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.
LETTER TO THE EDITOR

Controversy on another possible risk of preterm delivery after cervical conization: time interval between conization and conception
S. Matsubara, R. Usui, A. Ohkuchi - Tochigi, JAPAN
A letter to the Editor that points out the interval between conization and pregnancy.

ORIGINAL ARTICLES

The prognostic significance of lymphovascular space invasion in laparoscopic versus abdominal hysterectomy for endometrioid endometrial cancer
Lymphovascular space invasion is a poor prognostic factor regardless of a theoretic impact of uterine manipulator during laparoscopic surgery.

Outcome of fertility-sparing treatment with medroxyprogesterone acetate for atypical hyperplasia and endometrial carcinoma in young Japanese women

The correspondence between abnormal transformation zone Grade 1 and Grade 2 colposcopic parameters and histology. Clinical implications
L. Di Stefano, F. Patacchiola, S. Necozione, G. Paolone, G. Di Febo, G. Carta - L’Aquila, ITALY
In cervical oncologic pathologies, colposcopy cannot substitute histology, but can improve the definition of the diagnosis.

An evaluation of immune system cell infiltrate in the cervical stroma of patients with grade III cervical intraepithelial neoplasia after treatment with intralesional alpha-2B interferon
F.A. Machado, D.R. Abdalla, L. Montes, R.M. Etchebehere, M.A. Michelin, E.F.C. Murta - Minas Gerais, BRAZIL

Clinical analysis of sentinel lymph node identification in patients with cervical cancer
Z. Zhang, Q. Chang - Yinchuan, P.R. CHINA
The use of methylene blue injection for sentinel lymph node biopsy has a high detection rate.

The impact of epithelial ovarian cancer diagnosis on women’s life: a qualitative study
L. Mangone, V.D. Mandato, R. Gandolfi, C. Tromellini, M. Abrate - Reggio Emilia, ITALY
Assessment of psychological problems due to the diagnosis of ovarian cancer and the consequent changes in daily life.
Assessment of the tumor-associated trypsin inhibitor (TATI) marker in patients with carcinoma of the uterine body 17 years after treatment

B. Kozakiewicz, M. Chądzyńska, E. Dmoch-Gajzlerska - Warsaw, Poland

The tumor-associated trypsin inhibitor factor was studied as a possible predictor of recurrence in case of uterine body carcinoma.

Expression of budding uninhibited by benzimidazoles-1 and mitotic arrest deficient-2 in endometrial carcinoma and its significance

Q. Zhao, A.P. Bian, Y. Zhang, L. Qin, H.R. Shi, K. Su - Zhengzhou, China

The possibility that benzimidazoles-1 protein expression may be correlated with the prognosis of endometrial carcinoma is investigated.

Evaluation of primary prophylaxis with granulocyte colony-stimulating factor for epithelial ovarian cancer


A retrospective examination of the value of primary prophylaxis using granulocyte colony-stimulating factor for epithelial ovarian cancer is referred.

Accuracy and diagnostic value of outpatient hysteroscopy for malignant and benign disease

T. Issat, J. Beta, M.A. Nowicka, A.J. Jakimiuk - Warsaw, Poland

Office hysteroscopy, as highly accurate and clinically useful method, is proposed for the diagnosis of endometrial cancer for women with or without abnormal bleeding.

Cervical dysplasias 1982–2010 in the Republic of Panama. Diagnosis, treatment, and evolution

J.L. Garrido - Panama City, Republic of Panama

The experience of 18 years of diagnosis, treatment, and evolution of cervical dysplasia is reported.

Evaluation of the effect of GnRH agonist on menstrual reverse in breast cancer cases treated with cyclophosphamide


GnRH agonists are effective in the protection of ovarian function in breast cancer patients during chemotherapeutic treatment.

Comparison of hematologic toxicity between 3DCRT and IMRT planning in cervical cancer patients after concurrent chemoradiotherapy: a national multi-center study


The impact of three-dimensional conformal radiotherapy and intensity modulated radiotherapy on hematologic toxicity in patients with cervical cancer, treated with concomitant chemoradiotherapy, are assessed.

Evaluation of serum CA 125 level and its relation to surgical, histopathologic and ultrasonographic findings in patients with pelvic mass


The relationship among serum CA 125, histopathologic, and ultrasonographic finding in patients with pelvic masses are investigated.

