (Cytometric bead array) in a BD FACS Calibur TM flux cytometer, in accordance with the Becton Dickinson (BD CBA) protocol.

The BD CBA protocol uses the sensitivity of amplified immunofluorescence detection through cytometry to measure elements based on immunossay and allows for multiple detections within a small volume. Human Inflammation Kits (IL-8, IL-1β, IL-6, IL-10, TNF, IL-12) and the Th1/Th2 Kits (IL-2, IL-4, IL-6 IL-10, TNF, IFN-γ) were provided by Becton & Dickson (BD). The cytokines captured were incubated with antibodies and formed complex “sandwiches” that work in the BDA CBA, and the results were obtained in the form of graphs (as shown in Figures 1 and 2). Concentrations were obtained using the BD CBA software. The entire methodology was based on the product catalog.

Evaluation of high-risk HPV viral load

The hybrid capture technique was used for the HPV research. The entire collection procedure was performed according to the guidance of Digene of Brazil, which furnished the kits and equipment used in the hybrid capture test. The Captura Hibrida II System DML 2000 brand microplate system with signal amplification was used for chemiluminescence. The information and methodology described below conform to the instructions provided by Digene of Brazil. Samples were collected from all patients prior to the start of treatment and at the conclusion of the treatment.

Evaluation of clinical response

For clinical response criteria after biopsy, complete disappearance of a high-grade lesion, that is reduction of moderate (CIN 2) or severe (CIN 3) dysplasia to mild (CIN 1) dysplasia or no apparent lesion as confirmed by histological study, was considered a response. Therapy was considered to have failed when a high-grade lesion persisted or progressed. All patients who showed persistence of a high-grade lesion (CIN 2 or CIN 3) were immediately taken for complementary surgical treatment (loop diathermy or conization).

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 4 program and Microsoft Excel. Cytokine levels in each group of patients (responsive vs failed) were analyzed by the Friedman test, followed by Dunn’s test, to define the differences between samples (initial sample, 6th application, 12th application, and 18th application). The Mann-Whitney test was used to analyze differences between responses and failures in each sample. Wilcoxon’s test was used to compare viral load before and after treatment in the two groups. Statistical significance was established at p < 0.05.
Results

Eight patients participated in this study, with a minimum age of 22 years, a maximum age of 50 years, and a median age of 31.5 years. In terms of the habits and lifestyles queried in the initial protocol (parity, smoking, and number of sexual partners), 75% (n = 6) of the patients were multiparous, 62.5% (n = 5) were smokers, 62.5% had a history of three or more sexual partners, and the average age of sexarche was 16.75 years (minimum 14, maximum 18).

Initially, 62.5% (n = 5) of the patients were diagnosed with CIN 2 and 37.5% (n = 3) were diagnosed with CIN 3 (Table 1). Colposcopic findings of large lesions were observed in 100% of the patients; these lesions consisted of dense acetowhite epithelium (100%, n = 8), an irregular mosaicism (50%, n = 4), irregular punctuation (62.5%, n = 5), and atypical vessels (37.5%, n = 3). In patients with a good clinical response (62.5%), colposcopic analysis showed signs of lesion regression by the second week after commencement of the injections. The following resolution could be observed: fragmentation of the mosaic and gradual attenuation of the areas of dense acetowhite epithelium with their substitution by metaplastic squamous epithelium.

When clinical response was evaluated, 62.5% (n = 5) of the patients showed a good response, while 37.5% (n = 3) showed therapeutic failure. All three patients with therapeutic failure were smokers. In terms of the lesion size to clinical response, patients with lesions occupying more than one quadrant (n = 6) had a 50% (n = 3) response rate. Thus all patients with therapeutic failure had a lesion occupying more than one quadrant. Comparing lesion size to clinical response, patients with lesions occupying only one quadrant (n = 2) had a 100% response rate to treatment, whereas patients with a lesion occupying more than one quadrant (n = 6) had a 50% response rate and a 50% rate of therapeutic failure. Thus all patients with therapeutic failure had a lesion occupying more than one quadrant.

We observed a significant drop in HPV viral load in patients who responded to treatment (p = 0.0313 vs before treatment, Wilcoxon’s test) (Table 2). On the contrary, patients in whom the treatment failed showed an increase in viral load after IFN treatment. All eight (100%) of the patients experienced secondary effects from the medication, including myalgia, low fever (~38 ºC), and asthenia. These symptoms were confined to the day of application, beginning, on average, two hours after application and lasting for up to eight hours. In no case was it necessary to interrupt or suspend treatment. No patient showed altered liver, kidney, or coagulation function, and the examinations performed during treatment (lactate dehydrogenase, hemoglobin, leukocytes, plaques, alkaline phosphatase, prothrombin activation time, and activated partial thromboplastin time, urea, creatine, AST and ALT) showed no changes with treatment.

Table 1 shows the initial and final diagnosis (before and after treatment) of each patient, as well as their clinical response to treatment and the actions taken in each case. Patients with good response were sent for trimesterly tracking by the colposcopy outpatient service, while those who experienced treatment failure (n = 3), one underwent loop diathermy and two conization (with the treatment indicated based principally on the size of the lesion).

Table 3 shows the results of the cytokines analyzed in the vaginal secretion samples. In women with therapeutic failure, there was an increase in the average concentration of IFN-γ over the first three treatments and a decline at the time of the final application (p = 0.5243, Friedman test). In those with good response, the levels were essentially constant throughout the treatment (p = 0.6522, Friedman test). The patients with therapeutic failure were smokers.
failure had a more elevated average concentration of IL-10 in the first sample than patients who responded to treatment ($p = 0.5714$, Mann-Whitney test). During treatment, patients with failure had falling levels of this IL-10 ($p = 0.7274$, Friedman test), while those who responded maintained essentially constant levels ($p = 0.5610$, Friedman test). The average concentration of IL-6 was significantly higher at the sixth application in the patients with therapeutic failure ($p = 0.0357$, Mann-Whitney test). The average concentration of IL-4 stayed essentially constant in the patients with therapeutic failure ($p = 0.9097$, Friedman test) and in the good responders ($p = 0.1066$); a similar pattern was also observed with IL-2. Patients with therapeutic failure had very high concentrations of IL-1β throughout treatment ($p = 0.7274$, Friedman test), while among those who responded, IL-1β concentrations stayed lower and virtually flat ($p = 0.5206$, Friedman test). With respect to IL-12, no significant variation in concentrations were observed in either the patients whose treatments failed ($p = 0.6076$, Friedman test) or the good responders ($p = 0.8566$, Friedman test). A stable average IL-8 concentration was observed in patients with treatment failure ($p = 0.3420$, Friedman test) and falling levels were observed in the good responders ($p = 0.6522$, Friedman test). At the sixth application, the average concentration of TNF-α was significantly higher for the group with therapeutic failure than for group that responded well ($p = 0.0357$, Mann-Whitney test).

Discussion

Evidence shows that various factors increase the risk of HPV infection and the development of CIN or cancer, among them smoking, parity [20], the number of sexual partners and sexual activity before 25 years of age [21]. In our study, all of these factors were observed: 75% were multiparous (3 or more births), 62.5% of the patients were smokers, 62.5% had a history of three or more partners, and the average age of sexarche was 16.75 years old.

The use of IFN to treat CINs started in the 1980s, and perhaps because good clinical results were obtained at that time, most studies examining this therapy date from that decade. One study [16] administered perilesional IFNα and IFNβ to CIN patients and observed that IFNα induced complete remission of the lesion in 85.7% of the patients; of these, 55% occurred 12-24 months after treatment. With IFNβ, complete response occurred in only 40% of the cases. Similar results with the use of IFN were also obtained by DUNHAM et al. [17]. Another study [18], which used a methodology similar to ours, obtained complete response in 33% of the cases, partial regression in 58% of cases, and therapeutic failure in 8% of cases. The cure of a patient with invasive vaginal neoplasia using intralesional IFNα-2b has also been reported [19]. However, other studies [22-23] that applied IFNα gel topically and IFNα-2b intralesionally obtained results similar to a placebo.

In our study group ($n = 8$), we observed that 62.5% of the patients showed good response to treatment, with the high-grade lesion disappearing, while 37.5% of the patients had therapeutic failure, though we did not track the patients over a long period of time. In terms of colposcopic evidence of lesion regression, observed during treatment (fragmentation of the mosaic and gradated attenuation of the dense aceto-white areas of the epithelium with substitution by metaplastic squamous epithelium), our findings are similar to those of Choo et al. [16]. Those authors state that they observed changes from the third day of treatment onward, but in our study, we observed these changes later (after the second week of treatment). With respect to lesion size, our findings suggest that smaller lesions have a greater probability of complete response, but the presence of large lesions (those occupying more than one quadrant) does not mean the treatment is contraindicated since 50% of the patients with this type of lesion achieved therapeutic success.

The minor side-effects (fever, headache, myalgia and asthenia) that were observed in all of the patients in this study cohort were consistent with those in the literature [18]. We did not observe any major side-effects (changes in the central nervous system, cardiopathy, myelosuppression). Nor were there any cases of changes in hemogram, coagulogram, or liver and kidney enzymes.

Increases in the production of IL-10 and IL-4 may be a mechanism that tumor cells use to escape recognition by the immune system [24, 25]. Indeed, IL-10 and TGF-β inhibit the maturation of dendrite cells and can indirectly block the response of T cytotoxic lymphocytes [26]. The biological effects of IL-10 stem from its capacity to inhibit the functions of activated macrophages, in addition to inhibiting the expression of class II major histocompatibility molecules in macrophages, which, in turn, reduce the activation of T cells and cell immunity. IL-4, the principal stimulus for the development of Th2 cells, can prejudice IFN-γ’s effect on activating macrophages and thereby inhibit cellular immune reactions. In our study, higher initial concentrations of IL-10 were observed in patients with treatment failure. Although these concentrations fell during treatment, they remained elevated compared to the group of patients who responded well to therapy. While it has not been statistically proven, this pattern of observations suggests that higher levels of IL-10 may be related to treatment failure. Low levels of IL-4 were observed in all patients during treatment, with no significant difference in concentration between the responder and non-responder groups.

IL-6, which acts on both innate and acquired responses, was present in relatively high concentrations in patients whose therapy failed (on average > 500 pg /ml) and at lower levels in patients whose therapy was efficacious ($p \approx 0.357$ Mann Whitney test, after the sixth application). A recent study observed an increase in the concentrations of IL-6 and IL-8 in the vaginal secretion of CIN patients [27]. It has already been shown that the expression of IL-6 in cervical cancer is related to tumor size (> 2 cm) and that this expression promotes tumor angiogenesis and
encourages the development of cervical cancer [28]. In our study, all patients whose IFN therapy failed had a lesion that occupied more than one quadrant, which might explain the increase in the expression of this cytokine in this group.

An increase in the concentration of IL-8 in the vaginal secretion of patients with cervical cancer has already been observed, with this cytokine being considered pro-inflammatory [29]. Some evidence suggests that this IL-8 may play a fundamental role in angiogenesis and may be associated with advanced cervical tumors [30]. Although our study did not note a significant difference in the concentration of IL-8 between the groups (failed vs responsive), it did show higher levels of IL-8 concentration in both groups (on average > 4000 pg/ml), which is consistent with the literature [27].

Some studies have noted an increase in the concentration of TNF-α in patients with CIN [31, 32]. However, other studies [33-35] have observed a systemic decrease of the Th1 cytokines (IFN-γ, TNF-α, and IL-2), which is correlated to an increase in CIN grade. Scott et al. [4] suggest that persistent HPV infection leads to a failure to express Th1 cytokines. In our study, we observed low levels (on average < 15 pg) of IFN-γ, TNF-α, IL-2, and IL-12 in both patients whose therapy failed and in those who responded well to treatment. These findings reinforce the idea that a decrease in Th1 cytokines occurs in high-grade lesions, as all of our patients had a high-grade CIN diagnosis.

We observed that IL-1β levels were higher in the group with therapeutic failure than in the group that responded well to treatment, but they were not statistically significant. IL-1β is produced by different cell types, including keratinocytes, and acts on local inflammation. On the basis of the present study, we suggest that very high levels of this IL are a factor in the failure of intralesional IFN-γ. Further studies, involving a larger number of patients, are needed to better clarify the pattern of immune response involved in treatment with IFNα-2b, although thus far it seems likely that the levels of more inflammatory cytokines may correlate with intralesional IFNα-2b treatment failure than with treatment success.

Acknowledgment

We thank the CNPq, FINEP and FAPEMIG for support.

References

[25] Seo N., Hayakawa S., Takigawa M., Tokura Y.: “Interleukin-10 secretion of patients with cervical cancer has already been observed, with this cytokine being considered pro-inflammatory [29]. Some evidence suggests that this IL-8 may play a fundamental role in angiogenesis and may be associated with advanced cervical tumors [30]. Although our study did not note a significant difference in the concentration of IL-8 between the groups (failed vs responsive), it did show higher levels of IL-8 concentration in both groups (on average > 4000 pg/ml), which is consistent with the literature [27].

Some studies have noted an increase in the concentration of TNF-α in patients with CIN [31, 32]. However, other studies [33-35] have observed a systemic decrease of the Th1 cytokines (IFN-γ, TNF-α, and IL-2), which is correlated to an increase in CIN grade. Scott et al. [4] suggest that persistent HPV infection leads to a failure to express Th1 cytokines. In our study, we observed low levels (on average < 15 pg) of IFN-γ, TNF-α, IL-2, and IL-12 in both patients whose therapy failed and in those who responded well to treatment. These findings reinforce the idea that a decrease in Th1 cytokines occurs in high-grade lesions, as all of our patients had a high-grade CIN diagnosis.

We observed that IL-1β levels were higher in the group with therapeutic failure than in the group that responded well to treatment, but they were not statistically significant. IL-1β is produced by different cell types, including keratinocytes, and acts on local inflammation. On the basis of the present study, we suggest that very high levels of this IL are a factor in the failure of intralesional IFN therapy. Further studies, involving a larger number of patients, are needed to better clarify the pattern of immune response involved in treatment with IFNα-2b, although thus far it seems likely that the levels of more inflammatory cytokines may correlate with intralesional IFNα-2b treatment failure than with treatment success.


Address reprint requests to:
E.F.C. MURTA, M.D., Ph.D.
Oncology Research Institute (IPON)
Discipline of Gynecology and Obstetrics
Federal University of the Triângulo Mineiro (UFTM)
Avenida Getúlio Guaritá
s/n° Uberaba (MG), Brazil
CEP 38025-440, Bairro Abadia
e-mail: eddiemurta@mednet.com.br

23rd Conference of the Society
Medical Innovation and Technology
Tel Aviv, Israel - September 13-16, 2011

Target Conferences: Tel. +972(3)5175150 - Fax +972(3)5175155
E-mail: smit2011@targetconf.com - Website: www.smit2011.org
Importance of office hysteroscopy screening to diagnose endometrial carcinoma in menopausal women

A. Tripodi¹, C. De Salvo¹, C. Ermio², D. Manuzio¹, G. Romeo¹, P. Vadalà¹

¹Gynaecology and Obstetrics, “Bianchi-Melacrino-Morelli” Hospital, Reggio Calabria
²Gynaecology and Obstetrics, Lamezia Terme Hospital, Reggio Calabria (Italy)

Summary

Uterine cancer is today the upcoming neoplasia in gynaecological oncology. In Western countries endometrial cancer is mostly diagnosed after menopause and often becomes apparent with atypical uterine bleeding. Because of the great importance of such disease a series of accurate diagnostic analyses which require an adequate expenditure by hospital structures become necessary. Transvaginal sonography (TVS) remains the first choice to diagnose atypical bleeding because it is less invasive and highly bearable by the patients. TVS exam allows the selection of all patients who have an endometrial thickness more than 5 mm and/or with an inhomogeneous endometrial line thickness, who would then undergo further analyses. To achieve the diagnosis, office hysteroscopy carried out in an outpatient departments, is the most useful exam. Such exam allows a complete overview of the uterine cavity with possible detection of smaller lesions and a specific sampling of histological material. Hysteroscopy is today an indispensable aid in last resort diagnosis of endometrial cancer and is highly tolerated by patients.

Key words: Office hysteroscopy; Endometrial carcinoma; Menopause.

Introduction

In the last 20 years endometrial carcinoma has been the main genital female neoplasia after breast carcinoma. Such neoplasia strikes mostly women aged 55-65 years old. In the European Union there are 16 cases out of 100,000 women per year diagnosed (range 13-24).

Concerning mortality there are four to five deaths out of 100,000 cases per year [1]. The risk of developing endometrial cancer in a lifetime is 1.7-2% and the incidence rates due to age have increased in the majority of developed countries [2].

Every year in Italy around 5,000 new cases of endometrial carcinoma occur (15% of all neoplasias) in relation to 3,500 new cases per year of cervical cancer and gynaecological pelvic neoplasias [2].

By underlining such rates a reversal of the ratio between the two uterine neoplasias can be noticed.

The impossibility of a simple and systematic secondary prevention, as the one today performed for cervical carcinoma, and the intolerance and cost of more invasive tests of women justify the ongoing increase in endometrial carcinoma cases.

In addition, there is a probable increased population at risk: a) increased female population; b) increased longevity; c) increase of exogenous estrogens (HR therapy) in postmenopause which implies a likely related condition of hyperestrogenism.

Moreover environmental, biological and socio-cultural factors have been considered to be responsible for the growth and prognosis of uterine tumours. Ethnic differences have been noted, especially concerning the prognosis. There is, in fact, a higher incidence of endometrial cancer in Caucasian women, but a better survival rate compared to black women [3], even when groups of women of both races, received similar treatment [4].

The aim of this work was to evaluate whether office hysteroscopy is a valid screening method for early diagnosis of endometrial carcinoma in menopausal women.

Materials and Methods

The study was carried out from January 2005 to December 2008 in the outpatient department for menopausal women of the Gynaecology and Obstetrics “Bianchi-Melacrino-Morelli” Hospital in Reggio Calabria in cooperation with the Diagnostic Hysteroscopy Outpatient Department of the same Hospital.

We examined 95 women ranging in age between 46 and 69 (average 57.3 years old) whose last menstrual period dated back at least one year before they came to our department. Each woman had symptomatic for atypical uterine bleeding consisting of spotting or frank metrorrhagia and presented high risk factors for uterine neoplasia.

These patients were started on screening management for the early diagnosis of endometrial carcinoma.

The first step, after a very accurate analysis and a general and gynaecological exam, was an echography with transvaginal sonography (TVS) to measure the thickness of the endometrium.

An endometrium larger than 5 mm in thickness and/or with nonhomogeneous endometrial echopatterns indicated suspicion of uterine disease.

At a later stage these women received cytologic sampling with the endocyte and microcurettage on the four quadrants of the uterine cavity (Novak). At the end, office hysteroscopy was performed to inspect the uterine cavity including the tubal ostium, the site, and spread of the potential lesions.

Hysteroscopy performed in the outpatient department without any pharmacological treatment allowed us to evaluate the
endometrial features with the aim of achieving a macroscopic diagnosis of the lesion. In each case hysteroscopy was completed with a focused biopsy for surgical staging of the lesion.

Results

TVS allowed us to determine three groups of patients according to the thickness of the endometrial stripe (Table 1).

Of 95 patients in total we found:
- 18 women with endometrium < 5 mm in thickness;
- 30 patients with endometrium between 5 and 6 mm in thickness;
- 47 patients with endometrium > 6 mm in thickness.

For cytologic sampling using the endocyte we found no pathology in 18 patients with negative TVS (endometrium < 5 mm in thickness). In contrast, in 77 women with positive TVS (endometrium > 5 mm in thickness) the following were diagnosed:
- 22 hyperplasias, 13 simple glandular hyperplasias (SGH), six complex glandular hyperplasias (CGH), three atypical glandular hyperplasia (AGH);
- seven endometrial carcinomas, five of which had a stripe > 6 mm in thickness at TVS.

No pathology was found in the remaining 48 patients.

Histological evaluation results of the endometrial tissue sampled with microcurettage by Novak are shown in Table 2. Hysteroscopy was completed with a focused biopsy for histological diagnosis (Table 3).

The pathological exam showed the definitive diagnosis of atypical uterine bleeding (AUB), leading the patients to request medical consultations at our outpatient departments.

Hysteroscopy is a very accurate exam for early diagnosis of uterine diseases.

Discussion

According to clinical and epidemiological data, endometrial carcinoma can be divided in two pathogenetic categories: type I dependent estrogens and type II independent estrogens. Type I is represented by endometrioid adenocarcinoma which seems to arise on an endometrial hyperplasia substrate showing an inferior grade of differentiation [5]. Such neoplasia is clinically characterised by a favourable prognosis. It occurs most frequently in women without previous pregnancies [6], overweight/obese women, and in peri-postmenopausal age [7] where women are exceedingly exposed to exogenous estrogens (substitute non balanced estrogen therapy) [8] and/or endogens [9].

Eventual ovulation disturbances as polycystic ovary syndrome (PCOS) represent one of the most common causes of infertility in women predisposed to endometrial carcinoma [10, 11].

Diabetes mellitus and hypertension are related to obesity and could be considered as independent risk factors [8].

Substitutive estrogen therapy in perimenopause and menopause is the hexogen risk factor for hyperestrogenism. Protracted estrogen therapy, not balanced with progestosterone, increases the risk of endometrial hyperplasia and then frank cancer [12]. The risk increases with the growth of the duration of treatment and with the rise of dosage of estrogens.

Often an increase in the incidence of endometrial adenocarcinoma has been noted in women who have been operated on for breast cancer and who undergo adjuvant therapy with antiestrogens, among these SERMs (tamoxifen) have been the most investigated [13, 14].

Endometrial carcinoma becomes apparent most frequently with irregular vaginal bleeding (atypical uterine bleeding, AUB) in peri- or postmenopausal women, or in women with spotting in premenopausal age.
Importance of office hysteroscopy screening to diagnose endometrial carcinoma in menopausal women

Because of the lack of routine screening for endometrial carcinoma, when those symptoms appear, a great number of tests need to be performed to exclude the neoplasia [15].

Different diagnostic tools to evaluate the causes of AUB in pre-, peri-, and postmenopausal women are used: echotomography with TVS, endometrial cytology, sample of endometrial histological material (biopsy) and office hysteroscopy (outpatient diagnostic hysteroscopy).

As a first level examination, TVS is an excellent diagnostic instrument to evaluate patients with AUB. An endometrial stripe, whose average thickness in postmenopausal age is inferior/equal to 5 mm, can be detected through the TVS exam.

Many scientific works underline that diffuse endometrial thickening and/or irregular stripe patterns of echo structure can show malignancy even in asymptomatic women; in a few cases, postmenopausal AUB can occur in women whose endometrial thickness is inferior to 5 mm [16].

Although TVS is useful in screening endometrial carcinoma, also because of its favourable cost/benefit rate, other tools are needed to confirm the diagnosis. There are cytologic and histological tests that allow discovery of variations of endometrial gland cells which contribute to the diagnosis of a potential malignant lesion.

Historically the most common procedure has been cervical enlargement with fractional sounding, but today uterine material sampling can be obtained with different kinds of sounds or cannulas introduced into the uterine cavity: jet wash, cytobrush, endocyte, curette (Perma, Novak), Vabra aspirator, etc.

Cytological exams have been used for many years up to today because they are highly tolerated by patients due to the simple execution and also the the low cost.

The endocyte is a very good instrument in terms of diagnostic accuracy to sample cytologic material for first level screening of endometrial adenocarcinoma and is more favourable compared to the traditional cytologic diagnosis. It allows distribution of cells on a glass slide generally in a good state without any tainted material such as blood or mucus [17].

Moreover, Zarcone et al. reported the reliability of sampling with an endocyte comparing it in more than 90% cases with endometrial biopsy [18]. Nevertheless such sampling shows some limits essentially due to the “blind” technique and in some cases to the interpretation of the examined material.

Techniques based on the sampling of endometrial material by means of curette (Novak, Perma) or suction (Vabra) in uterine neoplasia diagnosis vary from 67% to 96%, when used as the unique diagnostic means [17].

With respect to endometrial cytology, with such techniques there is no problem of sample interpretation, but at the same time evidence of focal endometrial lesions is not always found.

Since the beginning of the 1980s hysteroscopy has been the preferred diagnostic procedure in menopausal women with AUB. Hysteroscopy is the most accurate diagnostic method to detect neoplastic pathology in women with AUB with endometrium larger than 5 mm in thickness. In addition it is efficacious in detecting focal endometrial anomalies which do not appear on echography, and those areas that can arise on an atrophic endometrial substrate [19]. Such method became essential for an early and accurate diagnosis of uterine neoplasias because of its minimal invasiveness and because it can be performed directly in outpatient clinics (office hysteroscopy) without anaesthesia, and at the same time it offers the possibility of performing a focused biopsy.

With diagnostic hysteroscopy sample material can be reached in all areas of the endometrial cavity, such as the tubal ostium, which cannot be reached with previous histological sampling techniques, where very often there are preneoplastic (hyperplasia) and cancerous lesions.

An endoscopic view helps in evaluating the features of the endometrium and the cervical canal, thus obtaining a macroscopic diagnosis of the lesion, and defining the site and the spread of the tumour in the uterine cavity.

<table>
<thead>
<tr>
<th>HISTOLOGICAL EXAM (95 PATIENTS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO EVIDENCE OF PATHOLOGY</td>
</tr>
<tr>
<td>(54 patients)</td>
</tr>
<tr>
<td>NO PATHOLOGY DIAGNOSED AFTER CYTOLOGIC EXAM</td>
</tr>
<tr>
<td>22 SGH</td>
</tr>
<tr>
<td>6 CGH</td>
</tr>
<tr>
<td>3 AGH</td>
</tr>
<tr>
<td>ENDOMETRIAL HYPERPLASIAS</td>
</tr>
<tr>
<td>(31 patients)</td>
</tr>
<tr>
<td>ENDOMETRIAL CANCERS</td>
</tr>
<tr>
<td>(10 patients)</td>
</tr>
</tbody>
</table>

Table 2. — Outcome of microcurettage and histological exam.
Conclusions

Irregular vaginal bleeding during menopause could be suspicious for endometrial cancer which can be accompanied with single or multiple risk factors. Thus early tests need to be performed in order to diagnose the cancer in early stage or precancerous.

Still today, unlike cervical uterine carcinoma, it is not possible to discuss a secondary prevention because there are no accepted routine screening methods to detect endometrial carcinoma.

In all clinical environments as, for instance ours, a diagnostic approach can be developed by using procedures with an acceptable cost-benefit rate in order to obtain a correct diagnosis in a short time.

Office hysteroscopy is a highly specific method and shows a very high diagnostic sensibility for uterine carcinoma in menopause.

References


Address reprint requests to:
C. DE SALVO, M.D.
Via Torrione, 6
89100 Reggio Calabria (Italy)
E-mail: descla85@hotmail.com
Copper and zinc concentrations in Nigerian women with breast cancer

G.O. Ajayi

Department of Obstetrics & Gynaecology, Prenatal Diagnosis and Therapy Centre, College of Medicine, University of Lagos, Lagos (Nigeria)

Summary

Trace elements are accepted to be involved directly or indirectly in the process of cancer formation. In this study, serum selenium, copper and zinc were measured in three groups of patients using atomic absorption spectrometer. A total of 29 Nigerian women were included: group I consisted of nine age-matched healthy controls without breast problems; group 2 included nine women with benign breast disease; and group 3 was comprised of women with breast cancer. The serum concentration of copper (Cu) was significantly higher in patients with breast cancer compared to the control group (1.43 ± 0.31 μg/ml vs 0.91 ± 0.18 μg/ml). The zinc (Zn) concentration was significantly lower in breast cancer patients than in the other two groups (0.74 ± 0.21 μg/ml vs 1.14 ± 0.31 μg/ml; p < 0.05). The study shows alteration in the concentration of copper and zinc in serum of patients with breast cancer, which may indicate abnormal copper and zinc metabolism in Nigerian females with breast cancer.

Key words: Zinc; Copper; Breast cancer; Nigerian women.

Introduction

Trace elements like zinc and copper are accepted to be involved directly and or indirectly in the process of cancer formation since they are important in activation and/or inhibition of enzymatic reactions which are important in many biological processes [1-12].

Several studies of either trace elements or oxidative stress in various clinical pathological conditions have been reported but none from Nigeria [1, 9, 12, 13]. Many Nigerian women are not able to afford a normal diet. Epidemiological studies in our environment have shown a high incidence of breast cancer patients at advance stages.

Therefore in this study, alterations in these trace elements were examined in the serum of Nigerian women with benign breast disease and breast cancer in comparison to healthy women.

Materials and Methods

A total of 27 patients presenting at the pre-pregnancy class of the Prenatal Diagnosis and Therapy Centre of the College of Medicine, Lagos – who after evaluation were transferred to the Gynaecological Outpatient Clinic of the Lagos University Teaching Hospital for further follow-up and therapy – were recruited for this study.

The ages ranged between 28 and 55 years. The women were divided into three groups. Venous blood samples were collected from all patients in serum separation tubes.

Group 1 consisted of nine age-matched healthy patients (control). In group 2 there were nine patients with benign breast disease diagnosed after fine needle biopsy or tissue biopsy by cytology or histopathology, and in group 3 were also nine patients with breast cancer diagnosed by histopathology.

The blood samples were centrifuged, and after separation stored at –20°C until assayed using an atomic absorption spectrophotometer (AAS).

Written informed consent was obtained from all patients participating in the study. Routine statistical methods (Student’s t-test, Newmann-Keuls procedure and Wilcoxon paired tailed test were used. Data are expressed as mean ± SD (standard deviation) and median ± SD.

Results

A significantly higher concentration of serum copper (p < 0.05) was found in patients with breast cancer (1.43 ± 0.31 μg/ml) compared with both patients with benign breast disease (0.91 ± 0.18 μg/ml) and age-matched healthy controls (0.94 ± 0.10 μg/ml). The zinc concentration was significantly lower in breast cancer patients than in the other two groups (0.74 ± 0.21 μg/ml vs 1.14 ± 0.31 μg/ml; p < 0.05). The study shows alteration in the concentration of copper and zinc in serum of patients with breast cancer, which may indicate abnormal copper and zinc metabolism in Nigerian females with breast cancer.

Discussion

All women in the breast cancer group showed a significant reduction in serum zinc concentrations and significantly higher serum copper concentrations when compared to both those with benign breast disease and age-matched healthy controls. These results confirm other reports which showed increased serum concentrations of zinc in patients with breast cancer [1, 13].

Trace elements have been shown to contribute to the antioxidant status although in this study no antioxidants were investigated [1, 2, 13].

Zinc has been shown to be present at active sites of some enzymes, particularly in the regulation of function and also in the modulation of growth of both normal and pathological cells [8]. Copper on the other hand is a
cofactor of many enzymes and has been shown to catalyze production of reactive oxygen species in abnormal oxidative conditions. Oxidative stress has been shown to play a role in various cancer types including breast cancers [13, 14] although the importance of antioxidants in human breast cancer has recently been highly discussed and remains unclear.

Although the results of this study indicate a relationship between serum concentrations of trace elements and breast cancer, there is still considerable debate on the role of antioxidant activity in cancer genesis.

To answer the question as to whether these changes are a result or cause of cancer, more studies are needed and the possibility of replacement therapy also has to be investigated.

References


Table 1. — Zinc and copper levels (ng/ml) in controls, benign breast disease and breast cancer.

<table>
<thead>
<tr>
<th>Type group</th>
<th>Zinc</th>
<th>Copper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>0.74 ± 0.21</td>
<td>1.43 ± 0.31</td>
</tr>
<tr>
<td>Benign breast disease</td>
<td>1.14 ± 0.31</td>
<td>0.91 ± 0.18</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>0.74 ± 0.21</td>
<td>1.43 ± 0.31</td>
</tr>
</tbody>
</table>

Table reprint requests to: G.O. AJAYI, M.D.
Department of Obstetrics & Gynaecology
Prenatal Diagnosis and Therapy Centre
College of Medicine
University of Lagos
P.M.B. 12003
Idi-Araba, Surname/Lagos (Nigeria)
e-mail: prenataldiagnosiscentre@hotmail.com
p16 and retinoblastoma protein expression in endometrial carcinoma and clinical significance

V. Mue Koh¹, Y.X. Shi², Q.H. Tang²

¹University of Douala, University Teaching Hospital Yaounde (Cameroon), ²Wuhan University, Hubei Medical Institute (China)

Summary

Objective: To investigate the clinical significance of p16 expression, a product of the cyclin dependent kinase inhibitor CDKN2 (also known as MTS1, multiple tumor suppressor 1) and assess its relationship with retinoblastoma protein expression in the pathogenesis of endometrial cancer. Method: p16 and pRb expression were histochemically evaluated, using p16 and RB polyclonal antibodies on paraffin sections of 27 primary endometrial adenocarcinomas with no therapy prior to surgery, through the streptavidin peroxidase conjugated method. Further analyses were carried out using the polymerase chain reaction for exon 1 gene amplification to investigate the mechanism of abnormal p16 expression. Result: p16 expression was detected in 100% of normal endometriums and in 74.04% of endometrial carcinomas (p < 0.05). This was significantly associated with tumor cell grade (p < 0.05). PCR analyses of exon 1 in five cases with no detectable p16 expression revealed four homozygous deletions. Additionally, the inverse correlation between RB and p16 expression was confirmed in this study, with 71.42% of tumors demonstrating inverse expression of p16 and RB (p < .005). Conclusion: p16 expression decrease is a significant event in endometrial carcinoma pathogenesis, and it is inversely correlated to tumor cell grade. Exon 1 homozygous deletion might be one of the mechanisms of loss of p16 expression. The p16/pRb growth suppressor pathway is targeted in human endometrial carcinoma.

Key words: Endometrial carcinoma; p16 protein; Retinoblastoma protein; Immunohistochemistry; Polymerase chain reaction.

Introduction

Cancer genesis and progression involve numerous factor interactions with the final result being activation of proto-oncogenes and/or inactivation of tumor suppressor genes. The multiple tumor suppressor gene 1 (MTS1), locus on the 9p21 chromosome band, has three exons which encode the p16 protein, whose molecular weight is 15,845 kD.

Since its discovery in 1994, its tumor inhibiting action has been widely proved in several malignant tumors including the ovary, brain, lung, bladder and skin malignancies [1]. In China and abroad, little has been done to investigate its relationship with endometrial carcinoma, the leading female genital tract malignancy in Western developed countries [1], the second in China after cervical cancer, and unlike in Cameroon, a disease still increasing throughout the whole world.

The cells of adenocarcinoma of the endometrium are mostly well differentiated, with a relatively low malignant degree compared to other gynecological cancers. It therefore shows a slower clinical pattern, revealing relatively obvious tumor suppressing factor potential, making this disease a strong candidate for evaluation.

Some studies have reported that malignancies that express a normal (wild type) Rb gene product are likely not to express the p16 gene product and vice versa, but this has never been done before with regard to endometrial cancer. This inverse correlation in protein expression between the Rb and p16 gene product is important in view of the known function of the p16 protein as an inhibitor of cyclin-dependent-kinase4 (CDK4)-mediated phosphorylation of pRb [2].

To investigate the relationship between p16 and Rb gene product expression and endometrial carcinoma, we examined 49 paraffin-embedded tissue sections, including 27 endometrial cancers, seven endometrial polyps and 15 normal endometriums. The polymerase chain reaction (PCR) technique was further used in five p16 protein expression immunohistochemically negative adenocarcinomas for exon 1 status to assess the bio-molecular mechanism of loss of expression.

Material and Method

Tissues

Twenty-seven surgically resected endometrial carcinoma specimens were fixed in 10% formalin, routinely processed and paraffin embedded. They were randomly selected from patients who underwent total hysterectomy between 1990 and 1997. Seven endometrial carcinoma specimens, 15 normal endometriums and seven polyp specimens were obtained from the first Teaching Hospital, two endometrial cancer specimens were from the second Teaching Hospital of Hubei Medical University, 14 cancer specimens were from the Cancer Hospital of Hubei Province and the remaining four cancer specimens were from the Xiehe Teaching Hospital of Wuhan. All were primary adenocarcinomas with no radiotherapy or chemotherapy prior to surgery, including 21 adenocarcinomas, 2 mucinous carcinomas, one papillary serous adenocarcinoma, two adenoacanthomas and one adenosquamous carcinoma. Tumor grade according to FIGO standard showed seven grade 1, 15 grade 2 and five grade 3.

Fifteen normal endometrial tissues (10 proliferative and 5 secretory phase) and endometrial polyp tissues (7) were also

Revised manuscript accepted for publication May 10, 2010
included, the former exclusively for p16 protein expression analysis. Those tissues also went through 10% formalin fixation, routine processing and were paraffin embedded.

The youngest patient was 41 years old and the oldest 71, with the mean age being 55, 53 ± 2.98.

Materials

Immunohistochemistry: Rabbit polyclonal anti-human p16 antibody (ref. RAB-0233) and rabbit anti-polyclone retinoblastoma antibody (ref. RAB-0186) were purchased from Fuzhou Maxim Biotech Inc. (Fuzhou, China) as was the large spectrum immunostain sp kit, including:

A: endogenous peroxidase blocking solution
B: normal (10%) non-immune serum
C: biotin-conjugated second antibody
D: streptavidin-peroxidase

The detection reaction also used the diaminobenzidine (DAB) kit and positive control slides (Fuzhou Maxim Biotech Inc’s).

Polymerase chain reaction

- SMMC (liver cancer cell line) as a negative control
- LD2 (normal liver cell line)
- Escherichia coli p16 recombinant plasmid DNA
- 10 x PCR amplification buffer (Promega Co.)
- dNTP (HUA MEI Co., Shanghai)
- 6 PCR DNA Markers (Sabc, China) maker size 1543, 994, 695, 515, 377 and 237, respectively.
- Gene Amp PCR system 1109 (Beijing New tech application research institute)
- TGL-168 centrifugator (Shanghaï ANHAO Co.)
- DYW for electrophoreses
- ZF-1 UV reflect and transmit analyze (Shanghaï Guang Electronic Apparatus Factory)
- Exon I (as described by Kamb et al.)
- S1 (Sense) 5’ GAAGAAAGAGGGGGCTG3’
- A51 (antisense) 5’ GCGCTACCTTATTCAATTC3’
- AS1 (antisense) 5’ GCGCTACCTTATTCAATTC3’

Immunohistochemical labeling for p16 and PRB proteins

1) All slides were submerged in basic and acidic media, respectively, for 24 hours, washed and put up to dry for 60 min at 60°C, and later on coated poly-L-lysine solution (10%), further dried again at 60°C for an hour.

2) Tissue sections fixed in 10% formaldehyde solution and embedded in paraffin were cut at 5 μm, placed on the above-mentioned slides, deparaffinized in graded alcohol, and then washed three times with phosphate buffered saline (PBS, pH7.4) at room temperature for 4 min.

3) After incubation with normal rabbit serum (37°C/10 min) to block non specific binding, the p16 group reacted with the p16 antiseraum at a 1:1200 dilution or with control immunoglobulin at 4°C overnight but there was no antigen retrieval, whereas Rb protein in which antigen retrieval was done through a microwave unmasking step (10–20 min in 0.01M citrate buffer, pH 6.0) method, reacted with Rb protein antibody at 1 mg/ml for one hour. Negative control slides were treated with non-specific rabbit IgG1 at equivalent conditions.

4) Sections washed with PBS (pH, 7.4) three times at room temperature for 4 min each time.

5) 50 μl of endogenous peroxidase blocking solution was added to each slide (reagent A) to deactivate endogenous peroxidase, and then left to incubate for 10 min at room temperature.

6) Step 4 repeated.

7) 50 μl of normal non-immune serum (reagent B) was added to each slide followed by incubation for 10 min at room temperature.

8) Step 4 repeated.

9) 50 μl of biotinylated second antibody (reagent C) was added to each sample with incubation for 10 min at room temperature.

10) Step 4 repeated.

11) 50 μl of streptavidin-peroxidase (reagent D) was added to all the samples followed by incubation for 10 min at room temperature.

12) Step 4 repeated.

13) 100 μl of newly made up DAB solution was dropped on each sample and then kept out of light at room temperature, and further washed 10 min later with water for 5 min. The sections were then counterstained with hematoxylin, washed again with water and mounted with a cover slip.

Positive staining criteria

p16 positive staining was cytoplasmic or nuclear dark yellow coloration. We counted 200 malignant cells and reactivity was graded as follows: negative if less than 10% were stained.

+ if 10~30% were stained
++ if 30~50% were stained
+++ if > 50% were stained.

pRb tumors were scored as pRb negative if all malignant cells had no nuclear dark yellow staining and pRb positive if any malignant cells had nuclear staining.

Nucleic acid analysis

DNA was extracted from deparaffinized p16 protein expression negative endometrial carcinoma samples, and in one pRb+/p16+ grade 1 adenocarcinoma and three normal endometria as a control, to perform exon 1 status assessment. PCR was performed using 5 μl of DNA in a 50 μl volume of 10X Taggense. Amp PCR buffer 8 μl, 25 pmol of sense and 25 pmol antisens primer, 10μM MOL/L dTNP 1μ of ampli Taq DNA polymerase and 2.5 μl DMSO. Then an amplification condition included an initial cycle of 5 min at 94°C, followed by 35 cycles of 30 sec at 94°C for denaturation, 55°C x 30 sec (annealing T°C) 30 sec at 72°C and final extension at 72°C 3 min for incubation.

PCR products (10 μl) and the above buffer solution were mixed and loaded onto 1.5% agarose gel for electrophoresis (80V), and visualized by ethidium bromide staining. Visible absence of a signal at the 340 bp site was interpreted as an Exon 1 homozygous deletion.

Statistics

The association between loss of p16 or pRb and categorical variables were analyzed by the chi-square test or Fishers exact test as appropriate. The significant level chosen was p < 0.05, and all tests were two-sided. All immunohistochemical studies and PCR procedures were done without knowledge of the clinical data.

Results

p16 protein expression in benign and malignant endometrial sections

Immunohistochemical analysis of p16 expression showed that all the 15 normal endometrial samples had high p16 expressions, no p16 protein was detectable in
Additionally, 57% (12/21) of p16 positive endometrial cancer sections had undetectable pRb protein; 19% (4/21) had lost both; 14% Rb positive had no p16 protein expression and in 9% (2/21) both could be identified. Fifteen cases (71.43%) showed an inverse correlation and this rate was statistically significant when compared to p16+/pRb+ and p16-/pRb (<0.05). This difference was not related to tumor grade or histological type (Table 5).

Exon 1 analysis

We chose five adenocarcinomas with no p16 staining and two p16 positive. The p16 negative group included two grade 2 and three grade 3 with one pRb positive mucinous adenocarcinoma. The p16 positive adenocarcinoma sample which was also pRb positive was grade 1. Four lacked exon 1 amplification and were all p16 negative, one grade 2 and three grade 3, including the pRb positive specimens. The fifth p16 negative sample, the only p16 positive adenocarcinoma, and all the three normal endometriums showed an electrophoresis band at the 340 bp site.

Discussion

Lack of p16 protein expression has already been shown to be a common event in a variety of human malignant tumors [3]. However since Takafumi et al. [4], using a PCR method for endometrial carcinoma allelotype analysis discovered that the chromosome band 9p21, locus of the p16 gene (also known as multiple tumor suppressor, MTS1, or CDKN2), was frequently involved, little has been done for the assessment of the role played by this gene in the pathogenesis of this frequent malignancy, and so far there is no known study of the p16 and pRb protein relationship in that process.

Recent analyses of mammalian cell-cycle machinery have implicated several key regulators, prominent among

---

### Table 1. Immunohistochemical analysis of p16 protein expression in benign and malignant lesions of the endometrium.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>n</th>
<th>p16 protein</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal endometrium</td>
<td>15</td>
<td>15</td>
<td>100–</td>
</tr>
<tr>
<td>Endometrial polyp</td>
<td>7</td>
<td>0</td>
<td>0–</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>24</td>
<td>17</td>
<td>71 0.030</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>21</td>
<td>15</td>
<td>71 0.030</td>
</tr>
<tr>
<td>Mucinous carcinoma</td>
<td>2</td>
<td>1</td>
<td>1–</td>
</tr>
<tr>
<td>Papillary serous adenocarcinoma</td>
<td>1</td>
<td>1</td>
<td>1–</td>
</tr>
<tr>
<td>Adenoacanthoma</td>
<td>2</td>
<td>1</td>
<td>100 NS</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1</td>
<td>1</td>
<td>100 NS</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>49</td>
<td>35</td>
<td>71.42</td>
</tr>
</tbody>
</table>

*PS: NS: non significant.*

### Table 2. Tumor grade and p16 protein expression.

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>n</th>
<th>p16 protein</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>6</td>
<td>85.71 NS</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>13</td>
<td>86.66 NS</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>1</td>
<td>20.00 &lt; 0.001</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>27</td>
<td>20</td>
<td>74.07</td>
</tr>
</tbody>
</table>

*PS: NS: non significant.*

### Table 3. Tumor grade and p16 protein expression.

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>n</th>
<th>p16 protein</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>22</td>
<td>19</td>
<td>86.36 –</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>1</td>
<td>20.00 0.0089</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>27</td>
<td>20</td>
<td>74.07</td>
</tr>
</tbody>
</table>

*PS: NS: non significant.*

### Table 4. Immunohistochemical analysis of Rb protein expression in normal endometrium and endometrial carcinoma.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>n</th>
<th>p16 protein</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal endometrium</td>
<td>15</td>
<td>0</td>
<td>0–</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>21</td>
<td>5</td>
<td>23.8 NS</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>19</td>
<td>5</td>
<td>5–</td>
</tr>
<tr>
<td>Adenoacanthoma</td>
<td>1</td>
<td>0</td>
<td>0–</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1</td>
<td>0</td>
<td>0–</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>26</td>
<td>5</td>
<td>19.23</td>
</tr>
</tbody>
</table>

*PS: NS: non significant.*

### Table 5. p16 and pRb protein expression correlation in endometrial carcinoma.

<table>
<thead>
<tr>
<th>p16 protein</th>
<th>Rb protein</th>
<th>Total</th>
<th>Inverse correlation</th>
<th>X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>–</td>
<td>12</td>
<td>14</td>
<td>71.42</td>
<td>4.27</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>5</td>
<td>16</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

Additionally, 57% (12/21) of p16 positive endometrial cancer sections had undetectable pRb protein; 19% (4/21) had lost both; 14% Rb positive had no p16 protein expression and in 9% (2/21) both could be identified. Fifteen cases (71.43%) showed an inverse correlation and this rate was statistically significant when compared to p16/pRb+ and p16-/pRb (p < 0.05). This difference was not related to tumor grade or histological type (Table 5).

### Exon 1 analysis

We chose five adenocarcinomas with no p16 staining and two p16 positive. The p16 negative group included two grade 2 and three grade 3 with one pRb positive mucinous adenocarcinoma. The p16 positive adenocarcinoma sample which was also pRb positive was grade 1. Four lacked exon 1 amplification and were all p16 negative, one grade 2 and three grade 3, including the pRb positive specimens. The fifth p16 negative sample, the only p16 positive adenocarcinoma, and all the three normal endometriums showed an electrophoresis band at the 340 bp site.

### Discussion

Lack of p16 protein expression has already been shown to be a common event in a variety of human malignant tumors [3]. However since Takafumi et al. [4], using a PCR method for endometrial carcinoma allelotype analysis discovered that the chromosome band 9p21, locus of the p16 gene (also known as multiple tumor suppressor, MTS1, or CDKN2), was frequently involved, little has been done for the assessment of the role played by this gene in the pathogenesis of this frequent malignancy, and so far there is no known study of the p16 and pRb protein relationship in that process.
them are cyclin and cyclin-dependent kinases (CDKs), whose sequential activation and deactivation regulate the cell cycle. It is believed that cyclin D, through association with CDK4 or CDK6, activates the cell cycle, especially in the mid/late G1 phase, most likely via intimate interplay with the retino-blastoma protein, stimulating cell cycle progression from the G1 to S phase. These kinases are in turn negatively regulated by several inhibitory polypeptides which include the p16 protein [5].

**p16 protein expression and endometrial carcinoma**

Our study illustrates the relationship between p16 protein expression and some clinicopathological characteristics. It appears that there is a significant loss of detectable p16 protein expression in the process of this malignancy. This decrease is higher with poor differentiation, especially with tumor grade 3, thus one interpretation of those data is that loss of CDN2 expression may be important in the pathogenesis of this cancer.

The role of histological types could not be properly apprehended because of the limited number of adenocanthoma and adenosquamous carcinoma samples (2 and 1, respectively) although adenocarcinoma p16 protein expression was significantly lower compared to normal endometrium (Table 1).

These results are similar to those of a recent study by Xumiao et al. [6]. They immunohistochemically analyzed 50 cases of endometrial cancer of which 48% were positively stained for p16 protein, while 100% of control tissues were stained. This decrease not only was present with worse tumor grade but was also associated with advanced surgical stage and poorer prognosis. They concluded that loss of p16 protein expression was rather a
late event in its pathogenesis. Due to fewer data for convincing staging, we could not confirm this assertion.

Four mechanisms are currently viewed as the cause of loss of p16 protein expression: MTS1 deletion, point mutation, CPG island methylation and down regulation.

CDKN2 deletion, the commonest mechanism, includes hetero and homozygous deletion. It has been widely proven in other malignancies [6-12] but due to fewer reports, there is a great variation of data as far as endometrial carcinoma is concerned [13]. Hatta et al. [13] using the Southern blotting procedure analyzed 15 cases but no single deletion could be detected. Another study by Peiffer et al. [14] using the same technique found an 8.8% (31/32) deletion.

In China, Shao et al. [15], using a polymerase chain reaction method, reported that 18.8% had homozygous deletion, four were adenocarcinomas, one adenocarcinoma, one adenosquamous carcinoma (all five being grade 3), and one grade 2 clear cell carcinoma. No grade 1 or benign tissue had a deletion. As in our study the four cases showing exon 1 deletion were grade 2 and 3: three adenocarcinomas and one mucinous carcinoma. The grade 1 p16 protein positive adenocarcinoma used as an internal control and normal endometrium showed normal exon 1 amplification.

These results show that CDKN2 deletion invariably affects different histological types. It was impossible to evaluate its relationship with surgical stage and prognosis due to lack of reliable data. Those important factors need to be focused on by further research, nevertheless, the fact is that MTS1 deletion, precisely Exon1 deletion in our case, is a cause of loss of P16 protein expression in endometrial cancer.

CDKN2 point mutation is also a regular feature in several human malignancies [3, 16], exon 1 and 2 being the mostly affected [17].

Mori et al. [18], using the PCR-SSCP procedure for exon 2 DNA sequence analysis reported that nearly half of esophageal squamous cell carcinomas (14/27) showed CDKN2 somatic mutation, among them, eight were frameshift mutations due to 1- 2- or 5-base pairs, and six were missence mutations involving various types of nucleotides. For example, a missence mutation with a GAC to AAC shift led to aspartic acid substituting for asparagine at codon 66, or deletion of one base pair at codon 97(GTGGACGTG to GTGACGTG); GCG to ACG missense mutation at codon 60 resulted in a change from alanine to thyrosine, etc.

Nagakawa et al. [19] using the same method analyzed 54 non small lung cancer (NSCLC) samples. Point mutation rate was 7% all in exon 2, characterized by G:C to T:A transversion on the coding strand. No mutation was found in tumor samples from patients with Stage I or II, compared to three of 23 (13%) tumor samples from Stage III and IV patients, and there was also no mutation in 28 primary tumors compared to 15 (20%) tumors from metastatic lesions. Two patients with no mutation in their primary tumor showed CDKN2 point mutations in metastatic lesions.

Subsequent DNA sequence analysis [14] of 34 endometrial carcinoma samples showed two point mutations. This mechanism is therefore believed to be a distinct pathway in loss of p16 protein expression.

De novo methylation of promoter region CpG islands, an area rich in CpG dinucleotides, has been increasingly associated with transcriptional inactivation of important genes in neoplasia [20, 21], including CDKN2 and it is as a matter of fact the only known CDKN2 aberration in colorectal cancer [22]. These CpG isands normally lack DNA methylation regardless of the expression status of the gene [23]. Promoter methylation when present is usually associated with irreversible inhibition of gene transcription [24] leading to transcription block of full length p16.
Decreased expression with no deletion, mutation or de novo methylation has recently been put forward [25] and is believed to be caused by down regulation by CDK4 gene amplification leading to relative decrease of p16 protein.

**p16 protein expression and endometrial polyps**

Our research showed total lack of p16 expression in all the seven endometrial polyp samples. The etiology of this pathology is unknown and its malignancy rate is 0.5 to 3.7% [26]. Epidemiologic analysis has shown that the probability of a patient with an endometrial polyp to contract endometrial carcinoma is twice the general population rate [27]. Endometrial polyps consist of projection of endometrial glands and stroma into the uterine cavity; 80% are inactive, not responding to circulatory hormones. Such polyps do not undergo cyclic changes, and they show fibrosis of the stroma and blood vessels leading to slow and chronic degeneration. They are askew, appear inactive and often cystically dilated as were most of our samples, with mitoses lacking [26].

This study was conducted on seven mostly inactive polyps. These are too few for any convincing conclusion, but whether lack of p16 protein expression is due to chronic degeneration of non functional endometrial cells remains to be fully investigated since they can not all have premalignant behavior, knowing the low malignancy rate of those lesions.

**p16 and pRB protein correlation in endometrial carcinoma**

The Rb gene, the first discovered tumor suppressor gene, is assigned to chromosome band 13q 11, and it encodes three nuclear phosphoproteins: the retinoblastoma tumor suppressor protein pRB and related p107 and p130. Most of the growth suppressive properties of pRb are mediated through interaction and modulation of the activity of transcription factors involved in cell cycle progression and cell differentiacion [28]. Alteration of the Rb gene can lead to uncontrolled cell proliferation and malignancy.

In our study, the pRb protein immunohistochemical detection rate was 23% and there was no statistical difference with the control group (Table 5). This conclusion is similar to Ambros et al’s [29], but due to the limited number of studies concerning pRB protein expression and endometrial carcinoma, the role of the Rb gene in its pathogenesis remains to be established.

Of the sections 71.42% showed an inverse correlation between p16 and pRb expression with a statistical significance, but were not related to tumor grade or histological type. This finding of the p16 and Rb gene alteration in a mutually exclusive way in endometrial carcinoma suggests that the gene products do interact. Therefore it shows strong evidence for the existence of a common pathway which includes both of these cell cycle regulators.

To investigate the mechanism of negative p16 expression and its link with this inverse correlation, Shapiro et al. [30] analyzed a series of non small cell lung cancer cell lines among which Rb positive had little or no detectable p16 protein and pRb negative had abundant p16 protein. They found that among nine Rb-positive cell lines, four had homozogous deletions, three had point mutations, and the remaining showed CpG island de novo methylation. Reintroduction of the p16 gene into cell lines by retroviral transfer resulted in increased abundance of hypo-phosphorylated Rb, reduced growth rate, and accumulation of cells in G1 which prevented S-phase entry of Rb/p16-cells, but not Rb deficient cell lines [31]. Additionally inverse expression of p16 and Rb protein was seen with increasing pathological stage in NSCLC [32].

These findings reveal that the mechanism of loss of p16 protein expression with regard to pRb status is an active, and yet classically well known one. The p16 protein seems to play an upstream role in counter balancing Rb proteins. They also suggest that Rb may be a major substrate for the inhibitory activity of the p16 gene product.

Our results show that 28.58% were either Rb+/p16+ or Rb-/p16-. There is no report investigating Rb gene status in pRb negative samples to clarify which role is active and which one is passive in this interaction. This pathway seems therefore to be more complicated than p16 and pRb interaction in a direct and mutually exclusive way.

The intimate mechanism is still not totally understood, but it is widely believed that it comprises Rb, p16 proteins, cyclin D and CDK4 and CDK6 which are very important for G1-S phase transition [33]. The proposed mechanism is: in specific conditions or situations, like loss of p53 function or its downregulation causing a p21 protein decrease, the cyclin D- and CDK4/6 complex are activated and phosphorylate pRb protein in the G1 phase; this phosphorylated form of one previously bound to transcription factors (such as E2F) pRb, releases the captured factors. The transcription of proteins needed for cell cycle progression follows. The result is subsequent entry of previously quiescent cells into the S-phase [34]. The decrease of Rb tumor suppressor ability leads to a compensatory p16 protein increase, which in turn effectively inhibits the complex cyclin D-CDK4/6, causing a negative feedback control over the pRb protein.

This theory still can not completely explain why some samples were p16+/Rb+ or p16-/Rb-, suggesting that this interaction might be more complicated than our understanding so far, at least in the case of endometrial carcinoma.

**Conclusions**

We used an immunohistochemical method to analyze 49 paraffinized sections of normal endometrium, endometrial carcinoma for p16 and pRb protein detection, and endometrial polyps for p16 protein evaluation. We went on with further analyses using polymerase chain reaction for exon 1 amplification in six cancer tissues.
The findings indicate that there is a decrease in multiple tumor suppressor gene product p16 protein expression in the pathogenesis of endometrial cancer and it is related to poorer tumor cell grade.

p16 and pRb protein expressions are inversely correlated, showing that they interact in a common regulatory pathway.

Exon 1 homozygous deletion is one of the mechanisms of p16 gene inactivation in endometrial carcinoma.

References


Recombinant human endostatin, Endostar, enhances the effects of chemo-radiotherapy in a mouse cervical cancer xenograft model

Y. Jia¹, M. Liu¹, L. Cao², X. Zhao¹, J. Wu¹, F. Lu¹, Y. Li², Y. He³, S. Ren¹, Y. Ju³, Y. Wang⁴, Z. Li⁶

¹Oncology Department, Hebei General Hospital, Shijiazhuang, Hebei
²Department of Histology and Embryology, Hebei Medical University, Shijiazhuang, Hebei
³Centre of Animal Experiments, Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei
⁴Department of Radiotherapy, Fourth Hospital, Hebei Medical University, Shijiazhuang, Hebei
⁵Cancer Institute of Hebei Province, Shijiazhuang, Hebei
⁶Second Department of Surgery, Fourth Hospital, Hebei Medical University, Shijiazhuang, Hebei (China)

Summary

Background: The effects of recombinant human endostatin, Endostar, combined with concurrent chemo-radiotherapy (CCRT) on tumor growth, angiogenesis and lymphangiogenesis in a mouse xenograft model of cervical cancer was investigated. Methods: HeLa cells were injected subcutaneously to establish mouse xenograft models and mice were treated with normal saline (control), CCRT with cisplatin (CDDP), Endostar, or a combination of Endostar and CCRT. Growth, metastasis, and angiogenesis of tumors was monitored. Results: Tumorogenic activity of tumor cells in the CCRT, Endostar and combination Endostar-CCRT treatment groups was markedly decreased compared with the activity in the NS group (p < 0.05). The most significant inhibition of tumor growth was observed in the Endostar with CCRT group. Lymph node metastases in the Endostar with CCRT group (12.5%) and Endostar alone group (25%) were lower compared to the CCRT group (42.8%) and NS group (66.7%; p < 0.05). Endostar was also found to inhibit tumor angiogenesis. Endostar induced apoptosis of HeLa cells in vitro, and inhibited expression of VEGF and HIF-1α in vivo and in vitro. Conclusion: Endostar enhanced the anti-cancer effect of CCRT in a mouse xenograft model of cervical cancer. These findings thus provide a new strategy to treat cervical cancer.

Key words: Angiogenesis; CCRT; Lymphangiogenesis; Recombinant human endostatin; Cervical cancer.

Introduction

Cervical cancer is the second most common malignant fatal disease among women worldwide. The morbidity of cervical cancer is still on the rise. Current conventional therapies after surgery include either radiation alone or concurrent chemo-radiotherapy (CCRT) using cisplatin (CDDP) [1, 2], however, these therapies have proven to be relatively ineffective and have significant side-effects [3, 4]. Therefore it is necessary to develop new approaches for cervical cancer treatment.

It has been established that angiogenesis (the generation of new blood vessels) occurs in the microenvironment of many solid tumors. Angiogenesis is controlled by angiogenic factors. Among these factors, vascular endothelial growth factor (VEGF) is crucial for angiogenesis, vascular permeability, and metastasis during tumor development [5]. HIF-1α is a hypoxia-induced transcription factor that regulates gene expression in critical pathways involved in tumor growth and metastasis, and it is a major trigger for the “angiogenic switch” [6]. Production of VEGF in tumor cells is mediated by HIF-1α activation [7]. Some cervical adenocarcinomas have been shown to be enriched in expression of VEGF and HIF-1α. Moreover, ectopic expression of HIF-1α could enhance the resistance of human cervical cancer HeLa cells to radiation [8-10].

Anti-angiogenic therapy has recently emerged as a treatment of malignant diseases. Bevacizumab (anti-VEGF antibody) has been reported to be able to shrink cervical tumors and delay tumor progression and are therefore being studied in a Gynecologic Oncology Group phase III trial. Other intracellular tyrosine kinase inhibitors of angiogenesis are also promising. Further studies of angiogenesis and its inhibition are ongoing [3]. endostatin is another type of tumor angiogenesis inhibitor, and has demonstrated inhibition of VEGF expression [11]. A synergistic effect has been shown to occur between Endostatin and cytotoxic medications [12]. Researchers have recently shown that endostatin also sensitizes established tumors to radiotherapy [13].

Endostar, a novel recombinant human endostatin, which is expressed and purified from E. coli, is also an inhibitor of angiogenesis. It was approved by the State Food and Drug Administration of China (SFDA) for the treatment of non-small cell lung cancer in 2005 [14]. The effects of Endostar on cervical cancer were not well characterized at the start of this study, and it is not clear whether Endostar combined with CCRT would have the most effect on cervical cancer.

In the present study, we established a cervical cancer xenograft mouse model and evaluated the effect of Endostar combined with CCRT on tumor growth, metastases, and angiogenesis, with the goal of providing a new prospect for targeted therapy of cervical cancer.
Materials and Methods

Mice

Nude mice (BALB/c nu/nu, 5-6 weeks old), obtained from the Institute of Laboratory Animal Science of the Chinese Academy of Medical Sciences (Beijing, China), were used for these studies. Animals were housed under specified pathogen-free conditions. Animals received sterile rodent chow and water ad libitum. Animal experiments were conducted in accordance with Institutional Animal Care and Use Committee guideline, which has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Cell culture

A luciferase-expressing human cervical tumor cell line HeLa (a gift from Longmed Incorp., USA) was maintained in Minimum Essential Medium (Gibco, US) supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO2. When cells reached 90%-95% confluence, they were trypsinized and resuspended in passage medium at 2.5 × 10^6 cells/ml. All cells were maintained by serial subcultivation.

Mouse xenograft models

HeLa cells were briefly treated with trypsin-EDTA and washed twice with serum free medium. Single-cell suspensions (5-6 × 10^6 cells in 200 μL serum-free medium) were injected subcutaneously into the right hind flank of the mice. Tumors were allowed to grow untreated until they reached 7 mm in diameter, and then mice were randomly divided into four experimental groups (n = 8). Group 1 (control group) was treated with normal saline (NS) (200 μl for each mouse) by subcutaneous injection around the tumor every other day. Group 2, (CCRT group) was treated with CDDP (5 mg/kg) by intraperitoneal injection every three days and by radiation, with the mice immobilized on a platform 100 cm from the radiation source. The vertical beam was adjusted to project a 5 × 5 cm field and the tumor-bearing leg was extended into this field. Approximately 20 Gy of X-rays were delivered per fraction at a dose rate of 2 Gy/min. The radiation was given only once at the beginning of the treatment regimen. Group 3 was treated with Endostar (10 mg/kg) by subcutaneous injection around the tumor every other day. Group 4 (Endostar+CCRT group) mice were treated with Endostar combined with CCRT. Endostar treatment was initiated two days before CCRT. All these treatments were maintained for four weeks.

Xenogen IVIS Imaging System

After one week, tumors were around 7 mm in length, at which point the mice underwent initial imaging. Before imaging, mice were given a 150 mg/kg dose of D-luciferin (Xenogen) by intraperitoneal injection. Mice were imaged 15 min after luciferin administration using the IVIS system to determine total flux (photons/sec) of emitted light as a measure of the relative number of viable tumor cells in the tumor. All experimental mice were imaged once a week in this way. Data were analyzed using Xenogen Living Image Software.

Tumor and tissue sample harvesting

A week after the treatment regimen completed, all mice were weighed and then sacrificed by application of chloral hydrate anesthesia. A complete autopsy, including lymph node dissection, was performed. The samples were evaluated by two pathologists in a double-blinded manner on the basis of hematoxylin and eosin stained sections, and the autopsy samples were evaluated histopathologically for the presence of metastases.

Determination of blood vessel density

To visualize tumor blood vessels, CD31 was detected by immunohistochemistry. Sections were stained with anti-CD31 antibody (rat anti-mouse monoclonal antibody, 1:200, ebioscience, Inc. US). Biotinylated anti-rat antibody (Beijing Zhongshan-Golden Bridge Biological Technology CO. Ltd, Beijing, China) was used as a secondary antibody. Three or four fields per tumor were scored for tumor microvessel elements at x200 magnification by two double-blinded observers, and tumor microvessel densities (i.e., number of microvessel elements/field) were calculated.

Detection of tumor cell apoptosis

Apoptosis was analyzed by triphosphate nick end labeling (TUNEL) for tumor cells. TUNEL reaction solution was prepared by mixing the enzyme solution (with TdT) and labeling solution (with Biotin-dUTP) at a ratio of 1:9, and staining was carried out according to the manufacturer’s manual. Five slides were selected from each group and the number of apoptotic cells per field of view were counted in ten fields of view per treatment group. The apoptosis rate was calculated based on the formula: AI (%) = apoptotic cells/total tumor cells × 100%.

VEGF and HIF-1α expression in vivo and in vitro

Expression levels of VEGF and HIF-1α in the four groups were detected by immunohistochemistry. 5μm thick sections were deparaffinized and rehydrated using graded alcohol concentrations. Sections were incubated with anti-VEGF antibody (rabbit anti-human polyclonal antibody, 1:100; Santa Cruz, US) or anti-HIF-1α antibody (rabbit anti-human polyclonal antibody, 1:200,
Upstate, Inc. US) at 25°C for 48 h. The optical density value of tumor cells that stained positive for VEGF and HIF-1α were examined with a Motic 6.0 digital medical image analysis system (Motic Inc, China).

Expression levels of VEGF and HIF-1α in vitro were determined by Western blotting with actin as an internal control. Proteins were separated on a 10% SDS-PAGE gels and transferred onto PVDF membranes. Equal loading and transfer of proteins was confirmed by Ponceau S staining the membranes. The membranes were incubated overnight at 4°C with specific primary antibodies against VEGF and HIF-1α, respectively. Control membranes were incubated in PBS without primary antibodies. After incubation with primary antibodies, the membranes were

Figure 2. — Changes in the activity of tumor cells as evaluated by Xenogen IVIS Imaging System in vivo.
Recombinant human endostatin, Endostar, enhances the effects of chemo-radiotherapy in a mouse cervical cancer xenograft model

incubated with secondary antibodies conjugated to horseradish peroxidase (anti-rabbit, 1:20,000; Santa Cruz) and immunoreactive proteins were detected by the enhanced chemiluminescence system (ECL, Sigma) using serial exposures on radiographic film (Hyperfilm ECL, Amersham International, UK). The blots were analyzed and quantified by MCID imaging software (Imaging Research Inc., St. Catharines, Ontario, Canada).

Statistical analysis

Differences in metastasis formation between the groups were analyzed using a \( \chi^2 \) test and confirmed with the Fisher’s exact test. The other data were expressed as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to assess the statistical significance of differences between groups using SPSS 15.0 software; \( p < 0.05 \) was considered significant.

Results

During the treatment regimen, two mice in Group 1 (control group) and one mouse in Group 2 (CCRT group) died. All of the other mice survived until the experiment was completed.

Tumor growth evaluated by the Xenogen IVIS Imaging System

In order to determine the viability of tumor cells, photons emitted by luciferase expressing tumors were quantified by the Xenogen IVIS Imaging System, and tumor growth curves were plotted. All experimental mice were imaged once a week. The photons of Groups 2, 3 and 4 were markedly decreased when compared with the control group (\( p < 0.05 \)). In these groups, the most significant inhibition of tumor growth was observed in Group 4 (Endostar+CCRT group) (\( p < 0.05 \); Figures 1 and 2).

Effects of Endostar on lymph node metastasis

Enlarged lymph nodes were found at the inguinal and axis area. By hematoxylin and eosin staining, these enlarged lymph nodes were confirmed to be metastatic lesions. Lymph node metastases of Endostar+CCRT group and Endostar group were 12.5% (1/8) and 25% (2/8), respectively, which was markedly lower compared with the CCRT group (42.8%, 3/7) and the control group (66.7%, 4/6; \( p < 0.05 \); Figure 3).

Angiogenesis in a mouse xenograft model

Immunohistochemical detection of CD31 was used to visualize the tumor blood vessels (Figure 4a, b, c, d). A significant reduction in intratumor microvessel density was observed in mice that received either Endostar therapy alone (10.82 ± 0.34) or the combination of Endostar+CCRT (6.36 ± 0.47) when compared with animals in the CCRT group (15.09 ± 0.94) and in the control group (20.09 ± 0.91) (\( p < 0.05 \)). Furthermore, the density of blood vessels in the Endostar+CCRT group was markedly reduced compared to the Endostar group (\( p < 0.05 \); Figure 5).

Apoptosis of tumor cells in vivo

The evaluation criteria were as follows: with diaminobenzidine (DAB) as the substrate, cells that stained brown in the nucleus were considered positive.
False-positive results were observed in a few necrotic cells, which had a lighter stain. The apoptosis rate in the CCRT group, Endostar group, and Endostar+CCRT group were 8.01 ± 0.48%, 11.08 ± 0.46% and 19.58 ± 1.17%, respectively. These values were higher than the value exhibited by the control group, 3.56 ± 0.30%. Moreover, the apoptosis index of the Endostar+CCRT group was significantly higher than the CCRT group and Endostar group ($p < 0.05$; Figures 6 and 7).

VEGF and HIF-1α expression in vivo and in vitro

To investigate the anti-angiogenesis mechanisms of Endostar, we further evaluated the effect of Endostar on the expression of VEGF and HIF-1α in vivo and in vitro.

In tumor tissues, VEGF was expressed in the cytoplasm while HIF-1α was expressed in the cytoplasm and nucleus. The optical density value of VEGF positive staining cells in the Endostar group and Endostar+CCRT group (0.126 ± 0.003, 0.118 ± 0.008, respectively) decreased significantly compared with CCRT group and control group (0.295 ± 0.012, 0.282 ± 0.007, respectively) ($p < 0.05$) (Figures 6 and 8). The optical density value of HIF-1α positive staining cells in the control group, CCRT
Recombinant human endostatin, Endostar, enhances the effects of chemo-radiotherapy in a mouse cervical cancer xenograft model

Figure 6. — Representative staining for apoptosis (TUNEL), and VEGF, HIF-1α (immunohistochemistry) in mouse xenograft tissues of cervical cancer (× 400).

Figure 7. — Comparison of apoptosis rates in mouse xenograft tissues of cervical cancer.

* p < 0.05; ** p < 0.01 vs the control group.

Figure 8. — Expression levels of VEGF in mouse xenografts tissue of cervical cancer.
The expression of VEGF in Endostar+CCRT group and Endostar group was significantly lower compared with the control group and CCRT group.

* p < 0.05 vs the control group or the CCRT group.
Y. Jia, M. Liu, L. Cao, X. Zhao, J. Wu, F. Lu, Y. Li, Y. He, S. Ren, Y. Ju, Y. Wang, Z. Li

322
group, Endostar group and Endostar+CCRT group was 0.181 ± 0.008, 0.223 ± 0.017, 0.132 ± 0.008, and 0.138 ± 0.006, respectively. Endostar inhibited the expression of HIF-1α significantly (p < 0.05) (Figures 6 and 9).

In HeLa cells, expression of VEGF and HIF-1α in the Endostar group was 1.3-fold and 1.26-fold lower than in the control group, respectively (Figure 10).

Discussion

In the present study, we found that Endostar enhanced the effects of chemo-radiotherapy in a mouse xenograft model of cervical cancer. Endostar inhibited angiogenesis and induced apoptosis in tumors. Endostar also inhibited expressions of VEGF and HIF-1α in vivo and in vitro.

Cervical cancer is the second most common cause of cancer-related mortality globally among women, causing approximately 234,000 deaths annually in developing countries and 40,000 deaths in developed nations [1]. Most of these deaths occur in women with bulky or locally advanced cervical cancer. Current therapies used after surgery include either radiation alone or CCRT using CDDP. When patients have inoperable masses, CCRT treatment followed by taxol plus CDDP is necessary [1, 15]. Unfortunately the common treatment modalities used for cervical cancer, including surgery, chemotherapy and radiation, are still insufficient to eliminate tumor burden. It is therefore necessary to develop new approaches for the treatment of this disease.

It is well known that angiogenesis is essential for the development, growth and advancement of solid tumors, including those associated with cervical cancer. HeLa cells were originally derived from a patient with cervical adenocarcinoma. However, the manner of tumor progression, response to conventional therapy, clinical prognosis, and angiogenic factors involved in adenocarcinoma are all very different from those associated with squamous cell carcinoma. For example, the levels of VEGF protein expressed in adenocarcinomas were significantly increased compared to those occurring in squamous cell carcinomas of the uterine cervix [8, 16]. In contrast, the expression of platelet-derived endothelial cell growth factor (PD-ECGF) in squamous cell carcinomas was significantly higher than that in adenocarcinomas [17].

Endostar is a novel recombinant human endostatin expressed and purified in E. coli. It differs from endostatin in that it has an additional nine-amino acid sequence with zinc binding activity that increases Endostar’s half-life [18]. The current data revealed that Endostar might exert anti-angiogenic effects via a similar mechanism as endostatin. However, the role of Endostar in the treatment of cervical tumors is still unclear.

We attempted to analyze the roles of Endostar in xenograft tumors in nude mice. We found that Endostar combined with CCRT resulted in delayed growth of tumors compared to either CCRT or single Endostar treatments. Apoptosis is one of the major forms of tumor death after radiation therapy, and in the present study it was found that Endostar could enhance the apoptotic induction effect of CCRT on tumor cells. Previous studies have shown that tumor cell apoptosis may occur directly by radiation-induced DNA damage to tumor cells or indirectly by endostatin and radiation-induced damage to endothelial cells [19]. On the other hand, radiotherapy also up-regulated the expression of VEGF in tumor tissue, resulting in rapid regrowth of the tumor [20, 21]. A combination of radiotherapy with Endostar can efficiently antagonize this effect. The ability of Endostar to suppress the rapid regrowth of cervical tumors postradiotherapy is consistent with previous reports [8, 22, 23].

In our study, we found Endostar reduced the blood vessel density in cervical tumor tissue. Previous studies have indicated that the growth and metastasis of tumors depend on circulation and vascularization, and tumors exceeding 1-2 mm in diameter halt their growth once there is no longer support from de novo blood vessels [24]. Recent studies showed expression of VEGF in many tumor cells is regulated by the activated HIF-1α mediated system. An increase in VEGF level subsequently promotes tumor growth and metastasis by angiogenesis [25-28].
While the mechanism for Endostar to sensitize tumors to chemotherapy and radiotherapy is currently unknown, it is clear that Endostar works in multiple ways. Sensitivity to radiation and chemotherapy can be influenced by factors extrinsic to the cancer cell. Apart from the tumor cells, neighboring cells such as endothelial cells may influence radiosensitivity [29, 30]. Researchers have found that expression of HIF-1α in patients with advanced cervical cancer correlates to the oxygenation of tumor cells, and its expression level can be considered as a biological index of hypoxia in cervical tumor cells, thereby predicting the sensitivity of cancer cells to radiation [9]. It has been reported that the level of VEGF directly corresponds to the resistance of the tumor to irradiation [31]. Some researches have shown that inhibition of HIF-1α and VEGF can not only reduce angiogenesis, but also enhance the sensitivity of tumor cells to radiotherapy and cytotoxic agents, including CDDP [32, 33]. More recently, it was reported that knocking-down HIF-1α could increase the sensitivity of HeLa cells to radiation [10]. Ohnuma and colleagues found that downregulation of VEGF enhances the effects of radiation on tumor growth and tumor-associated angiogenesis [34].

In addition, some researches have shown that Endostar also enhanced the sensitivity of tumor cells to radiation by inhibition of cellular proliferation, inducing apoptosis, and redistribution of cell cycles in human nasopharyngeal carcinoma and human lung adenocarcinoma xenografts [35, 36]. Previous research has shown that cells in the G1 and G2/M phase exhibit moderate to high sensitivity to radiation [34]. Some studies have shown that Endostar arrested the cells in the G0/G1 and decreased the S phase cells. The decreased number of S cells and increased G0/G1 cells might improve the efficacy of radiotherapy. Cells were most vulnerable to CDDP during the G1 phase, which may be one of the reasons for Endostar-induced sensitivity to chemotherapy [38]. It is necessary to examine whether or not Endostar has any influence on cell cycles of cervical cancer cells.

In summary, using a mouse human cervical cancer cell xenograft model, we have confirmed that Endostar enhanced the anti-tumor effect of CCRT. Our findings thus provide a new strategy to develop novel treatments for cervical cancer.

Acknowledgements

We thank Dr. James S. Martin (School of Medicine, University of Pennsylvania) for preparing the article. We also thank Dr. Jingguo You for the technical assistance. This research was supported by the Natural Science Foundation of Hebei province of China (05547008D-3).

References


Recombinant human endostatin, Endostar, enhances the effects of chemoradiotherapy in a mouse cervical cancer xenograft model 323


Address reprint requests to:
Z. LI, M.D., Ph.D.
The Second Department of Surgery of the Fourth Hospital
Hebei Medical University
Shijiazhuang, Hebei (China)
e-mail: lizhongxin99@yahoo.com.cn
Lymphoepithelial-like carcinoma of the uterine cervix; a case report


Department of Obstetrics and Gynecology, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto (Japan)

Summary

Lymphoepithelioma-like carcinoma (LELC) of the uterine cervix is a rare variant of squamous cell carcinoma of the uterine cervix. This tumor is characterized by nests of poorly differentiated epithelial cells surrounded by a prominent lymphocytic infiltration. Despite the poorly differentiated pathological findings, it appears to have a better outcome than the usual squamous cell carcinoma of the uterine cervix. Therefore, it is quite important to differentiate this tumor from poorly differentiated squamous cell carcinoma and lympho-proliferative disorders of the cervix. LELC arising from the nasopharynx has been suggested to be associated with Epstein-Barr virus (EBV), whereas the involvement of EBV in LELC of the uterine cervix is still controversial. In addition, the role of high-risk human papilloma virus (HPV) in this type of tumor remains unknown. We report a case of LELC of the cervix with diagnosis on the basis of histopathology in a 52-year-old Japanese woman who presented with a history of continuous bleeding post menopause. We also examine the association of EBV and HPV in this case.

Key words: Lymphoepithelial-like carcinoma; Uterine cervical cancer; EBV; HPV.

Introduction

Lymphoepithelial-like carcinoma commonly occurs in the nasopharynx. This tumor is characterized by nests of poorly differentiated epithelial cells surrounded by a prominent lymphocytic infiltration. Similar tumors, which have been termed by lymphoepithelial-like carcinoma (LELC), have been reported in several organs including the salivary gland, thymus, stomach and uterine cervix [1, 2]. LELC of the uterine cervix is a very rare variant of squamous cell carcinoma and appears to have a better outcome than the usual SCC of the cervix. Therefore, it is quite important to differentiate LELC from poorly differentiated SCC and lympho-proliferative disorders of the cervix. LELC arising from the nasopharynx has been suggested to be associated with Epstein-Barr virus (EBV) [3-5]. So far, the role of EBV in LELC of the uterine cervix is still controversial because its presence differs between Asian and European women [6-8]. On the other hand, high-risk human papilloma virus (HPV) has been established as the etiological factor in the carcinogenesis of conventional SCC of the uterine cervix and several authors have suggested it may play an important role in the pathogenesis of LELC of the uterine cervix [6, 9].

Here we report a case of a 52-year-old Japanese woman who was diagnosed a LELC of the uterine cervix with the examination of the presence of EBV and HPV.

Case

A 52-year-old Japanese woman, gravida 3, para 1, presented for metrorrhagia which occurred post menopause. Past medical and family histories were unremarkable. Her last gynecologic examination and cervical smears had been performed seven years before and were considered as normal. There were no visible tumors at the uterine exo-cervix but the scraping by cervical smear made it bleed easily. Examination by colposcopy showed an unsatisfactory colposcopic finding (USF). The cervical smear showed positive as shown in Figure 1. Transvaginal ultrasound detected a low echoic lesion 21 x 13 mm in size and magnetic resonance imaging (MRI) also showed the low intensity area of 26 mm in size at the uterine cervix. Serum CEA antigen and SCC antigen levels were 9.1 ng/ml and 1.6 ng/ml, respectively. Biopsy was performed and after histopathologic diagnosis of a carcinoma of the cervix, the patient was clinically staged as FIGO Stage Ib1, and a radical hysterectomy was performed. Histology showed no evidence of residual tumor. No lymphatic metastasis was found and no adjuvant therapy was administered. After six years of observation, no recurrence of the disease has occurred in this patient.

Figure 1. — Cytological findings from cervical smears showing solid nests or dissociative form of atypical squamous type cells. The background is necrotic.
LELC of the uterine cervix is an uncommon neoplasm. This rare variant represents only 0.7% of all primary cervical malignancies. A review of the literature demonstrates that the incidence of cervical LELC in Asia is higher, representing 5.5% of all cervical malignancies, than in Western countries (0.7%), just as LELC of the nasopharynx is more common in Asia than in the West. This ethnic factor might affect the carcinogenesis of this tumor.

This type of tumor usually presents with vaginal bleeding. The tumor typically appears arising in the endocervical canal and shows superficial ulceration as seen in this case. The prognosis of LELC of the uterine cervix appears to be better than that of SCC. One of the reasons is that there is less vascular invasion and lymph node metastasis in LELC than in SCC. In this case, lymph node metastases after surgery were all negative and the disease-free survival period has been more than 90 months.

This tumor is microscopically characterized by a sheet structure of undifferentiated cells with indistinct cellular borders growing in a dense lymphoid stroma. It is important to clinically differentiate LELC from poorly differentiated SCC and lymphatic disorders of the cervix. The morphological similarities between nasopharyngeal and cervical tumors suggest that the Epstein-Barr virus (EBV), a common oncogenic virus frequently recognized in nasopharyngeal LELC, may play an important role in the pathogenesis of LELC of the uterine cervix. Indeed, Tseng et al. reported EBV was detected in 73.3% (11 of 15) of cervical LELC in Taiwanese women [6]. On the other hand, the association of EBV with the pathogenesis of LELC in Western countries has not been described [7]. In our case, EBV was not detected by in situ hybridization.

HPV infection is strongly associated with the pathogenesis of uterine cancer of the cervix. A possible involvement of HPV in the pathogenesis of LELC of the uterine cervix has also been suggested. Indeed, HPV-16 was detected in 20% (3 of 15) of Taiwanese women with LELC of the uterine cervix.
LELC of the uterine cervix [6]. HPV-16 and HPV-18 were found in two cases of European women with LELC of the uterine cervix [11]. In our case, the presence of HPV-16 and HPV-18 was not detected by in situ hybridization. Pathogenesis may thus be different between Western and Asian women. In summary, according to our negative result, it seems that HPV and EBV did not play any etiologic role in LELC of the uterine cervix in a Japanese patient.

References

Address reprint requests to:
T. MORI, M.D., Ph.D.
Department of Obstetrics and Gynecology
Kyoto Prefectural University of Medicine
465 Kajii-cho, Kawaramachi Hirokoji
Kamiyogo-ku, Kyoto 602-8566 (Japan)
e-mail: moriman@koto.kpu-m.ac.jp

Figure 3. — No virus genomes were detectable in the tumor cells (x 40, a. EBV, b. HPV-16, c. HPV-18).
Laparoscopic total fallopian tube removal at the time of bilateral salpingo-oophorectomy in BRCA2 positive women

J. Wabersich1, G. Artioli2, R. Giordano3, F. De Lorenzi4, G. Azzarello1, F. Garbin1

1Department of Gynecology and Obstetrics, Mirano; 2Department of Medical Sciences, Oncology-Haematology, Mirano
3Department of Pathology, Mirano (Italy)

Summary

About 10% of all serous ovarian cancer has BRCA1 and/or BRCA2 mutations. Recent data showed that following the SEE FIM protocol it is possible to evidence more fimbriae cancers. Due to those studies, fallopian tube cancer in recent years has become the predominant site of cancer in BRCA1 and/or 2 mutation carriers. The pathological study of the fallopian tube is not complete during salpingo-oophorectomy because a small part (intramural site) is situated inside the uterus. In this case report we demonstrate how it is possible to remove the tubes entirely for pathological analysis without hysterectomy by laparoscopic surgery.

Key words: Fallopian tube carcinoma; BRCA; Laparoscopy.

Introduction

The lifetime risk of developing an ovarian cancer (OC) in BRCA positive women is 35-60% for BRCA1 and 10-27% in BRCA2 by the age of 70. Most of those OCs are situated in the tubes and account for approximately 0.5% of all gynecologic malignancies in the general population [1, 2]. Recently data show that 60% of gynecological cancers due to BRCA1 and/or BRCA2 mutations take origin from the distal part of the tubes and then spread to the ovaries and peritoneum.

Consequently, surgeons asked themselves how to remove all of the tubes because a small part of the fallopian tubes are inside the uterus (hostium). The part of the oviduct that crosses the uterine wall, the intramural segment, is 1-2 cm long and constitutes the uterine-tubal junction. This section extends through the wall of the uterus and the ostium opens within the uterine cavity. The wall of the oviduct has the same basic components as the wall of the uterus.

Although in this residual tissue where columnar cells can be found as in the distal part of the tubes, all these cells are at risk of developing a cancer because the BRCA mutation is present in all cells [3]. To the best of our knowledge there is no report in the literature of cancer at this site.

Since a surveillance program with transvaginal ultrasound (TVS), pelvic examination and CA125 serum is not able to reduce mortality and its efficacy is not proven [4], risk-reducing bilateral salpingo-oophorectomy (RRSO) seems to be the only way to reduce ovarian and fallopian tube cancer by up to 96% [5] and also breast cancer up to 50% [6].

In this case report we describe how to perform a laparoscopic RRSO removing the entire fallopian tube without performing hysterectomy because the residual proximal tubal epithelium could be the site of tumor origin. The aim of the study was to assess the surgical results, complications and pathological findings of laparoscopic salpingo-oophorectomy without hysterectomy.

Key words: Fallopian tube carcinoma; BRCA; Laparoscopy.

Case Report

The case of 49-year-old premenopausal Caucasian women affected by BRCA2 mutation is presented. When she was 37 years old she underwent left lumpectomy and axilla node dissection for a lobular breast cancer, Stage pT1c N1a Mo (AJCC Staging System) with positive estrogen and progesterone receptors. She was treated with adjuvant chemotherapy and tamoxifen for five years and remains disease-free.

She undergoes breast surveillance annually with breast RMI, mammography and breast ultrasound (Health-Istitute protocol, Italy), CA125 serum dosage and TVS.

In October 2010 she underwent her annual TVS and a cyst with septum on her left ovary of 5 cm was found. After a multidisciplinary meeting with the patient and counselling on surgery treatment the patient agreed to undergo a modified RRSO.

Surgical technique

Usually during laparotomic RRSO the proceedings include the ligation of the fallopian tubes and the uteroovarian ligament, which are then dissected. In a second step the gynecologist performs a double sequence procedure and then section of the infundibulum. During laparoscopic surgery, the infundibulum and the uteroovarian ligament with the emergence of the fallopian tubes are dissected by diathermocoagulation.

Even during the standard surgical laparotomic procedure for RRSO, the uterine cornua is left behind the residual fallopian tubes. In this particular case we performed resection of both ovaries and fallopian tubes completely with the cyst after peritoneal washing. The fallopian tubes were resected until the intramural site dissecting the uterine cavity where the ostium is situated (Figure 1).

The surgical specimens were placed in a large bag which was brought out through the intraabdominal incision with trocar clamps and scissors (Figure 2).

During the surgical procedure of this case anatomical isolation of both fallopian tubes was performed with section of the intramural site. The steps of the surgery procedures were: 1) isolation of the pelvic infundibulum prior to identification of ureter, as described by Crum et al.; 2) diathermocoagulation of the pelvic-infundibulum and uterus-ovarian ligament to reduce mesosalpinx vascular flow; 3) isolation and dissection of the mesosalpinx;
4) removal of the total tube with the intramural portion by endobag;
5) pressure points;
6) removal of the ovaries (Figure 3).

Pathological findings

The sectioning and extensively examining the fimbria protocol was developed to ensure maximal examination of the fimbria by sectioning and extensively examining the fimbriated end. Recent reports indicate that the fimbria is the site for most tubal cancers irrespective of BRCA status [7-9]. In recent studies of ovarian and peritoneal serous carcinoma, approximately 50% had a plausible origin in the fimbria [10, 11]. Based on the hypothesis that the fimbriated end is unique and susceptible to tubal neoplasia [8-12] we analyzed the composition of the intramural fallopian tube to assess if it was composed of ciliated cells at the distal part.

The findings were: Peritoneal washing was negative. Fallopian tubes were both 8 cm length and the intramural portion was 1 cm in length. The cyst was a mucinous cystadenoma of the ovary.

The intramural tubal epithelium was not stratified and consisted of two cell types: ciliated cells with a nucleus placed centrally and secreting type with cubic-cylindrical cells. The two cell types were equally represented.

The tissues from tubes to the uterus were defined by the presence of endometrial stroma and loss of tubal epithelium. Immunocytochemical assay was performed by p53 and Ki-67 tests and was negative.

Discussion

The literature about the origin of cancer in the fallopian tube in BRCA positive woman shows a greater frequency of early malignant lesions in the distal fallopian tube which can spread to the ovarian surface and peritoneum. The reason early cancer is often found in the fimbria is not yet clear. Some authors suggest that it may be due to the increased surface area of this site, or potential differences in characteristics of the cells from this region versus more proximal sections of the tube. Carlson et al. described 79% of endosalpingeal involvement [13]. Many literature reports describe fallopian tube cancer in the fimbria and in the midportion in BRCA women [14].

There have been no reported cases of tubal carcinoma occurring in the tubal remnant following RRSO. Whether this residual proximal tubal epithelium could be the site of tumor origin in the rare cases of primary peritoneal carcinoma that follow RRSO is unknown at this time [3].

The issue related to the residual fallopian tubes have induced some surgeons to perform hysterectomy as well at the time of surgery. Hysterectomy seems to simplify the use of tamoxifen in reducing the risk of breast cancer and its related increased risk of developing endometrial cancer [15, 16].

The role of hysterectomy is important in decision making, as well as the use afterwards of hormone
replacement therapy (HRT). Performing total abdominal hysterectomy (TAH) at the time of RRSO implicates the use of HRT with estrogen alone to reduce menopause symptoms [17].

However we have to consider that TAH at the time of RRSO adds some risk of slightly higher morbidity and prolonged time of hospitalization, and consequently costs. TAH does not seem to contribute to reducing the risk of serous ovarian carcinoma.

Consequently TAH at the time of RRSO still remains a controversial issue. TAH may simplify HRT for women who decide to take it, but its role in reducing ovarian/fallopian cancer risk by removing the small remnant of fallopian tubes left attached to the uterine wall is not clear yet [17, 18].

Some authors agree that TAH in women who underwent RRSO could be performed later.

However, in this particular patient we decided not to use HRT because of her previous history of hormone positive breast cancer [19].

Conclusion

Whether the residual intramural tubal epithelium could be the site of tumor origin in the rare cases of primary peritoneal carcinoma that follow RRSO is still unknown [5, 20, 21].

The optimal prophylactic surgical procedure for BRCA mutation carriers at this time is RRSO with or without hysterectomy. The reasons to perform hysterectomy at the time of surgery could be the use of tamoxifen to reduce breast cancer risk and the use of HRT without progesterone, but data suggests that the risk of carcinoma from residual tubal tissue following RRSO is the least compelling reason for hysterectomy [3]. Women who do not need to use tamoxifen and HRT maybe do not need to have hysterectomy performed. Laparoscopic RRSO modified with the removal of the total fallopian tube could be a safety option with short hospitalization and with a favorable cosmetic outcome and minor cost. To answer the question whether the intramural site is the place where cancer originates after RRSO as published by Cass et al. [3], we should be familiar with its anatomopathology, and this surgical technique could be a way to study it.

References


Virilizing ovarian Krukenberg tumor in a 27-year-old pregnant woman. A case report and literature review

E. Papakonstantinou1, A. Liapis2, E. Kairi-Vassilatou3, C. Iavazzo2, C.K. Kleanthis3, A. Kondi-Pafiti1

1Pathology Laboratory, 2nd Department of Obstetrics and Gynecology, Aretaieion University Hospital, Athens (Greece)

Summary

A case is reported of a 27-year-old pregnant woman with ovarian tumors, measuring 12 cm and 11.5 cm in the greatest diameter, discovered during investigation for virilization symptoms. Termination of the pregnancy at the 22nd week of gestation and tumorectomy with both adnexa were performed, with the provisional diagnosis of arrhenoblastoma. Pathological examination of the tumors showed typical Krukenberg neoplasms and subsequent upper GI tract endoscopy revealed a gastric cancer that was excised. The pathological examination revealed a diffuse type gastric adenocarcinoma with signet ring morphology, similar to ovarian tumors. In any case of ovarian tumor with unusual hormonal manifestations, in addition to hormonally active sex cord-stromal neoplasms, metastatic ovarian tumors must be considered as well, especially in cases of bilateral tumors.

Key words: Virilization; Pregnancy; Ovarian metastatic tumor; Gastric cancer; Krukenberg tumor.

Introduction

Virilization caused by ovarian tumors with functioning stroma during pregnancy is extremely rare [1] and has been reported in cases with mucinous cystadenomas and cystadenocarcinomas, Brenner tumors, Krukenberg tumors, mature cystic teratomas and dysgerminomas [2, 3]. These types of ovarian tumors have been reported to be associated with endocrine abnormalities mainly of estrogenic, or rarely androgenic or progesteragenic type or of combinations thereof.

A few cases of virilizing ovarian tumors with functioning stroma have occurred in pregnant women, and among these, one third were Krukenberg tumors, one fifth were primary mucinous cystic tumors, mostly benign, and the rest were cases of metastatic carcinomas from the large intestine, dermoid cysts, strumal carcinoid tumors and other miscellaneous tumors [4, 5].

Krukenberg tumors are metastatic ovarian adenocarcinomas with a distinctive histological appearance first described by Krukenberg in 1896, in five tumors which he classified as primary neoplasms of the ovary. Since then this term has been variously interpreted as: i) a pathological entity in which solid, usually bilateral, ovarian tumors are formed of mucus producing signet-ring cells set in hyperplastic cellular stroma, ii) a purely histological diagnosis which is applicable to any tumor showing the above histological characteristics irrespective of its macroscopic appearances, iii) any metastatic ovarian tumor, iv) any ovarian metastasis from a primary gastric carcinoma, v) any ovarian metastasis from a primary tumor in any part of the gastrointestinal tract [6].

Case Report

A 27-year-old woman presented at approximately the 22nd week of pregnancy with signs of virilization. The patient had no other endocrine symptoms and her past medical history was otherwise unremarkable. Menstrual cycles were regular and the pregnancy was uneventful until she reported facial acne and onset of prominent facial hair, which was obvious during the physical examination at admission. Abdominal ultrasonography revealed bilateral large compact ovarian tumors and arrhenoblastomas were suspected. Laboratory investigation showed that testosterone (1.37 μg/ml) and progesterone (44 nmol/ml) and 17-H-progesterone (9.42 ng/ml) levels were elevated. Estradiol, androstendione, LH, FSH, prolactin and dehydroepiandrosterone sulphate levels were within normal range.

A cesarean section was performed because of fetal distress and a male stillborn fetus was delivered, showing pulmonary immaturity, meconium inspiration and intraparenchymal pulmonary hemorrhage. Both adnexa showed large compact tumors (Figures 1 and 2), measuring 12 cm (right) and 11.5 cm (left) in the greatest diameter with a lobulated external surface and bilateral tumorectomy was performed, with the provisional diagnosis of arrhenoblastoma. Histological examination of the specimen (surgical report no. 15131) and of multiple paraffin sections of formalin-fixed tissue stained by hematoxylin-eosin showed cords of neoplastic cells with distinct signet ring morphology, in a loose stroma, with many theca-like cells (Figures 3 and 4). Histochemical examination for mucicarmine and PAS stains was positive for intracytoplasmatic mucin and the diagnosis of Krukenberg tumors was made with extensive luteinization of the stroma.

In order to detect the primary site of this tumor, further examinations were performed after the operation. Endoscopy of the upper GI tract revealed an ulcerative lesion in the body of the stomach. Gastric biopsies showed adenocarcinoma with signet-ring cells. Colonoscopy showed no abnormal findings. There was no evidence of other metastatic lesions in the abdomen or liver, according to abdominal Ultrasonography and computed tomography studies.

Total gastrectomy was performed and a gastric adenocarcinoma of diffuse type infiltrating the gastric wall with lymph...
node metastasis was diagnosed. The patient received adjuvant chemotherapy and radiotherapy but was lost to follow-up 12 months after surgery. The acne on the face cleared up and the hair growth returned to normal. The androgen serum levels were normal after the operation.

Discussion

Krukenberg tumors are metastatic neoplasms from primary lesions in the gastrointestinal tract. These tumors originate in the stomach in the majority of cases [7]. Sometimes the gastric cancer may be small and remains undetected for several years after oophorectomy. Much less frequently the primary tumor is the large intestine, breast, gallbladder, uterine cervix, appendix, or urinary bladder. In rare cases the site of origin of the primary tumor remains unknown [7].

It is widely accepted that the diagnosis of a “primary” ovarian Krukenberg tumor has been proposed for those cases in which either the patient survives in good health for at least five years or longer after removal of the ovarian neoplasm, or has died and been subjected to a thorough autopsy which fails to reveal an extra ovarian primary tumor [7].

Krukenberg tumors, which account for 3-4% of all ovarian neoplasms, are bilateral in over 80% of cases and range in size from 5-20 cm in diameter. They are usually solid with a smooth nodular or bosselated outer surface and on section are composed of firm, white, yellow or pinkish tissue. The tumor is sometimes locally myxoid or mucinous in texture and occasionally Krukenberg tumors are predominantly cystic [7].

The age of women with an ovarian Krukenberg tumor tends to be unusually low for patients with metastatic carcinoma, most are between 30 and 50 years of age at the time of initial diagnosis, and the mean age is about 45 years [7].

The symptoms of a Krukenberg tumor usually are non specific and related solely to the presence of a pelvic mass, abdominal discomfort or pain, and accompanying ascites being the most commonly encountered complaints. Although the symptoms are usually not specific, endocrine manifestations, such as virilization during pregnancy, may result from stromal luteinization [7-10]. It is worth noting, however, that pregnant women with a Krukenberg tumor show a particular tendency to become virilized. This is thought to be due to stimulation of the luteinized cells by hCG and the maternal virilization may

Figure 1. — Gross view of the compact ovarian tumor (right ovary) showing smooth outer surface.
Figure 2. — Gross view of the cut surface of the ovarian tumor (left ovary) showing compact surface with myxomatous areas.
Figure 3. — Histological section of the left ovarian tumor with signet-ring cells (center) (H&E x 250).
Figure 4. — Histological section of right ovarian tumor with signet-ring cells in a loose stroma (H&E x 120).
be associated with partial masculinization of female children resulting from such pregnancies [6]. The signs of masculinization of such patients usually regress or disappear after removal of the ovarian tumor or termination of pregnancy, supporting the perception that the luteinized ovarian stromal cells are responsible for the androgen production. Virilizing ovarian tumors may cause mild erythrocytosis via production of androgenic steroids [8]. Pseudo-Meigs syndrome of metastatic ovarian tumor from gastric cancer is very rare [9]. Soon after the removal of the tumor, the levels of serum testosterone decrease and the patient gradually loses the virile type of body hair [7]. Indeed, in our patient the virilizing manifestations disappeared after the removal of the ovarian neoplasm.

Only between 20-30% of patients with an ovarian Krukenberg tumor have a history of a previously removed extra ovarian neoplasm [6]. Krukenberg tumors are at least 80% bilateral. Occasionally, the gastric primary cancer is not discovered until after the ovarian tumor has developed, exemplifying the well known propensity for Krukenberg tumors of gastric origin to herald the presence of disease [4]. Krukenberg tumors may also show glandular differentiation and this presents problems in the differential diagnosis from other primary or metastatic ovarian tumors but the predominance of signet-ring cells suggests the gastric origin [7].

It is important not to be misled by the presence of endocrine manifestations into making an erroneous diagnosis of a sex cord-stromal tumor that usually acts as a feminizing tumor secreting female sex hormones and as a virilizing tumor, secreting male sex hormones [10-12]. It is to be noted that all hormonally active sex cord-stromal tumors are potentially malignant [7].

Microscopic examination shows stroma that may be cellular or relatively acellular often edematous with signet-ring cells. Occasionally, small or large cysts lined by minimal atypical-appearing mucinous epithelium or completely flattened benign appearing epithelium are present [13, 14]. Blood vessel and lymphatic invasion is common. Krukenberg tumor when associated with stromal luteinization can be confused with a Sertoli-Leydig cell tumor, however, signet-ring cells are not a feature of the latter tumor [7]. Immunohistochemical expression of hCG is more frequently found in tumors with a functioning stroma than in those with an inactive one [15]. Stromal steroid cell reaction in ovarian tumors is more frequent, more extensive and more broadly distributed at the periphery of tumors in pregnant patients than in non pregnant patients [10, 12, 15].

The prognosis for a woman with a Krukenberg tumor is extremely poor, and it worsens if the primary tumor is identified after the metastasis to the ovary is discovered. The median survival after the diagnosis of Krukenberg tumor is reported to be 7-14 months [16-18]. Resection of the primary lesion may have a role in the management of Krukenberg tumor of stomach origin, but no effective therapy has been reported so far [19].

References
Unexpected synchronous follicular lymphoma of paraaortic and pelvic lymph nodes in a patient with endometrial carcinoma: a case report

H.B. Lee¹, J.C. Park², Y.S. Lee¹, I.C. Jeung¹, E.K. Park¹

¹Department of Obstetrics and Gynecology, College of Medicine, The Catholic University of Korea, Seoul (South Korea)

Summary

Background: Multiple neoplasms in a patient occur rarely. There has only been one case report about synchronous endometrial carcinoma and follicular lymphoma of the paraaortic and pelvic lymph node (LN) until now. Case report: The patient was 64 years old and had vaginal spotting for four months. She was diagnosed with endometrioid endometrial carcinoma by endometrial biopsy. In intraoperative inspection, the whole paraaortic and pelvic LN had formed into a massive tumor bundle following the aorta and iliac vessels. The diagnosis was endometrial carcinoma FIGO Stage IB with synchronous follicular lymphoma Stage III. We performed adjuvant chemotherapy and radiotherapy. Currently, the patient has no evidence of recurrence for either carcinoma. Conclusion: Lymph node dissection was included in the staging and debulking operation of the endometrial carcinoma. An inaccurate result of the frozen section can not rule out metastasis of endometrial carcinoma and surgeons can fall into a dilemma regarding treatment.

Key words: Multiple neoplasms; Endometrial carcinoma; Lymphoma.

Introduction

Multiple primary cancers associated with hematological malignancies are divided into the following three groups: Synchronous malignant neoplasms, metachronous cancer following hematological malignancy, and metachronous hematological malignancy following carcinoma [1]. The differential diagnosis of metachronous and synchronous malignant neoplasms is separated by time, the metachronous neoplasms usually develop after six months, and the synchronous neoplasms may be diagnosed simultaneously or within a short time of each other, usually two to six months [2]. The most common hematological malignancies seen in patients with multiple cancers are acute leukemia, non Hodgkin’s lymphoma (NHL), multiple myeloma and chronic myelogenous leukemia [1]. Multiple neoplasms in patients occur rarely, ranging from 1.2-4.5% of cancer patients in autopsy and clinical studies [2, 3]. Furthermore endometrial carcinoma with synchronous lymphoma is rare. We report such a case.

Case Report

A 64-year-old Asian woman had postmenopausal uterine bleeding for four months. She was a virgin and a nun. She did not have any other symptoms, including pelvic pain, night sweats, weight loss, or fever. She also did not have any other underlying disease nor operation history. In her family history her grandmother and cousin had died of stomach cancer. We performed an endometrial biopsy and the result was endometrioid adenocarcinoma. PET computed tomography (CT) and pelvic magnetic resonance imaging (MRI) were performed. On PET CT, a uterine lesion showed moderately increased FDG uptake, and multiple massive lymphadenopathies involving the paraaortic region, both iliac, both axillary and both inguinal regions (Figure 1). Transabdominal hysterectomy, bilateral salpingo-oophorectomy, paraaortic and pelvic lymph node dissection, partial omentectomy and appendectomy were performed. On intraoperative inspection, the aorta and iliac vessels were surrounded by bundles of hard tumor mass. Thus we had difficulty distinguishing the lymph node (LN) from the great vessels. A frozen biopsy was done on the tumor mass of multiple paraaortic and iliac chains. The result was malignancy for all tumors, or metastatic carcinomas. Cytoreductive surgery was performed and there were no complications postoperatively. In the final pathologic report, the estimated extent of invasion of the endometrial carcinoma was the outer half of the myometrium. The thickness of the uninvolved myometrium was less than 0.1 cm. There was lymphatic and vein invasion but tumor metastasis to other sites was not observed. However cytology of the peritoneal fluid included malignant cells of endometrial carcinoma. The whole mass of the paraaortic and pelvic LN dissection showed malignant lymphoma without metastasis of endometrial carcinoma. Thus, the final diagnosis was endometrioid endometrial adenocarcinoma (poorly differentiated, grade 3) FIGO Stage IB [4] with synchronous follicular lymphoma Stage III (grade 2). Immunohistochemical studies were positive for CD10, CD20, CD21a and LCA confirming NHL and B-cell phenotype. Bcl-6 expressed by the follicles was also positive. The patient received three courses of cisplatin and doxorubicin at 3-week intervals with radiation therapy. She refused further treatment. PET/CT after treatment was clear. Nine months after surgery, the patient is currently in a state of complete remission for both carcinomas.
Discussion

Multiple primary cancers can occur in the same patient. Colon, ovarian, and breast cancers have previously been reported to be the malignancies most commonly associated with endometrial cancer. In the recent literature, the prevalence of lymphoma, breast, and ovarian cancers were reported to be greater than expected at the time of surgery for endometrial cancer [5]. Multiple synchronous malignant tumors were found at different sites or organs. In our patient, the diagnosis of NHL was made after the staging surgery for the endometrial carcinoma. Synchronous carcinoma and NHL have been reported previously. The sites of the primary carcinoma associated with synchronous NHL were in order starting from the colon, prostate, lung, breast and stomach [2]. Synchronous NHL and uterine carcinoma are relatively rare. The presenting sites of NHL are various. However one case of NHL involved in pelvic LN of a patient who had endometrial carcinoma has been reported. The number of case reports are limited so a standard treatment is difficult to define for our case. The surgical treatments of endometrial carcinoma are removal of the tumor mass and surgical staging. In our case, the unexpected bulky mass of paraaortic and pelvic LN during the staging operation of the endometrial carcinoma was necessary to exclude involvement of metastatic carcinoma from the known primary. However, intraoperatively the differential diagnosis between poorly differentiated carcinoma and NHL can be difficult by frozen section. The ambiguous frozen result of the malignant tumor for LN led to debulking surgery and the complete excision of the tumor. In preoperative PET CT, increased FDG uptake in axillary LN is an unusual finding of endometrial carcinoma, but we missed it. The initial treatment of a patient with follicular lymphoma includes combination chemotherapy. Our patient had deep myometrial invasion, grade 3, and had malignant cells in the peritoneal fluid for endometrial carcinoma, so adjuvant therapy was performed. In our case, NHL was discovered incidentally during the staging operation of the known endometrial carcinoma. NHL may involve adjacent site of patients who have carcinoma of the female genital tract. A rare situation can lead to difficulty of diagnosis and treatment. Although a rare modality, the relation of lymphoma during surgery of endometrial carcinoma should be kept in mind by the surgeon.

References


Address reprint requests to:  
E.K. PARK, M.D.  
Daejeon Jung-gu Deaheung-dong 520-2  
The Catholic University of Korea  
Seoul (South Korea)  
Daejeon St. Mary Hospital  
e-mail: guevara614@catholic.ac.kr
Leiomyosarcoma of ovarian vein compression as a cause of hydronephrosis

S.H. Yang¹, J.C.W. Chien¹, C.L. Chen², W.P. Chan¹,³
¹Department of Radiology, Taipei Medical University-Wan Fang Hospital
²Department of Pathology, School of Medicine, Taipei Medical University
³Department of Radiology, School of Medicine, Taipei Medical University, Taiwan (Republic of China)

Summary
We present a case of leiomyosarcoma of the right ovarian vein with MRI findings. The patient was a 52-year-old woman who had suffered from right flank pain for one week. Abdominal ultrasound and excretory urography revealed hydronephrosis of the right kidney. Ureteroscopy showed external compression at the right upper third of the ureter. CT and MRI of the abdomen revealed a retroperitoneal mass with compression of the right ureter. The retroperitoneal mass proved on histology to be a leiomyosarcoma arising from the right ovarian vein.

Key words: Computed tomography (CT); Leiomyosarcoma; Magnetic resonance imaging (MRI); Ovarian vein.

Introduction
Vascular leiomyosarcomas are rare tumors, accounting for less than 2% of all leiomyomas [1]. The most common site of the tumor is the inferior vena cava, followed by the central veins and the long saphenous vein [1]. To our knowledge, only five cases of leiomyosarcoma of the ovarian vein have been reported [2, 3]. We present the magnetic resonance imaging (MRI) and computed tomography (CT) findings of a leiomyosarcoma of the right ovarian vein in a 52-year-old woman.

Case Report
A 52-year-old woman had suffered from right flank pain for one week, without any history of urolithiasis. Abdominal ultrasound and excretory urography revealed hydronephrosis of the right kidney. Ureteroscopy showed external compression at the right upper third of the ureter. There was no mucosal lesion or stone. A double-J catheter was inserted into the right ureter. CT scan of the abdomen showed a 3.8 cm retroperitoneal mass compressing the upper third of the ureter (Figures 1A/B), resulting in obstructive uropathy. The tumor was located adjacent to the right psoas muscle and lateral to the right upper ureter, displacing the ureter medially. An enhancing vessel at the central zone of the tumor was noted. MRI clearly delineated the ureter, the vessel and the mass (Figures 1C/D). After gadolinium administration, a small vessel was shown connected to the tumor, and the vessel could be traced to the inferior vena cava, indicating that the vessel was an ovarian vein. At surgery, there was an elastic mass in the right upper third of the perireteral region (Figure 1E), with adhesion to the ureter and connection to the right ovarian vein. Histological diagnosis revealed leiomyosarcoma of the ovarian vein (Figure 1F).

Discussion
Leiomyosarcoma of the vein arises from the smooth muscle cells of the tunica media. It has three major growth patterns: completely extravascular (extraluminal) (62% of cases), completely intravascular (intraluminal) (5%), and extramural and intraluminal mixed (33%) [4]. The tumor predominantly occurs in middle-aged women and seldom presents symptoms until it is large enough to compress regional organs. Its growth pattern is usually to expand into the less resistant tissue plane and eventually invade the adjacent tissues and metastasize distantly. Aggressive surgery remains mandatory in retroperitoneal sarcoma, but radiotherapy for local control is controversial.

Because leiomyosarcoma of the ovarian vein is extremely rare, radiological findings mainly are of heterogeneous solitary masses adjacent to the ureter associated with obstructive uropathy, as in our case, or just incidental findings. Only one case, reported by Cho et al. [5], had a bi-armed appearance due to the tumor extending through the ovarian vein to the inferior vena cava. These cases usually present as an eccentric mass adjacent to the ureters resulting in hydronephrosis. Ureteroscopy should be performed to rule out a mucosal lesion of the ureter.

The most important aspect of diagnosis of leiomyosarcoma of the ovarian vein is identifying the relationship between the tumor, ovarian vein, ureter and psoas muscle. In our case, with the advantage of multiplanar capability, the relationships of the ureter, ovarian vein and psoas muscle were clearly delineated with use of various pulse sequences on MRI. The high-signal-intensity of the ureter on T2-weighted MR images was due to urine content. The coronal images clearly showed the obtuse angle and external compression between the tumor and ureter, indicating a non-ureteral origin. On coronal gadolinium-enhanced images, the connection of the tumor and ovarian vein was well depicted, and the center of the tumor...
A 52-year-old woman had suffered from right flank pain for one week.

(A) Axial contrast-enhanced CT scan shows a heterogeneous enhancing mass with central zone of low density (large arrow). Note the inserted double-J catheter (small arrow) indicating the medially displaced ureter.

(B) Axial contrast-enhanced CT scan at the upper margin of the tumor depicts central enhancing vessel within the tumor (large arrow). Small arrow indicates double-J catheter.

(C) Coronal T2-weighted MRI image depicts smooth tapering of the ureter due to external compression by the retroperitoneal mass (large arrow). Note high-signal-intensity of urine content within ureter (small arrow).

(D) Coronal gadolinium-enhanced T1-weighted image shows connection of the right ovarian vein (large arrow) and the tumor, and the eccentric position of the compressed ureter (small arrow).

(E) Gross specimen reveals a well-defined, lobulated, grayish and elastic tumor resected from the retroperitoneum.

(F) Microphotograph of the tumor reveals leiomyosarcoma of the ovarian vein. (H & E).
was closer to the ovarian vein than the ureter. We could also exclude an origin in the psoas muscle because the tumor was completely separated by the fat plane between the mass and psoas muscle. The signal intensity of the mass was intermediate on T1-weighted images, mixed intermediate and high on T2-weighted images, and heterogeneous on gadolinium-enhanced images, compatible with the signal changes of leiomyosarcoma [4].

In summary, the multiplanar capacity and various pulse sequences of MRI can clearly well delineate the relationships of the ureter, vessel, and muscle, and can differentiate the tumor origin from signal changes, thereby providing a powerful tool for surgical planning.

References

Address reprint requests to:
J. C.W. CHIEN, M.D.
Department of Radiology
Taipei Medical University-Wan Fang Hospital, 111 Hsing-Long Road, Section 3, Taipei 116 Taiwan (Republic of China)
e-mail: wp.chan@msa.hinet.net
Proximal-type epithelioid sarcoma of the mons pubis: report of a case

A. Andrisani¹, A. Serena¹, G. Ambrosini¹, G. Capobianco¹, S. Chiarelli²

¹Department of Gynecological Science and Reproductive Medicine, University of Padova, Padova
²Department of Medical Diagnostic Sciences and Special Therapies, Section of Pathology, University of Padova, Padova

Summary

Introduction: Proximal-type epithelioid sarcoma (PES) represents an extremely rare and aggressive form of soft tissue neoplasm, typically presenting as a painless subcutaneous nodule in the trunk often located in the genital area. Case report: A 46-year-old female was subjected to the excision of a growing soft tissue mass in the mons pubis that, at histology, was identified as PES. The tumor showed an extreme aggressiveness involving subsequently adjoining structures and lymph nodes despite subsequent wide surgical resections during the following months. Discussion: Gynecologists should pay careful attention to all soft tissue masses of the perineal area or external genitalia. It is important to know the possible genital localization of PES which, although rare, is an aggressive high-grade soft tissue tumor with a deceitful behavior, poorly sensitive to chemotherapy and radiotherapy. Surgery, though wide and demolitive, often fails to obtain the necessary radicality.

Key words: Sarcoma; Epithelioid sarcoma; Soft tissue neoplasms; Female genitalia.

Introduction

Proximal-type epithelioid sarcoma (PES), also called large-cell type [1], represents an extremely rare form of epithelioid sarcoma differing from the distal counterpart for an aggressive pattern of local recurrence despite negative margins and a stronger propensity for early metastasis [2, 3]. Epithelioid sarcoma is commonly cited as example of ungradable sarcoma [4, 5], due to the failure in capturing the essential histological information of this rare disease. If the distal form is infrequent, even much rarer is the proximal-type, with the perineum as a quite frequent location both in men and women. Particularly the vulvar site has been described [6].

The symptoms can be different according to the site of the neoplasm [7, 8], but PES typically presents between the 3rd and 5th decade of life as a painless subcutaneous nodule in the trunk (mainly located in the pelvic area, perineus, external genitalia) [3, 9].

This tumor shows sheets of large cells with prominent nucleoli resembling a poorly differentiated carcinoma [1], and immunohistochemical analysis is important for the correct diagnosis [10, 11].

Despite radical surgery, the prognosis for this tumor is generally poor and the role of adjuvant therapy remains unclear owing to the deficiency of evidence.

Case Report

The patient, a 46-year-old Caucasian female, who underwent laparoscopic uterine myomectomy one year before at our Gynecological Clinic, presented to our Department with a painless growing soft tissue mass in the mons pubis. The lesion, noticed three weeks before as a small pimple, was 5.5 x 4 cm at presentation. Considering it as a probable soft tissue sarcoma, we avoided a biopsy and the lesion was excised.

On cut sections, the lesion was ill defined, with multinodular confluent whitish masses, often with necrosis. The lesion extended to the deep margins. At histology, the neoplasm disclosed a granuloma-like pattern with nodules of epithelioid cells and frequent central necrosis. Clusters of lymphocytes and also follicles were present (Figure 1a) mainly peripherally, but also intermixed. On high power view, sheets of large cells, mainly rounded polygonal with amphophilic cytoplasm, enlarged vesicular nuclei, and prominent central nucleoli were observed. The tumor, resembling a poorly differentiated carcinoma, disclosed discohesive cells at the border of the nodules. Keratin stain (MNF116) was positive in some cells (Figure 1b); vimentin was widely positive. Other immunohistochemical markers were tested (AE1, AE3, CAM5-2,CK7 negative; EMA focally positive; CD34, CD56, CD79a negative; $100, MB45, NSE, chromogranin, synaptophysin, negative; lymphocytes were mainly CD20 positive). Hemorrhage and necrotic changes were common. Margins, mainly the deep ones, were positive. The patient underwent specialist supervision and a final diagnosis of PES was made.

Ten days later, since the surgical margin was positive, the patient underwent superior radical vulvectomy (preserving the clitoris, labia minora and majora and fourchette) and bilateral superficial and deep crural inguinal lymphadenectomy. The histological findings demonstrated the presence of several neoplastic foci – less than 3 mm in diameter – infiltrating the subcutaneous tissue close to the right resection margin; all 16 crural inguinal lymph nodes were negative.

During the following ten months the patient underwent five surgical resections performed by different surgeons at our Gynecological Clinic and later also at a General Surgery Service. Surgeons noticed a particular aspect of the margins, described as scattered “white spots” suggesting neoplastic infiltration and, despite subsequent cutting, a clean margin result is often not easy to reach. When apparently disease-free, the local...
recurrence of the sarcoma negated the radicality of the surgery (Figure 1c-f).

The tumor demonstrated an extreme aggressiveness involving subsequently adjoining structures as the fascial sheet, nerves and vascular wall, with extension to the genital, inguinal and thigh muscles and, finally, also the abdominal wall (Figure 1c-f). Despite this, lymphatic involvement, generally limited, was evidenced only histologically, with an isolate micro metastasis found in one of the 11 removed lymph nodes.

Table 1 reports timing of surgical excisions and main histological findings and Figure 1c-f shows tumor spread in relapses.

In association with surgery, the patient underwent adjuvant polychemotherapy with epirubicin and ifosfamide, but after six cycles the treatment was suspended due to the worsened clinical condition. This therapy was integrated with pelvic and inguinal radiotherapy (45 Gy in 5 fractions).

Computerized tomography, performed after three months, showed the presence of hypervascularized foci in the abdominal wall, inguinal area, anterior region of both thighs and a coarse neoplastic area surrounding the bladder.

Considering the severe clinical condition, only palliative therapy was possible, and the patient died two months later.

Discussion

This case underlines the relevance of paying careful attention to all soft tissue masses, in particular painless, even if the lesion does not show particular suspicious features. Epithelioid sarcoma is an uncommon aggressive high-grade soft-tissue tumor [12], typically appearing as a subcutaneous or deep dermal mass in distal portions of the extremities of adolescents and young adults [6, 13], whereas the proximal-type grows up in proximal parts of middle-aged and older people.

Correct histological diagnosis can be difficult considering the extreme rareness of the disease. Small superficially located tumors with nodular pattern are likely to be mistaken for an inflammatory process as necrotizing infectious granuloma. Some epithelioid sarcomas are hard to distinguish from a wide array of epithelioid-appearing malignant soft tissue neoplasms and poorly differentiated carcinomas [14-16]. The number of pathologists dealing with female genital disease, that can be named as expert in this particular kind of sarcoma is limited, and the main reference texts of gynecologic pathology do not even mention PES among the possible vulvar lesions or they only cite it [17]. For this reason it is essential to have at disposal a highly qualified histopathological center with experience to avoid incorrect diagnoses that can lead to wrong therapeutic decisions. Immunohistochemical examination provides a contribution to the diagnosis, but the pathologist’s specific experience in the field of soft tissue sarcoma is fundamental. Epithelioid sarcoma usually expresses epithelial membrane antigen, vimentin and cytokeratins, and is often positive for CD34, but several other antigens can be expressed. The ultrastructure displays epithelial and mesenchymal features including myofibroblastic differentiation. In addition, many cases display chromosomal abnormalities in the 22q region, but this can not be considered as specific [10, 18].

The mainstay of treatment in case of proximal-type epithelioid sarcoma is surgery and a radical resection is necessary as in all high-grade soft tissue sarcomas [19]. Extensive operations were performed in our case by different expert surgeons, but often surgery results only apparently radical and insufficient to improve prognosis, considering the extreme aggressiveness and the deceitful behavior of the disease.

Patients often develop multiple local recurrences with subsequent metastases in 30-50% of the cases [20]. As described, our case well exemplifies the propensity of PES to disseminate in a centripetal way, at first with local spread towards the aponeurosis and tendon sheaths, and successively infiltrating also vessels, nerves and the lymphatic system.

---

Table 1. — Timing of surgical excisions and main findings.

<table>
<thead>
<tr>
<th>Sequence of surgery</th>
<th>Site/resection</th>
<th>Histology</th>
<th>Margins</th>
<th>Tumour spread or neural invasion</th>
<th>Vascular, lymphatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>February</td>
<td>Mons pubis mass (5.5 cm)</td>
<td>Confluent nodules (5 x 4 cm)</td>
<td>+</td>
<td>Subcutis</td>
<td>–</td>
</tr>
<tr>
<td>February</td>
<td>Radical superior vulvectomy</td>
<td>Small foci (max 0.3 cm)</td>
<td>+</td>
<td>Subcutis</td>
<td>–</td>
</tr>
<tr>
<td>March</td>
<td>Local enlargement</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>May</td>
<td>Inner right tight nodule: 6 x 5.5 cm</td>
<td>Confluent multiple nodules</td>
<td>+</td>
<td>Infiltrating striated</td>
<td>–</td>
</tr>
<tr>
<td>May</td>
<td>Resection of: Inner right tight:</td>
<td>Confluent multiple nodules</td>
<td>+</td>
<td>Infiltrating striated</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>4 x 3 cm</td>
<td></td>
<td></td>
<td>muscle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inner left tight:</td>
<td>Confluent multiple nodules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 x 2.5 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>December</td>
<td>Resection of: Right tight:</td>
<td>Multiple nodules</td>
<td>+</td>
<td>Infiltrating striated</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>7 x 5 x 4 cm</td>
<td>(max 0.3 cm)</td>
<td></td>
<td>muscle</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Labia minora</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Gracilis muscle margin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>January</td>
<td>Resection of: Abdominal wall:</td>
<td>3 nodules</td>
<td>+</td>
<td>Subcutis, striated</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>10.5 x 9.5 cm</td>
<td>(max 5.2 x 2.5 cm)</td>
<td></td>
<td>muscle invasion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pubic tubercle</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Partial omentectomy</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Proximal-type epithelioid sarcoma of the mons pubis: report of a case

Figure 1. — a) First biopsy: multinodular pattern; aggregates of lymphocytes in follicles (H&E 12.5x).
b) First biopsy: at immunohistochemistry scattered groups of cells are positive for cytokeratins (MNF116 100x).
c) First surgical enlargement. Scattered microscopic foci (< 3 mm) of discohesive neoplastic cells, mainly in the deep right margin (H&E 31x).
d) Fifth surgical enlargement: neoplastic cells with large vesicular nuclei and prominent nucleoli surrounding and infiltrating a neural trunk (H&E 200x).
e) Last surgical excision: neoplastic cells infiltrating the muscle of the abdominal wall close to the resection margin (marked by India ink) (H&E 25x).
f) Detail of Figure 1e (H&E 200x).
Considering the insidious behavior of PES, the gold standard would be to ensure, with the first surgical approach, the best level of radicality, avoiding the necessity of a following intervention to enlarge the margins of resection. Some authors underline that the execution of needle or excision biopsy, with a diagnostic aim, can be dangerous considering the high risk of neoplastic cells spreading; this concept, important in all oncological surgery, is actually essential in case of high-grade sarcoma [5]. Moreover the literature reports that epithelioid sarcoma in cytology specimens shares many features with other neoplastic and non-neoplastic conditions and that the relatively low specificity in the classification is the main limitation of fine needle aspiration biopsy [21, 22].

Taking into account the difficulty for the surgeon to identify the limits of the mass and the underhanded way of spreading, the necessity to perform wide demolitive surgery as soon as possible is evident. Mass extirpation with wide margins (> 2 cm) should be integrated by locoregional lymphadenectomy, although, as in our case, the lymph nodes are usually involved later.

It is also important to underline that radical intervention, which should be fundamental as the first approach, is not common in case of unsuspected trivial small masses, all the more considering the absence of elements capable of addressing suspected malignancy.

Chemo- and radiotherapy were performed without effect, confirming that adjuvant therapy presents limited efficacy to control both local relapse and metastases [5]. Nevertheless, adjuvant radiation therapy is recommended in soft-tissue sarcoma, while the efficacy of chemotherapy is still unclear and only a few drugs result effective, even if only partially: anthracycline, ifosfamide, dacarbazine [5].

Few cases of this rare sarcoma localized in the female genital area have been described until now; a recent review considers about 20 cases of vulvar localization described in the literature [3]. The low clinical experience in dealing with this kind of pathology can lead to diagnostic errors due to the rarity of the disease and to lack of knowledge about soft tissue sarcoma in the scientific background of the gynecologist.

Considering the possible localization of PES in the genital region, though rare, awareness of the existence of this entity and knowledge of its deceitful behavior are important for a quick and correct management. Gynecologists should keep in mind PES as a possible differential diagnosis when a subcutaneous mass of the perineal area or external genitalia is observed during a visit [22, 23].

References


Off-midline retroperitoneal choriocarcinoma presenting as neurologic symptoms

J.C.W. Chien¹, Y.L. Hsiao², S.E. Lin³, W.P. Chan¹ ²
¹Department of Radiology, Taipei Medical University-Wan Fang Hospital
²Department of Radiology, School of Medicine, Taipei Medical University
³Department of Pathology, School of Medicine, Taipei Medical University, Taiwan (Republic of China)

Summary

A 28-year-old woman suffered from frequent headaches. She had a history of a dilatation and curettage for hydatidiform moles. This admission showed markedly elevated levels of human chorionic gonadotropin (hCG) and lactate dehydrogenase. Brain MRI showed a hemorrhagic mass in the left temporal area, with rapid growth. Histology of tumors obtained from multiple areas including retroperitoneum was consistent with choriocarcinoma.

Key words: Brain; Choriocarcinoma; Computed tomography (CT); Magnetic resonance imaging (MRI), Retroperitoneum.

Introduction

Choriocarcinoma has been reported to occur in 1 in 40,000 pregnancies [1]. It can present with variable clinical symptoms owing to the tendency to metastasis. We report the case of a 28-year-old woman with an initial presentation of neurological symptoms of an off-midline retroperitoneal mass.

Case Report

A 28-year-old woman suffered from frequent headache almost every day, but the symptoms could be controlled by painkillers. One month before admission, the symptoms had become worse, with intractable headache, nausea and vomiting that could not be relieved by medication. She had recent chest tightness.

The menstrual cycle of the patient had become irregular two years after this clinical manifestation. She had been pregnant three times, with two live births and one termination. Five years earlier, she received dilatation and curettage for hydatidiform moles. The level of human chorionic gonadotropin (hCG) had returned to the normal range after one year, without medication. The last menstrual period was two weeks before the presentation. As her pregnancy test was positive, she had transvaginal ultrasound, which showed no detectable mass in the uterus or adnexa. Her vital signs were normal. Laboratory test results revealed a markedly elevated level of hCG (185839m IU/ml) and lactate dehydrogenase (700 IU/ml).

During this admission, chest radiograph revealed a soft-tissue mass, measuring 11.5 × 9 cm, in the left upper lung zone, with pleural attachment. Echo-guided biopsy of the chest showed anaplastic carcinoma. Magnetic resonance imaging (MRI) of the brain showed a mass, 1.6 × 2.5 × 1.7 cm, in the left temporal area (Figure 1A). The tumor showed gradient-echo darkness and perifocal edema (Figure 1B), indicating focal hemorrhage. The mass had a high signal on T1-weighted images, a low signal on T2-weighted images, and heterogeneous enhancement on postcontrast images (Figure 1C).

On the 17th day after admission, she became drowsy. Computed tomography (CT) scan of the brain showed rapid growth of the mass (Figure 1D), 2.7 × 3 × 2.3 cm, with heterogeneous density in the left temporal lobe with prominent perifocal edema and a mass effect caused compression of the left lateral ventricle, and impending uncal herniation. CT of the abdomen showed a loculated hypodense mass, 9 × 9.1 × 7.5 cm, with central necrosis and peripheral intense enhancement, located in the infrahepatic region, with transverse abdominal muscle involvement (Figure 1E). There was no demonstrable tumor in the uterus. Diagnostic dilatation and curettage revealed only hyperplastic changes.

The patient received craniotomy with total removal of the brain tumor. A dark brownish blood clot was mixed with grayish tumor, and a well-encapsulated tumor at the temporal tip of the left petrosal bone. Microscopy showed choriocarcinoma with blood lakes surrounded by nests of neoplastic cells characterized by pleomorphic nuclei, prominent nucleoli, and amphiphilic cytoplasm, and scattered multinucleated cells. The tumor represented syncytial growth and marked central hemorrhagic necrosis (Figure 1F). The tumor cells were positive for hCG.

The patient received a chemotherapy regimen (bleomycin, etoposide, and cisplatin) three days after the brain surgery. After three courses of chemotherapy, the level of hCG was 7881 mIU/ml and the left lung tumor had become smaller.

The patient then underwent left upper lobectomy of the lung, wedge dissection of the left lower lobe and lymph node dissection. Histology revealed choriocarcinoma. There was no invasion at the hilar and mediastinal lymph nodes.

After that this patient received the fourth course of chemotherapy. The level of hCG decreased to 56183 m IU/ml but then the level persisted and increased slightly. A simple hysterectomy was performed first. Then the patient had a radical right hemicolectomy, which included the right retroperitoneal tumor, partial hepatectomy (segments 3, 5, and 6), partial resection of the inferior wall of the third portion of the duodenum, and ligature of Batson’s plexus. Histology of the uterine specimen showed no choriocarcinoma. The specimens of hepatic tumors and the retroperitoneal mass had total necrosis, and the specimens of the abdominal and duodenal walls showed infiltration by choriocarcinoma cells (Figure 1G).
Figure 1. — A 28-year-old woman suffered from frequent headaches.

(A) Axial T1-weighted (TR 1823/TE 7.7) MRI of the brain shows a mass in the temporal lobe with central isointensity and high signal intensity peripherally (arrow).

(B) Axial T2-weighted image (TR 3500/TE 86.5) shows low signal intensity in the central part of the tumor with extensive perifocal edema and spotty dots of dark signal intensity peripherally (arrows). The dark dots become darker on gradient-echo images, suggestive of hemorrhage. Hemorrhage was proven by histology.

(C) Gadolinium-enhanced axial image shows irregular enhancement (arrow), mainly at the periphery of the tumor.

(D) Follow-up CT scan of the brain 17 days after admission shows rapid growth of the tumor (arrow).
Off-midline retroperitoneal choriocarcinoma presenting as neurologic symptoms

Discussion

In choriocarcinoma, metastasis often develops early and is generally blood-borne because of the affinity of trophoblasts for blood vessels. Most have metastases in the lung (60-95%) and vagina (30-50%) [2]. Disease of the central nervous system is seen in 10% of patients, mostly in those with advanced disease. These patients almost always have concurrent pulmonary or vaginal involvement, or both. These lesions may undergo spontaneous hemorrhage leading to acute focal neurologic deficits [3]. Diagnosis of pulmonary embolization or metastasis is suspected in women with an abnormal chest radiograph after recent abortion or pregnancy in the presence of elevated serum hCG levels. These emboli or metastases manifest as multiple parenchymal nodules on radiography. Occasionally, a large intravascular tumor can also develop [4].

Pulmonary emboli or metastases are markedly vascular, as evidenced by extensive hemorrhage in tumor nodules and adjacent lung parenchyma. Symptoms are usually absent, although dyspnea may develop with extensive embolization. Hemoptysis may result from intrapulmonary hemorrhage [5]. Primary nongestational, extragonadal choriocarcinoma is very rare and always in or near the midline, because of primordial germ cell rests that failed to migrate properly [6].

As in our case, metastasis of unknown origin is the first impression. All of the reported cases have had similar features on imaging: low-density masses with heterogeneous enhancement and with an irregular margin. The clue to the nature of these tumors has been the brain MRI. Nonproportional perifocal edema of the brain mass, darker on gradient echo images, is suggestive of metastatic tumor with hemorrhage [7]. Rapid growth is another characteristic during the disease course. In a woman of childbearing age with multiple hypervascular masses with the characteristics of bleeding and rapid growth, choriocarcinoma should be considered.

Over 95% of malignant sequelae occur within approximately six months of evacuation of a hydatidiform mole [3]. The interval in our case was about five years, another unusual point. The other possibility is primary nongestational, extragonadal choriocarcinoma. But in our case, none of the masses were located in the midline. Because of the affinity of trophoblasts for blood vessels, blood-borne metastasis is not unusual. Although no pelvic mass was noted, the previous molar pregnancy could be the

![Fig. 1e](image1.png)

![Fig. 1f](image2.png)

![Fig. 1g](image3.png)

(E) Postcontrast abdominal CT scan shows a central necrotic tumor with irregular peripheral enhancement. The tumor invades the abdominal wall and anteriorly displaces the ascending colon (arrow), indicating its location in the retroperitoneum.

(F) Photomicrograph of the specimens obtained from the brain and duodenum. Metastatic choriocarcinoma of the brain with nests of tumor cells showing pleomorphic nuclei, prominent nucleoli, and amphophilic cytoplasm, intermingled with scattered multinucleated tumor cells. (H & E x 200).

(G) Choriocarcinoma cell infiltration admixed with blood clots in the serosa of the duodenum. (H & E x 200).
original cause. The probable mechanism may be that some malignant cells escaped to the retroperitoneum and, via venous return, to the lungs, and then spread to brain parenchyma, causing the clinical symptoms.

Multiagent chemotherapy in conjunction with whole-brain irradiation results in acceptable survival rate in patients with metastatic GTN in the brain. Craniotomy is often necessary in fulminant cases [8].

We conclude that in a childbearing woman with multiple disseminated hypervascular masses in the brain, frequent bleeding, central necrosis and a tendency to rapid growth, choriocarcinoma should be considered. Elevated serum hCG levels and careful tracing of any history of a clinical mole, especially early on can lead to a correct diagnosis.

References


Address reprint requests to:
W.P. CHAN, M.D.
Department of Radiology
Taipei Medical University-Wan Fang Hospital
111 Hsing-Long Road, Section 3
Taipei 116, Taiwan (Republic of China)
e-mail: wingchan@tmu.edu.tw
Conservative management of decidualized ovarian endometriotic cyst during pregnancy mimicking malignancy: case report and a review of the literature

T. Tohya, T. Tajima
Department of Obstetrics and Gynecology, Kumamoto Rosai Hospital, Yatsushiro, Kumamoto (Japan)

Summary
We report here the case of a 30-year-old woman with a decidualized ovarian endometriotic cyst (DOEC) during pregnancy mimicking malignancy occurring after fertility-preserving surgery for ovarian carcinoma arising from an endometriotic cyst. Intracystic excrescences appeared in the left ovarian endometriotic cyst at five weeks and three days of gestation. The serum CA-125 level rose to 676.7 U/ml (normal, 0-35). Based on these findings, ovarian carcinoma arising from the left ovarian cyst was strongly suspected. Frequent sonographic examinations revealed that the sizes and quality of the intracystic excrescences remained essentially unchanged. The cyst was evaluated for DOEC during pregnancy. The patient eventually delivered a male infant by normal vaginal delivery. After the delivery, the intracystic excrescences in the left ovarian endometriotic cyst disappeared. Close observation may be a reasonable alternative to antepartum surgery in patients with a DOEC during pregnancy.

Key words: Decidualization; Ovarian endometriotic cyst; Pregnancy; Endometrioma.

Introduction
Management of an ovarian tumor during pregnancy is complex and troubling, especially after fertility-preserving surgery for ovarian carcinoma. We were able to conservatively manage a case of decidualized ovarian endometriotic cyst (DOEC) mimicking malignancy occurring during pregnancy after fertility-preserving surgery for ovarian carcinoma arising from an endometriotic cyst. The literature regarding DOEC mimicking malignancy during pregnancy was reviewed [1-13].

Case Report
This patient was a 30-year-old woman, gravida-4, para-1, who had undergone laparoscopic oophorocystectomy for a right ovarian endometriotic cyst at the age of 21. Histologic examination of the endometriotic cyst revealed that it was benign. Thereafter, she did not undergo gynecologic follow-up examinations. At the age of 24 years, about three years after the first surgery, she visited our hospital complaining of abdominal swelling. Transvaginal ultrasonography and magnetic resonance imaging (MRI) examination revealed enlargement of the right ovary to about 12 cm in diameter and an endometriotic cyst with intracystic excrescences. Laparotomy revealed the diagnosis of right ovarian carcinoma arising from an endometriotic cyst, and right salpingo-oophorectomy, retroperitoneal lymphadenectomy, partial omentectomy and appendectomy were performed. A diagnosis was made of endometrioid adenocarcinoma of the right ovary, Stage I c(b) (pT1cN0M0). Cytology of the ascetic fluid was negative. The uterus and left adnexa were conserved. Thereafter, she successfully completed three courses of monthly paclitaxel and carboplatin combination chemotherapy, and underwent regular follow-up medical examinations.

Discussion
Ovarian carcinomas are a heterogeneous group of neoplasms and are traditionally subclassified based on type and degree of differentiation. It is becoming evident that each major histological type exhibits characteristic genetic defects that deregulate specific signaling pathways in the tumor cells. Moreover, among the most common histological types, ovarian carcinoma associated with endometriosis has recently received increasing attention, and numerous reports about this type of cancer.
are being published in the literature. One of the characteristic findings strongly suggestive of ovarian carcinoma associated with endometriosis is the presence of intracystic excrescences in an ovarian endometriotic cyst. However, the detection of intracystic excrescences in an ovarian endometriotic cyst during pregnancy is often a difficult diagnostic issue. Malignant transformation of ovarian endometriotic cysts is well known. By contrast, DOEC during pregnancy mimicking malignancy is not well recognized, and is a challenging diagnostic entity. Adnexal masses (excluding the physiological corpus luteal cysts of early pregnancy) are seen in about 0.5% to 1.2% of all pregnancies, and 11% of these are endometriotic cysts [14]. Despite the relative high frequency of detection of ovarian endometriotic cysts during pregnancy, decidualization resulting in an appearance mimicking malignancy may be very rare. There are a few reports in the literature of DOEC during pregnancy [1-13]. In our literature review, we identified 13 studies

Table 1. — Time-course of changes in serum CA-125 levels.

<table>
<thead>
<tr>
<th>CA-125 (U/ml) (normal value, &lt; 35 U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before the operation for ovarian cancer: 210.7</td>
</tr>
<tr>
<td>7 months before pregnancy: 33.4 (4 years after the operation for ovarian cancer)</td>
</tr>
<tr>
<td>3 months before pregnancy: 45.6</td>
</tr>
<tr>
<td>1 month before pregnancy: 67.6</td>
</tr>
<tr>
<td>5 weeks 3 days of gestation: 676.7</td>
</tr>
<tr>
<td>9 weeks 3 days of gestation: 173.2</td>
</tr>
<tr>
<td>12 weeks 3 days gestation: 129.4</td>
</tr>
<tr>
<td>16 weeks 3 days gestation: 220.7</td>
</tr>
<tr>
<td>20 weeks 3 days gestation: 255.8</td>
</tr>
<tr>
<td>27 weeks 3 days of gestation: 121.8 (5 years after the ovarian cancer operation)</td>
</tr>
<tr>
<td>31 weeks 5 days of gestation: 94.1</td>
</tr>
<tr>
<td>33 days after delivery: 39.8</td>
</tr>
<tr>
<td>2 months after delivery: 31.6</td>
</tr>
<tr>
<td>3 months after delivery: 26.9</td>
</tr>
<tr>
<td>4 months after delivery: 25.3</td>
</tr>
</tbody>
</table>
reporting on 25 cases of DOEC mimicking malignancy occurring during pregnancy [1-13]. The first case was reported in 1998 [1]. The subsequent reports were by Miyakoshi et al., 1998 (1 case), Tanaka et al., 2002 (1 case), Fruscella et al., 2004 (1 case), Sammour et al., 2005 (2 cases), Guerriero et al., 2005 (1 case), Iwamoto et al., 2006 (1 case), Asch and Levine, 2007 (1 case), Poder et al., 2008, (1 case), Takeuchi et al., 2008, (5 cases), Machida et al., 2008 (3 cases), Yoshida et al., 2008, (2 cases), Barbieri et al., 2009, (3 cases), and Ueda et al., 2010, (3 cases). Many such cases have undergone salpingo-oophorectomy during pregnancy on the basis of suspicious imaging findings, and the histologic examination of these ovarian cysts has revealed that the intracystic excrescences represented edematous vascularized decidualized endometrial tissue with abundant cytoplasm of the stromal cells. In retrospect, these surgical interventions might have been unnecessary. However, the differential diagnosis of a DOEC during pregnancy is difficult, and there are no definitive diagnostic guidelines. An ultrasonographic (US) guideline for DOEC during pregnancy will be necessary. We believe therefore that frequent US examinations before and during pregnancy are necessary in these patients. It is most important to have a high index of suspicion for this entity. Close observation may be a reasonable alternative to antepartum surgery in patients with a DOEC during pregnancy. The reporting of further cases of this entity should be encouraged, because analysis of even singular cases may provide important data for future research and development of treatment.

Serum CA-125 may be a useful marker for the diagnosis and monitoring of these cases. However, natural elevation of serum CA-125 during normal early pregnancy is a stumbling block. The present case may be the first of this entity occurring after fertility-preserving surgery for ovarian carcinoma arising from an endometriotic cyst.

References


Address reprint requests to:
T. TOHYA, M.D.
Department of Obstetrics and Gynecology
Kumamoto Rosai Hospital
Yatsushiro, Kumamoto 866-8533 (Japan)
e-mail: sanfu-tohya@kumamotoh.rofuku.go.jp
Primary squamous cell carcinoma of the endometrium: a case report

G. Bifulco¹, P. Giampaolino¹, V.D. Mandato¹, I. Morra¹, C. Nappi¹, L. Insabato²

¹Department of Gynecology and Obstetrics, and Pathophysiology of Human Reproduction, ²Department of Biomorphologic and Functional Sciences, Pathological Anatomy Section, University of Naples “Federico II” (Italy)

Summary

Background: Primary endometrial squamous cell carcinoma (PESCC) is a rare neoplasm. Squamous epithelium derived from endometrioid cancer or from cervical squamous cell carcinoma. The prevalence is about 0.1%. The genesis, histogenesis and biological behavior are unknown. Case presentation: A 48-year old woman in postmenopause, referring pelvic pain and vaginal bleeding. Transvaginal ultrasound showed a bulky uterus with the endometrium containing an hyperecogenic area. Endometrial biopsy found an epidermoid carcinoma. MRI showed a 4 x 2 x 1.2 cm mass occupying the uterine cavity. The patient underwent radical treatment. Pathological examination showed features of PESCC. The mutation of p53 tumor suppressor protein was disclosed in 15% of neoplastic cells. PCR revealed the absence of HPV DNA. Conclusions: The findings of our case move us to underline that the pathogenesis of this tumor is still unclear. Moreover, preoperative diagnosis and staging of PESCC is extremely difficult. Most patients do not show characteristic symptoms and predisposing factors, making it almost impossible to diagnose the precise localization of tumor origin.

Key words: Endometrium; Squamous cell carcinoma; Vaginal bleeding; Menopause.

Introduction

Primary endometrial squamous cell carcinoma (PESCC) is an exceedingly rare neoplasm, with fewer than 100 cases reported in the English literature [1]. Squamous epithelium in the uterine corpus is most commonly found as a form of differentiation in endometrioid carcinomas or as a result of extension of cervical squamous cell carcinoma [2], while only sporadic primary squamous cells are found in the endometrium. The prevalence of this neoplasm is about 0.1% [3] and there is no unanimous opinion about the genesis of this tumor and, due to its extreme rarity, its histogenesis, biological behavior and the optimal management strategy are still debated.

In this report we present the clinical and pathological findings of a case of PESCC in a 48-year old woman.

Case Report

A 48-year-old woman, in about one year of postmenopause, was referred to our department for pelvic pain and vaginal bleeding. The patient had no history of IUD use and pyometra. General physical examination was unremarkable and gynecological examination revealed a normal cervix, an enlarged uterus, and non-palpable adnexa. Transvaginal ultrasound showed a bulky uterus with the endometrium containing an hyperecogenic area. Subsequent hysteroscopy showed a polypoid mass arising from thick endometrium extending until the isthmus. Endometrial biopsy found an epidermoid carcinoma.

Magnetic resonance imaging (MRI) showed a 4 x 2 x 1.2 cm mass occupying and expanding the uterine cavity, and reaching the cervix. The serosal surface of the uterus was not disrupted. The vagina and adnexa appeared normal (Figure 1).

The patient underwent exploratory laparotomy; no extrauterine tumor was revealed. There was no pelvic or paraaortic lymphadenopathy.

A type II radical hysterectomy with bilateral salpingo-oophorectomy and bilateral pelvic lymphadenectomy, and paraaortic lymph node sampling were performed; peritoneal washing was taken for cytology.

Grossly the uterus measured cm 13 x 7 x 5; on cut section, in the endometrial cavity a friable and polypoid lesion, measuring 5 cm was observed (Figure 2); the lesion extended up to the internal uterine orifice. No lesions were found in the cervix.

Histologically, the polypoid lesion was characterized by a poorly differentiated no keratinizing squamous cell carcinoma of the endometrium, with a trabecular and papillary pattern of growth composed of large cells with slightly pleomorphic nuclei and conspicuous nucleoli. The tumor showed high proliferative activity and moderate necrosis (Figure 3). The lesion featured exophytic growth in the endometrial cavity, with early myometrium invasion (Figure 4).

The residual endometrium showed normal glandular architecture. The cervix was completely sampled, and only chronic cystic cervicitis was seen; no HPV infection was found. Both ovaries, vaginal flaps and lymph nodes (40 pelvic lymph nodes, 6 paraaortic lymph nodes) were unremarkable.

Immunohistochemically, the neoplastic cells were negative for estrogen and progesterone receptors.

Immunostaining with p53 tumor suppressor protein disclosed the mutation of p53 tumor suppressor protein as nuclear positivity in 15% of neoplastic cells. PCR revealed the absence of HPV DNA.

The molecular study with PCR in this report revealed the absence of HPV DNA. Final pathology diagnosed a primary endometrial squamous cell carcinoma in FIGO Stage IB, grade 3.

The patient was free of disease at one year of follow-up control.
Discussion

PESCC is a rare malignancy. The first description of this neoplasm was given by Gebhard in 1982 [4] and only sporadic cases have been published in the literature. The prevalence of this neoplasm is about 0.1% of endometrial carcinomas [5].

This neoplasm occurs in postmenopausal women and many factors predisposing its development have been suggested, such as pyometra, chronic inflammation, pelvic radiation, estrogen deficiency and estrogen excess [2]. The clinical manifestations of this neoplasm are postmenopausal bleeding, vaginal discharge, abdominal pain, weight loss, and pelvic mass.

Therefore, for a correct identification of this neoplasm, four criteria must be fulfilled: 1) there should be no co-existing adenocarcinoma of the endometrium; 2) there should be no demonstrable connection between the tumor

Figure 1. — Pelvic MRI. A 4 x 2 x 1.2 cm mass occupying and expanding the uterine cavity, and reaching the cervix can be seen.

Figure 2. — Macroscopic appearance of the excised mass. Gross features of the surgical specimen showing a 5 cm polypoid lesion in the uterine cavity.

Figure 3. — Histological examination of the excised mass. Microscopic appearance of the mass showing cords and trabeculae of large cells with moderate necrosis (HE, 4x).

Figure 4. — Enlarged view of the excised mass. Panoramic view of the lesion showing exophytic growth in the uterine cavity.
in the endometrium and the squamous epithelium of the cervix; 3) there is no co-existing primary squamous cell carcinoma of the cervix; 4) the tumor must exhibit keratinization and/or intercellular bridges [5].

Thus, the neoplasm must be carefully examined to exclude endometrial carcinoma with squamous differentiation and the cervix must be completely sampled to exclude the presence of squamous cell carcinoma extending to the endometrium [6]. The etiopathogenesis of PESCC is still unknown because of its rarity, but various controversial hypotheses have been proposed to determine pathogenetic mechanisms. In 1993, Horn and Bilek [7], based on immunohistochemical results with anticytokeratin (KLI), anti CEA and anti-vimentin, suggested that PESCC may be the result of a bidirectional differentiation of pluripotent endometrial precursor cell. Afterwards, Yamamoto et al. [8] suggested that this neoplasm arises from heterotrophic cervical tissue. Most recently, many authors proposed a direct role of human papilloma virus (HPV) infection, especially type 16 as is well known that high-risk HPV subtypes are strongly associated with squamous neoplasms of the cervix.

Furthermore the relationship of HPV antigen or HPV DNA has been provided in the squamous component of endometrial adenocarcinoma [5]. However, the results of these studies are controversial; in fact, some authors did not detect HPV in cases of PESCC by in situ hybridization and thus concluded that HPV infection may be a carcinogenic factor in the development of this neoplasm [9]. Another hypothesis was the malignant transformation of metaplastic endometrial cell that may result from various but nonelucidated processes such as chronic inflammation, HPV 6 and 11 infection, post radiation changes [8-10]. Other pathogenetic mechanisms which cause a mutation of p53 tumor-suppression gene, may have been involved in the development of PESCC [6]; indeed, the significance of p53 positivity would suggest that the tumor probably arises from a mutation of the p53 tumor suppression gene.

Our patient did not present any aforesaid predisposing factor, did not show any area of squamous metaplasia in the endometrium or abnormal endocervical glands in the uterine cavity.

According to the literature, immunohistochemical analysis revealed that the neoplasm did not contain estrogen and progesterone receptors.

In the index case HPV analysis failed to show the presence of HPV DNA and immunostaining with p53 tumor suppressor protein disclosed the mutation of p53 tumor suppressor protein as nuclear positivity in only 15% of neoplastic cells.

Moreover, we confirmed that preoperative diagnosis and staging of PESCC is really difficult. Most patients do not show characteristic symptoms and predisposing factors, making it impossible to diagnose the precise localization of tumor origin.

Conclusions

The findings of our case (negative immunostaining for HPV, weak positivity of p53 mutation, absence of squamous metaplasia) move us to underline that the pathogenesis of this tumor is still unclear. Moreover, at this time final treatment recommendations given so far include therapy usually consisting of surgical hysterectomy with salpingo-oophorectomy and radiotherapy in some cases.

Considering the rarity of this ominous tumor, multicenter trials should be planned to identify the risk factors, to clarify the pathogenesis until achieving a correct diagnosis and finally to suggest an adequate treatment to improve survival.

References

Angiomyofibroblastoma of the vulva: a clinicopathological and immunohistochemical analysis of a rare benign mesenchymal tumor

E. Kairi-Vassilatou1, C. Dastamani1, E. Vouza1, P. Mavrigiannaki1, D. Hasiakos2, A. Kondi-Pafiti1

1Pathology Laboratory and 2nd Department of Obstetrics and Gynecology, Aretaieion University Hospital, Athens (Greece)

Summary
Angiomyofibroblastoma is a rare benign distinctive mesenchymal tumor that occurs in the genital pelviperineal region, commonly the vulva and vagina. We report a case of angiomyofibroblastoma in a 42-year-old woman, presenting as a “cystic mass” located subcutaneously in the right labial area of the vulva. Recognition of this entity is based on specific histological and immunopathological features, and the correct treatment is important because of the aggressive behavior of other related mesenchymal tumors of the vulva and vagina, such as angiomyxoma and cellular angiofibroma.

Key words: Angiomyofibroblastoma; Immunohistochemistry; Vulva.

Introduction
Angiomyofibroblastoma (AMF) is a rare benign mesenchymal tumor of myofibroblastic origin that arises in the genital pelviperineal region. This tumor occurs in women of all ages (mean age 46 years) who present with a painless, slowly growing, well circumscribed subcutaneous vulvar, or less commonly vaginal mass. The clinical diagnosis is usually that of a labial or Bartholin gland cyst. According to the literature, most of these neoplasms are less then 5 cm in diameter and the size ranges from 0.5 cm to 12 cm [1-4].

We report the clinicopathological and immunohistochemical findings of a case of angiomyofibroblastoma in a 42-year-old Caucasian woman admitted to Aretaieion University Hospital in July 2009. The features that aid in the differential diagnosis from other related mesenchymal tumors of the vulva are summarized.

Case Report
A 42-year-old Caucasian woman presented with a painless mass in the right vulvar region that she had first noticed three years previously. The initial clinical diagnosis was that of labial cyst. The patient underwent tumorectomy. The excised tumor was well circumscribed and measured 4 x 3.5 x 2 cm. The cut surface was solid, homogeneous and gelatinous. It appeared light gray to tan in color and had a soft to rubbery consistency. Neither hemorrhage nor necrosis was seen.

Pathological findings
Microscopically, in multiple hematoxylin-eosin stained sections the tumor was well circumscribed but not encapsulated, and was composed of hypercellular and hypocellular areas (Figure 1). The tumor cells were spindle, plump spindle or oval in shape without any mitotic activity. Mild focal atypia was observed. Scattered among them, mast cells and a few lymphocytes were seen. The vascular component of the lesion was prominent and consisted of numerous small to medium sized vessels, predominantly of the capillary type, with open rounded luminae (Figure 2).

The stroma of the tumor consisted of loosely textured delicate wavy collagen fibers, which occasionally condensed to form more solid areas of fibrosis.

Immunohistochemical investigation by the Ventana immunohistochemistry automation system showed that the tumor cells were strongly and diffusely positive to desmin (Monosan – cl. D33), vimentin (Monosan – cl. V9), ER (Novocastra – cl. GF11), PgR (Neomarkers – cl. SP2) and negative to SMA (Biogenex- cl. 1A4), cytokeratins (Monosan – cl. 80), s-100 protein (Cell Marque – cl. 4C4.9), CD34 (Serotec – cl. MCA547G) and CD31 (Sckytek – cl. 1C/70A). The average labeling index of ki-67 was < 1% focally (Figure 3).

Discussion
A variety of mesenchymal tumors such as aggressive angiomyxoma, AMF, cellular angiofibroma, and superficial myofibroblastoma composed of morphologically bland fibroblastic and myofibroblastic cells have a propensity to occur within the soft tissues of the lower female genital tract [1, 2].

At least some of these tumors arise from hormonally responsive fibroblastic or myofibroblastic superficial stromal cells of the lower genital tract [5] that show variable expression for vimentin, smooth muscle markers and estrogen and progesterone receptors. Histogenetically related to these tumors are also the fibroepithelial polyps of the vagina and cervix.

This variety of histogenetically related mesenchymal tumors may present problems in the differential diagnosis because of their overlapping histological and immunohistochemical features.

AMF was first described in 1992 by Fletcher et al. [6].
The tumor arises in the superficial soft tissues of the vulva and vagina in women of reproductive and postmenopausal age (ranging in age from 23 to 86 years old [mean 45.8 years old]). It can also occur occasionally in the perineal region, the inguinal area within the canal of Nuck and the scrotal and inguinal area in males [1-4]. One case of AMF in the female urethra and one case in the fallopian tube have been reported [7, 8].

Patients usually present with a painless vulvar or less commonly vaginal mass that has existed from a few weeks to 14 years and the clinical diagnosis is often that of a Bartholin gland cyst.

Grossly the tumors are typically well circumscribed, but not encapsulated measuring 0.5 to 12 cm in diameter (mean diameter 4.5 cm).

Microscopically AMF is a well demarcated tumor, composed of alternating hypercellular and hypocellular edematous zones. Neoplastic cells are typically spindle-shaped, but are often oval, epithelioid or plasmacytoid in appearance with eosinophilic cytoplasm and are aggregated around blood vessels, forming nests or cords, or are loosely dispersed in the hypocellular areas. The nuclei of tumor cells are typically bland and occasionally multiple. The plasmacytoid cells have eccentric nuclei. In 40% of cases of AMFs, enlarged and hyperchromatic nuclei have been described. Mitoses are rare or absent although a mitotically active variant of AMF has been reported [9].

Numerous small to medium sized thin walled arborizing vessels, predominately of the capillary type, are irregularly distributed within a variably edematous or collagenous stroma. The stroma of AMF is edematous or fibrocollagenous with wavy strands or rarely thick bundles of collagen. Fat is present in some cases, and a lipomatous variant of AMF has been described [10]. Scattered in the stroma, mast cells and a few lymphocytes may be observed.

Immunohistochemically the neoplastic cells stain for desmin and vimentin, frequently for estrogen and progesterone receptor protein and occasionally for actin and CD34. The positivity of lesional cells for estrogen and progesterone receptors even in postmenopausal women might be a reflection of the normal presence of these receptors in the stroma of the subepithelial myxoid zone of the vulva Ultrastructurally, the tumor cells have features of myofibroblasts, with paranuclear clusters of intermediate filaments in the plasmacytoid cells [11].

The differential diagnosis of this neoplasm includes aggressive angiomyxoma, superficial angiomyxoma (cutaneous myxoma), cellular angiofibroma, and fibroepithelial polyps with cellular stroma as well as more common mesenchymal tumors such as leiomyoma, neurofibroma, neurilemmoma and solitary fibrous tumors [1-4].

AMF in contrast to aggressive angiomyxoma has circumscribed borders, more blood vessels that usually are...
thin walled, predominately of the capillary type. It is also more cellular with plump to epithelioid tumor cells that often occur perivascular condensation and has a lesser degree of stromal myxoid change. Mucin stains and immunohistochemical results have not proved particularly helpful in the differential diagnosis of these two entities [12, 13]. Superficial angiomyxoma (cutaneous myxoma) may recur and presents clinically as a painless papular or nodular mass that may be solitary or multifocal. The overabundant mucin in the lesions forms pools or clefts at the interface of the tumor nodule with the adjacent stroma. In contrast to AMF, the spindled and stellate-shaped cells scattered throughout the process do not concentrate around vessels. The vascular component of superficial angiomyxoma is also less pronounced than that of AMF. Cellular angiofibroma is an uncommon, usually well circumscribed soft tissue tumor, that most commonly arises in the vulva and vagina and rarely in the vagina, perineum, inguinal region or urethra. This tumor differs microscopically from AMF with its vascular pattern of medium sized vessels, often with thickened hyalinized walls. In addition, it lacks the perivascular clustering of epithelioid or plasmacytoid stromal cells. Immunoreactivity for desmin and negative staining for CD-34 favor angiomyofibroblastoma.

Unlike superficial myofibroblastoma cellular angiofibroma typically contains perivascular aggregates of epithelioid or plasmacytoid stromal cells and usually lacks the multipatterned architecture of myofibroblastoma. The immunoprofiles of these tumors are similar.

 Fibroepithelial stromal polyps occur primarily in the vagina, but also have been reported in the vulva and cervix [5]. AMF is usually well demarcated and is less likely to be polyoid or papillary such as a fibroepithelial stromal polyp. Histologically the latter may bear a superficial resemblance to AMF because of its composition of spindled, stellate and multinucleated stromal cells within a loosely textured stroma. The vascular component of a fibroepithelial stromal polyp is usually composed of dilated thick-walled vessels and the stromal cells exhibit variation in size and shape without any perivascular concentration. The immunoprofiles of the above tumors are similar except that a fibroepithelial stromal polyp is more likely to express CD34.

Leiomyomas of the vulva present clinically in the same manner as AMF. Histologically they are typically more cellular than AMF and usually show areas more characteristic of smooth muscle differentiation. Immunohistochemically they exhibit strong and diffuse reactivity of muscle specific actin and smooth muscle actin.

In conclusion AMFs of the vulva are rare benign tumors with distinct morphology and immunophenotype that permit the correct diagnosis and therapeutic procedures.

References

Address reprint requests to:
A. KONDI-PAFITI, M.D.
Department of Pathology
University of Athens
Aretaieion Hospital
76 Vassilisis Sophias Avenue, 1
11528, Athens (Greece)
e-mail: akondi@med.uoa.gr
Krukenberg tumor of gastric origin in pregnancy with dismal outcome

J. Stojnic, A. Stefanovic, K. Jeremic, S. Kadija, M. Jefitovic, J. Jeremic

Institute of Gynecology and Obstetrics, University of Belgrade, Clinical Centre of Serbia, Belgrade (Serbia)

Summary

Krukenberg tumors are mostly found as metastatic signet-ring cell adenomucinous carcinomas in young, premenopausal women. They are bilateral in 80% of the cases, and thus can be expected in pregnancy. A 31-year-old female was diagnosed by explorative laparotomy at 27 weeks of gestation with a Krukenberg tumor due to bilateral adnexal masses and a large amount of ascites. At surgery cesarean section with total abdominal hysterectomy, bilateral salpingo-oophorectomy, total omentectomy and pelvic lymphadenectomy was performed. The neonate died 24 hours later due to prematurity and respiratory distress syndrome. The primary site of the cancer was detected metachronously two months after surgery and postoperative chemotherapy, as stomach adenomucinous carcinoma. In spite of surgery and postoperative multiagent chemotherapy, the patient died six months from the diagnosis of Krukenberg.

Key words: Krukenberg tumor; Pregnancy; Therapeutic approaches; Prognosis.

Introduction

Krukenberg tumors are uncommon metastatic tumors of the ovary, initially described by Friedrich Ernst Krukenberg in 1896 [1] as fibrosarcoma ovarii mucocellulare carcinomatoises and assumed to be a primary ovarian tumor. Krukenberg tumors are mostly found as metastatic signet-ring cell adenocarcinomas in young, premenopausal women. They are bilateral in 80% of the cases, and thus they can be expected in pregnancy [2]. To date, more than 40 cases of Krukenberg tumors in pregnancy have been documented.

The diagnosis of Krukenberg tumor is rarely made before or even at laparotomy, particularly when associated with pregnancy [3]. The prognosis of Krukenberg tumor is very poor compared to primary ovarian cancer and generally with no treatment protocols yet established [4]. Early diagnosis and radical surgical resection of localized disease are the only hope for a better life quality and longer survival rate.

We report a case of ovarian metastatic signet-ring gastric carcinoma in a 31-old-pregnant female, diagnosed in the 27th week of pregnancy, after urgent laparotomy from intractable abdominal pain, bilateral ovarian masses and large amount of ascites. The final result was unfavorable for the fetus and for the mother, whose survival period was six months after laparotomy.

Case Report

A 31-old-female, gravida 2, para 1, was admitted to our clinic due to of recurrent abdominal pain and swelling, distension of the abdomen, occasional vomiting, and mild pregnancy-induced hypertension at 27 weeks of gestation. The patient presented a month prior to admission with symptoms of abdominal pain, fatigue, decreased appetite, elevation of blood pressure (to 140/90 mm Hg), and more frequent vomiting, with no gain in the body mass. The symptoms were not paid attention to, and they were assigned to aggravated pregnancy symptoms. The patient received symptomatic therapy with antihypertensive drugs and antacid medication. In the last few days prior to admission respiratory problems with dyspnea and problems with stool passage occurred.

Her past medical history included chronic hepatitis C infection. The course of the first pregnancy was expectable, with a term delivery of a healthy male child. Her family history revealed an ovarian carcinoma (sister), and gastric carcinoma (father).

On admission transabdominal ultrasound revealed bilateral asymmetrical ovarian tumors (diameter on the left side 200 x 140 x 80 mm and on the right about 190 x 150 x 95 mm), with heterogeneous echogenicity and massive abdominal ascites. The tumors consisted predominantly of a solid area centrally with dispersed tumor vessels and low impedance flow detected by color Doppler scan and mostly cystic areas peripherally. In the uterus a single live fetus was identified, morphologically and functionally developed as could be expected for the gestational age. Detailed ultrasound exam of the abdomen detected a mild enlargement of the liver and spleen, but without focal lesions.

Laboratory tests discovered mild anemia with a hemoglobin concentration of 112 g/l (normal range 120-150 g/l) and 82 g/l (normal range 60-80 g/l) as high levels of blood proteins. Liver function tests were normal. The patient’s coagulation tests (prothrombin time (PT), partial thromboplastin time (PTT), thrombin time) and fibrin degradation product (FDP) levels were within acceptable pregnancy range values. A platelet count of 580,000 x 10⁶ per liter was regarded as thrombocytosis with an addition of elevated fibrinogen levels to 5.7 g/l.

Since she suffered from dyspnea due to abdominal distension, 500-1500 ml of bloody ascites was aspirated daily through an abdominal drainage tube and cytological analysis was positive for malignant cells. A chest X-ray showed no pulmonary deposits with a small right pleural effusion. A detailed MRI of
the abdomen and gastrointestinal tract and liver was performed, but without a trace of any suspect secondary lesion or peritoneal deposits.

The level of CA 125 was 500 U/ml, alfa-fetoprotein 88.8 ng/ml, both highly increased, while levels of CEA were 3.5 ng/ml and CA 19-9, within normal range.

The tumors are suspected to be a primary ovarian malignancy and exploratory laparotomy is performed. About 6000 ml of ascites was drained and right salpingo-oophorectomy performed as a first step. The diagnosis of a malignant epithelial tumor was made intraoperatively by frozen section evaluation, leading to a decision for radical surgery. It was decided that cesarean section should be performed next and a male child weighing 1,000 g was delivered with Apgar scores of 1 and 2 at one and five minutes respectively. The child died 24 hours later due to prematurity and respiratory distress syndrome. The progressive course of surgery included total abdominal hysterectomy, left salpingo-oophorectomy, total omentectomy, appendectomy and bilateral pelvic lymphadenectomy. Gross inspection of the stomach, intestines, liver, spleen and peritoneum revealed no macroscopic visible signs of metastatic disease or peritoneal implants.

Definitive histopathology examination revealed a Krukenberg tumor with poorly differentiated metastatic adenocarcinoma of mucin-secreting type with large signet-ring cells. It was dispersed through both ovaries, the perimetrium, omentum and was present in three of 14 pelvic lymph nodes with invasion of vascular spaces. Mucincarmine stain demonstrated the large cytoplasmatic vacuoles to be mucin. Tissue specimens from both ovaries demonstrated immunohistochemical reactions – positive staining for cytokeratin CK 7, CK 20 and polyclonal CEA markers, indicating metastatic upper gastrointestinal carcinoma.

Postoperative recovery was without complications and the patient received several courses of multiagent chemotherapy with 5-fluorouracil, cisplatin, and epirubicine.

In search of primary carcinoma, gastroscopy and rectosigmoidoscopy were performed and revealed a gastric ulcer about 1.5 cm in diameter at the greater curvature of the stomach. A biopsy was performed and pathohistological examination of the biopsy specimen demonstrated a poorly differentiated adenocarcinoma with large mucin-secreting signet-ring cell type in the lamina propria. In spite of aggressive surgical management and postoperative chemotherapy, the patient died of advanced disease six months after diagnosis.

Discussion

Malignant ovarian tumors are uncommon during pregnancy, but not exceedingly rare, with an incidence of one in 10,000 to 25,000 deliveries [5, 6]. The most usual malignant ovarian tumors are germ cell tumors (about 40-50%), epithelial carcinoma (about 35%), metastatic tumors of the ovary (10%), sex-cord and stromal tumors (9%) and about 7% of miscellaneous tumors [7].

About 29% of all metastatic ovarian malignancies are Krukenberg tumors, and about 73% of them are of gastric origin. The Krukenberg tumor occurs in young patients (less than 40 years) more often than other common types of metastatic ovarian tumors [8]. Today, only a small number of cases are classified as primary tumors. Krukenberg is almost an exclusively metastatic, secondary tumor in the ovary, originating from gastric carcinoma (70%), the intestines (11%) – usually colon and rectum, breast (4%), biliary system (3%) and the remaining 3% include sites as the pancreas, uterine cervix, urinary bladder (including urachus), renal pelvis and non Hodgkin’s lymphoma [9].

A pregnancy-associated Krukenberg tumor is very rare (40 cases up to today) and the diagnosis in pregnancy is even more difficult [10]. The estimated 5-year survival of nonpregnant patients is 12.1% according to Jiang et al. [4], only 5.4% in 357 patients with cancer of gastrointestinal origin according to Webb et al. [11] and 10% were reported by Petru et al. [12] in a series of 82 patients where all the patients died within 58 months.

Prognosis of Krukenberg tumors is extremely poor compared to that of primary ovarian cancer. There have been only a few studies of Krukenberg disease since the discovery, because of its low incidence. Accordingly, the treatment approaches of this metastatic ovarian tumor are still a matter of controversy.

Detection of ovarian neoplasms in pregnancy is a diagnostic and therapeutic challenge [13]. Routine use of ultrasound in early pregnancy has increased the detection rate of associated ovarian tumors. Late clinical examination and ultrasound exams are less accurate. In cases where ultrasound results are suggestive of malignancy, an intraoperative diagnosis must be reached by use of frozen section. For all adnexal masses exceeding 6 cm with a complex structure or ascites and persistence or rapid enlargement, surgical intervention is important to obtain a final histological diagnosis and to rule out malignancy [14]. According to Lerner’s scoring system of sonographic criteria for ovarian tumors the risk of malignancy is high when the mass is solid (more than 5 cm in diameter) with nodules and thick septations [15]. Additionally color Doppler can be a useful tool to help differentiate between a benign or malignant ovarian mass. However, because of overlap in blood patterns such that the false-positive rate is nearly 50% [16], there is no advantage over use of ultrasound morphology indexing alone.

The management of each patient has to be individualized taking into consideration patient age, parity, stage of the tumor, duration of pregnancy, desire to conserve fertility and primary tumor location. The accurate definitive diagnosis of Krukenberg in pregnancy is usually at the time of laparotomy together with diagnosis of a primary tumor (synchronously) in two-thirds of cases. In most cases at the time of diagnosis of the Krukenberg, the primary tumor is in advanced stage. When diagnosis of a primary tumor is made before or after the diagnosis of Krukenberg (metachronous) there is significant impact on survival rate. However in about 5-10% of Krukenberg tumors the primary tumor site was not detected during the life time [17].

Acute Krukenberg syndrome is a suddenly emerged episode of abdominal pain originating probably with a rapidly growing tumor in the ovary. The most frequent symptoms and signs of Krukenberg in pregnancy according to the literature are abdominal pain and palpable mass (60.5%). A bilateral lesion is recorded in 64.1% and accompanied ascites in about 42% of cases [18]. Persistent abdominal pain (epigastric) in young pregnant women, with a suspected adnexal mass warrants a
detailed evaluation with endoscopy, computed tomography and ultrasound with color Doppler scans. The possibility of a Krukenberg tumor associated in pregnancy must be kept in mind to achieve a prenatal diagnosis before laparotomy. Growth of the fetus leading to abdominal distention masks the pressure of the metastatic ovarian tumor in the pelvic cavity. Thus, early diagnosis of the tumor may be delayed.

Surgery plays a critical role in the modern therapeutic approach in managing Krukenberg tumor in pregnancy. The diagnosis of Krukenberg tumor is rarely made before or even at laparotomy, particularly when associated with pregnancy [3]. Two-thirds of the primary carcinomas are detected synchronously with, or subsequent to, detection of the Krukenberg tumor, compounding the diagnostic difficulty posed by the cases [19]. Also, the clinical outcomes of surgical treatment for patients with Krukenberg tumors were influenced mainly by the origins of ovarian metastatic carcinoma. The prognosis of those patients with metastatic gastric carcinoma was poorer than those of other origin sites (colorectal cancer) [4].

There have been only a few reports regarding the place and timing of surgical treatment of Krukenberg tumors in pregnancy. Survival analyses confirmed a statistically significant advantage for patients with microscopic residual disease after metastasectomy compared to those with macroscopic residual disease, with a 5-year survival of 23.4% and 0%, respectively [20].

The extent of surgery depends on the intraoperative diagnosis and the extent of primary tumor and Krukenberg tumor involvement of the peritoneal cavity [21]. Timing of surgery for Krukenberg tumors is also a puzzle – whether the surgery should be performed synchronously or metachronously, and there is no significant difference in survival [4].

A role and benefit of intraperitoneal and/or intravenous chemotherapy as adjuvant chemotherapy has not been defined, but is usually provided. There are no sufficient studies regarding survival and potential benefits of chemotherapy [4].

The management of pregnant patients with a malignant ovarian neoplasm is similar to what is recommended in the non-pregnant state. The primary difference lies in the need to consider adjustments in the surgical and chemotherapy treatment to allow fetal viability if the patient desires such. In the setting of aggressive cancer (especially secondary), considerations can be given to preterm delivery and use of surgery with chemotherapy. Distinction between primary and metastatic ovarian tumors is important because misinterpretation of a metastatic tumor as a primary ovarian tumor may lead to inappropriate management and unsatisfactory treatment outcome.

However, the controversy over therapeutic approaches remains because prognosis for these patients is still pessimistic, especially if the Krukenberg originates from gastric cancer. Thus careful assessment should be performed as to whether patients can benefit from surgery and chemotherapy in pregnancy.

References


Address reprint requests to:
J. STOJNIC, M.D.
Institute of Gynecology and Obstetrics
Belgrade Medical School
26th Visegradka Street
11000 Belgrade (Serbia)
e-mail: jelence_01@open.telecom.rs
Alveolar soft part sarcoma of the uterine cervix in a woman presenting with postmenopausal bleeding: a case report and literature review

W.D. Kang¹, S.H. Heo², Y.D. Choi³, H.S. Choi¹, S.M. Kim¹

Departments of ¹Obstetrics and Gynecology, ²Radiology, and ³Pathology, Chonnam National University Medical School, Gwangju (Korea)

Summary

Introduction: Alveolar soft part sarcoma (ASPS) of the uterine cervix is a rare mesenchymal malignancy which occurs in adolescents and young adults. Case report: A 52-year-old postmenopausal woman presented with profuse vaginal bleeding of one month’s duration with severe anemia. The pelvic examination revealed a 3 cm mass on the posterior lip of the uterine cervix. On magnetic resonance imaging, the tumor had high signal intensity on T1- and T2-weighted images. A modified radical hysterectomy and bilateral salpingo-oophorectomy were performed. Immunohistochemical staining for TFE3 and electron microscopic examination revealed an ASPS of the uterine cervix. Discussion: The better prognosis of cervical ASPS, compared to the soft counterparts, may be related to early clinical detection, small size, resectability, and demarcation of the tumor.

Key words: Alveolar soft part sarcoma; Uterine cervix; TFE3; Magnetic resonance image; Postmenopausal woman.

Introduction

Alveolar soft part sarcoma (ASPS) is a rare mesenchymal malignancy which typically occurs on the extremities or thorax of young adults [1]. ASPSs are estimated to account for 0.5% to 1% of all soft tissue sarcomas. A MEDLINE search from January 1966 through July 2009 using the key words, alveolar soft part sarcoma and uterine cervix, resulted in ten English language articles [2-11]. There have been a total of 11 cases of ASPS originating in the uterine cervix. The case described herein is the first case of ASPS occurring on the uterine cervix of a postmenopausal woman.

Case Report

A 52-year-old postmenopausal woman (gravida 2, para 2) presented for evaluation of profuse vaginal bleeding of one month’s duration. The laboratory findings were all within normal limits, except a low hemoglobin concentration (3.8 g/dl). The pelvic examination revealed a 3 cm mass on the posterior lip of the uterine cervix without vaginal or parametrial involvement. A biopsy of the mass was interpreted as ASPS. Magnetic resonance imaging (MRI) revealed a well-circumscribed, hyperintense mass in the cervix on both T1- and T2-weighted images. There were some signal voids around and within the tumor, which corresponded to the enlarged or dilated peritumoral and intratumoral vessels (Figures 1A and B). On contrast-enhanced images, the mass showed homogeneous enhancement (Figure 1C).

A modified radical hysterectomy with bilateral salpingo-oophorectomy was performed. The gross specimen showed a 3.0 x 2.7 cm well-circumscribed yellow solid mass, which was obstructing the endocervix and confined to the cervical stroma. Microscopically, the tumor displayed a diffuse and nest-like growth pattern. The tumor cells were large and rounded with relatively uniform nuclei and prominent nucleoli arranged around blood vessels. Immunohistochemical staining was positive for periodic acid-Schiff (PAS), but negative for HMB-45, S-100, and diastase. The neoplastic nuclei were strongly positive for TFE3 (Figure 2A). Electron microscopy revealed numerous mitochondria, well-developed Golgi vesicles, and intracytoplasmic crystals with rhomboid- or rod shapes (Figure 2B). The final pathologic examination revealed ASPS of the uterine cervix.

Postoperative radiotherapy was administered at a dose of 5040 cGy external radiotherapy and 2400 cGy intracavitary radiotherapy. The patient is alive with no evidence of recurrent disease two years following the initial diagnosis.

Discussion

Most ASPSs occur in young adults and children, usually involving the extremities in the former and the head and neck region in the latter [1]. ASPSs are seldom detected at other sites. Although the uterine cervix is considered to be the most frequent region at which extraskeletal ASPSs occur, only 12 cases of cervical ASPS have been reported, including the case presented herein [2-11]. The 11 previously reported cases of cervical ASPS occurred in patients ranging in age from 8-39 years. The case described herein is the first case of ASPS occurring on the uterine cervix of a postmenopausal woman in the English literature.

Soft tissue ASPS usually presents as a slowly-growing, painless mass that almost never results in functional impairment [1]. Because of the relative lack of symptoms, ASPS is easily overlooked; in a number of cases, metastasis to the lung or brain is the first manifestation of the tumor. The most common clinical feature of cervical ASPS is abnormal uterine bleeding (9 of 12 cases [75%]); of the three remaining cases, two cases involved...
cervical nodules indentified during pelvic examination [3, 6] and the third case was an incidental finding on a hysterectomy specimen [9]. In 11 (92%) of the 12 cases, there was no evidence of tumor metastases or recurrence; one case had metastasis to an obturator lymph node [2].

Several investigators have described the MRI findings of ASPS and emphasized that high signals on T1- and T2-weighted images and numerous intra- and extra-tumoral signal voids are highly suggestive of ASPS [12, 13]. The high signal on the T1-weighted image has been attributed to slow-flowing blood in or around the tumor [13]. In the case presented herein, T1- and T2-weighted images showed high signal intensities and suspicious focal signal voids in the periphery of the mass. Also, the tumor margin was well-demarcated, which is a useful feature for clinicians in making the diagnosis of ASPS, in contrast to other soft tissue sarcomas of the uterine cervix.

Macroscopically, cervical ASPS is generally well-circumscribed and tends to be nodular with pushing rather than invading margins, in contrast to other soft tissue ASPSs, with tan-to-yellow cut surfaces. The 12 reported tumors ranged in size from 0.2-4.5 cm. Histochemical stains are useful for establishing the diagnosis of ASPS because the PAS preparation reveals varying amounts of intracellular glycogen and characteristic PAS-positive, diastase-resistant rhomboid- or rod-shaped crystals [1]. The typical crystalline material is present in at least 80% of the tumors. ASPSs have been extensively studied by immunohistochemical methods with no consistently positive findings. Recently, the majority of tumor cells show moderate-to-strong nuclear staining with antibody to the carboxy-terminal portion of TFE3 retained in the fusion protein, in contrast to most normal cells which show only weak-to-absent nuclear staining with this type of TFE3 antibody [1]. TFE3 immunohistochemistry is a very useful marker for the diagnosis of ASPS [14]. Electron microscopy shows that the cells contain numerous mitochondria, prominent smooth endoplasmic reticulum, glycogen, and a well-developed Golgi apparatus. Characteristically, there are rhomboid rod-shaped or spicular crystals with a regular lattice pattern and sparse electron-dense secretory granules [1].

There is no current consensus regarding the management of cervical ASPS, and the relatively slow growth of the tumor must be considered when one is assessing the effect of therapy. Of the reported cases, surgical excision providing adequate margins combined with radiotherapy or chemotherapy should be considered the treatment of choice.

Our case contributes to the reviewed literature in two ways. First, the cervical ASPS described occurred at an unusual age in a postmenopausal woman. Second, cervical ASPS should be considered when high signal intensities on both T1- and T2-weighted images are noted on MRI of a well-demarcated cervical mass with some signal void around and within the tumor.

The most important prognostic parameters of ASPS appear to be age at diagnosis, tumor size, and the presence of metastasis at presentation [1]. Although many cervical ASPS patients had relatively short follow-up periods, all of the 12 cases of cervical ASPS, including our case, are alive with no evidence of disease. The better
prognosis of cervical ASPS compared to the soft tissue counterparts may be related to early clinical detection, small size (< 5 cm), resectability, and demarcation of the tumor.

References


Address reprint requests to:
S.M. KIM, M.D., Ph.D.
Department of Obstetrics and Gynecology
Chonnam National University Medical School
8 Hakdong, Donggu, Gwangju (Korea)
e-mail:seokmo2001@hanmail.net
Tumor dissemination after laparoscopic surgery for an unsuspected endometrial stromal tumor

K. Pavlakis¹, I. Messini², C.A. Papadimitriou³, F. Zagouri¹, P. Yiannou², D. Mavrelos¹, T. Panoskaltis¹

¹Department of Pathology, Athens University Medical School, Athens
²Department of Pathology, “IASO” Hospital, Maroussi
³Department of Clinical Therapeutics, Alexandra Hospital, Athens University Medical School, Athens
⁴Department of Gynecological Oncology, “IASO” Hospital, Maroussi (Greece)

Summary

Background: The use of laparoscopic surgery in gynecologic oncology might be complicated by unsuspected side-effects for the patient. Experimental data suggest that the risk of tumor dissemination in the non traumatized peritoneum may be higher after pneumoperitoneum than after laparotomy, and they also show the importance of the surgeon’s experience and technique. Cases: We present two cases of uterine endometrial stromal tumors which were laparoscopically excised. In both cases, intraperitoneal tumor seedings were identified shortly after the initial operation. The first patient had a low-grade endometrial stromal sarcoma and succumbed from the disease two years after the initial operation, while the second patient who was diagnosed with endometrial stromal tumor remains disease free two years later. Conclusions: The laparoscopic excision of an endometrial stromal tumor might result in tumor dissemination into the abdominal cavity. A careful second-look examination of the abdomen or a radical surgical approach is proposed.

Key words: Endometrial stromal tumors; Intraperitoneal dissemination.

Introduction

The use of laparoscopic surgery has been proposed to offer potential benefit to patients since it is minimally invasive, secures an earlier recovery and improves the cosmetic result. However, when laparoscopy is performed for the resection of a malignant tumor, a high incidence of tumor seeding, both peritoneal and at port site has been reported, putting this approach into question [1-4].

Herein, we describe two cases of peritoneal dissemination after laparoscopic excision of unsuspected endometrial stromal tumors. To our knowledge, this is the first report focusing on the seeding of neoplastic endometrial stromal cells.

Case 1

A 36-year-old woman underwent laparoscopic surgery with the preoperative diagnosis of a uterine leiomyoma coexisting with adenomyosis. On histology, some of the morcellation fragments had the features of a benign smooth muscle tumor while the others consisted of myometrium that was infiltrated by a tumor with the features of a low grade endometrial stromal sarcoma. At the time of the initial operation, the abdominal cavity was free of disease.

One and a half months later an abdominal hysterectomy with bilateral salpingo-oophorectomy and omentectomy was performed. By the time of the second surgery, many grossly evident tumor masses were found in the omentum and the uterine serosa although the residual intrauterine tumor was not infiltrating the whole myometrial wall. One year later the patient presented with an intraabdominal recurrence. Two years later, the patient succumbed to disease.

Case 2

A 47-year-old woman presented to the gynecological clinic complaining of lower abdominal discomfort that had lasted for several months and was followed by metrorrhagia. On ultrasound, a well circumscribed intramural tumor was identified, and was preoperatively diagnosed as leiomyoma. A laparoscopic excision of the tumor was performed. The pathology examination revealed the presence of an endometrial stromal tumor, favoring an endometrial stromal nodule, since in some slides that comprised both tumor and normal myometrium, a “pushing margin” was identified.

One month later an abdominal hysterectomy with bilateral salpingo-oophorectomy, lymphadenectomy and omentectomy was performed. No residual tumor was identified in the myometrium, strengthening the diagnosis of an endometrial stromal nodule. Yet, small nodules composed of endometrial stroma with no nuclear atypia, were microscopically found on the uterine serosa as well as on the left ovary. A small but grossly identifiable nodule with identical histological features was also found on the bowel serosa. On immunohistochemical examination, these nodules were CD10 positive. There was also a strong diffuse nuclear positivity for estrogen and progesterone receptors.

The differential diagnosis included micronodular stromal endometriosis [5], which is usually an incidental microscopic finding unassociated with endometriosis elsewhere, and extratuminal low grade endometrial stromal sarcoma which nevertheless should have some, albeit mild atypia and tongue-like invasion, and an intraperitoneal seeding of benign neoplastic endometrial stroma. Although micronodular stromal endometriosis could not be excluded, taking under consideration the clinical background and location of the uterine nodule at port site serosa, the diagnosis of intraperitoneal seeding of benign neoplastic tissue was favored.

Despite the proposed diagnosis and for prophylactic reasons, the patient was treated for three months with an aromatase inhibitor (anastrozole). By the end of that period, a second-look laparotomy was performed. No residual tumor was found. One year after the initial operation, the patient is free of disease.
Discussion

Laparoscopic treatment of uterine leiomyomas is steadily increasing in gynecological surgery. This procedure has been shown to be an appropriate alternative to conventional surgery, since it is associated with less morbidity, allows a faster recovery, decreases the length of hospitalization and results in a better cosmetic result. Nevertheless, to prevent port-site metastasis or intraperitoneal spillage of tumor cells, in case of an unsuspected malignancy, the patients should be carefully selected.

Gynecologists should have in mind that tumors composed of endometrial stroma, either benign (endometrial stromal nodules) or of low malignant potential (endometrial stromal sarcomas), can mimic on ultrasound examination several benign conditions such as an endometrial polyp, leiomyoma or even adenomyosis [6]. The importance of a correct preoperative diagnosis, lies on the fact that endometrial stromal tumors can be cured by surgery alone (total hysterectomy with bilateral salpingooophorectomy). Most Stage I endometrial stromal sarcomas have a 5-year survival rate of 90% and a very low recurrence rate with some of the recurrences occurring ten or even 20 years after the initial surgery [7].

In our reported cases, both tumors presented with diffuse intraperitoneal disease shortly after the initial laparoscopic excision. Moreover, patient No 1 died of disease two years after the laparoscopic surgery, following a particularly aggressive clinical course for a low-grade endometrial stromal sarcoma. Most probably the intraperitoneal spillage of tumor cells could represent an undesirable side-effect of the laparoscopic procedure.

It has been indicated by experimental studies that change in the laparoscopic environment during surgery enhances the invasive potential of tumors and contributes to disease dissemination [8]. However, the risk factors that are associated with laparoscopic surgery are not yet elucidated. The use of insuffilatory agents such as carbon dioxide, the “chimney effect” or even changes of the local immune system have been implicated as potential causative mechanisms [9, 10].

Tumor seeding and port-site metastasis after laparoscopic surgery for an unsuspected endometrial stromal tumor remain to be answered.

References


Address reprint requests to:

F. ZAGOURI, M.D.
University of Athens
23 Irakleous str.
Kallithea, 17671 Athens (Greece)
e-mail: florazagouri@yahoo.co.uk
Chairman: Péter Bősze (Hungary)

Executive Board:
PIERLUIGI BENEDETTI PANICI (Italy)
CARLOS F. DE OLIVEIRA (Portugal)
GIUSEPPE DE PALO (Italy)
SANTIAGO DEXEUS (Spain)
WILLIAM DUNLOP (UK)
 STELIOS FOTIOU (Greece)
GERALD GITSCH (Austria)
A. PETER M. HEINTZ (Netherlands)
MICHAEL HOECKEL (Germany)
JAN JACOBS (UK)
JACQUES LANSAC (France)
TIZIANO MAGGINO (Italy)
HARALD MEDEN (Germany)
JOSEPH MONSONEGO (France)
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