CASE REPORTS

Squamous cell carcinoma arising in mature cystic teratoma of the ovary: report of two cases with molecular analysis


No modifications in the genes implicated in the development of malignancy were found in two cases of squamous cell carcinoma arising from mature teratoma.
Angiomyofibroblastoma of the vulva
M. Kanda, A. Sonoyama, H. Hirano, T. Kizaki, N. Ohara - Sanda, JAPAN
The differential diagnosis between angiomyofibroblastoma and aggressive angiomyxoma is discussed presenting a case report.

Mitotically active cellular fibroma of the ovary: a case report and a review of the literature
H. Wu, J. Xie, W. Huang, J. Wu - Nanjing, P.R. CHINA
The differential diagnosis between mitotically active cellular fibroma of the ovary and ovarian fibrosarcoma is discussed.

Laparoscopic radical trachelectomy (LRT) with round ligament and ascending branches of uterine artery preservation: case report
A case of cervical cancer Stage IA2 treated with laparoscopic radical trachelectomy with round ligament and uterine artery preservation is presented.

Placental site trophoblastic tumor on endometrial polyp: a case report
A very rare case of placental site trophoblastic tumor that developed in an endometrial polyp is discussed

Diagnostic laparoscopy identifies a peritoneal adenomatoid-like mesothelioma masquerading as ovarian cancer: a case report
T. Okuda, Y. Ogino, S. Yamashita, H. Ishii, S. Kin, A. Nagata, M. Otsubo, H. Kataoka, J. Kitawaki - Kyoto, JAPAN
The imaging technique must be coupled with laparoscopic surgery for an accurate diagnosis of peritoneal mesothelioma.

Bilateral Krukenberg tumor in a 16-week pregnant woman
M. Genç, B. Genç, A. Solak, E. Gür, C. Sezgin - İzmir, TURKEY
The management of a case of bilateral Krukenberg tumor during pregnancy is described.

A 29-year-old woman with complex atypical hyperplasia and polycystic ovary syndrome: a challenging issue
D. Caserta, E. Matteucci, E. Ralli, M. Mallozzi, G. Bordi, M. Moscarini - Rome, ITALY
Conservative therapy with norethisterone acetate in a young woman with complex atypical hyperplasia and polycystic ovarian syndrome is presented.

Primary carcinoid tumor of the ovary arising in a mature cystic teratoma: a case report
W.H. Ting, S. M. Hsiao, H. H. Lin, M. C. Wei - Taipei, TAIWAN
Diagnosis, treatment, and follow up of a case of primary carcinoid tumor of the ovary concomitant with mature teratoma is reported.

ERRATA - CORRIGE

Transition of low-grade to high-grade endometrial stromal sarcoma: a case report

Errata:
page 359
(Figure 1). High-grade ESS expressed the proliferation index, Ki-67 (MIB1) more frequently than low-grade ESS (Figure 2). Although in low-grade ESS the immunoreactive p53 was scarcely detected in the uterus and at 6.3% in the omentum, the positive rate of p53 in high-grade ESS was elevated to 17.5% (Figure 3).

Corrige:
(Figure 3). High-grade ESS expressed the proliferation index, Ki-67 (MIB1) more frequently than low-grade ESS (Figure 4). Although in low-grade ESS the immunoreactive p53 was scarcely detected in the uterus and at 6.3% in the omentum, the positive rate of p53 in high-grade ESS was elevated to 17.5% (Figure 5).
Controversy on another possible risk of preterm delivery after cervical conization: time interval between conization and conception

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We have read with the interest the article by Guo et al., “Effects of loop electrosurgical excision procedure or cold knife conization on pregnancy outcomes” [1]. Cold knife conization significantly increased the rate of preterm delivery (PD) of the subsequent pregnancy compared with the control, but loop electrosurgical excision procedure (LEEP) did not. Thus, the risk of PD may depend on the conization procedure. We wish readers to pay attention to another possible risk of after-conization-PD: the time interval between conization and conception.

Although the reason why conization increases the rate of PD is unclear, three possibilities have been suggested: 1) reduction of cervical collagen, reducing cervical strength, 2) removal of the cervical gland, weakening the cervical barrier function, and 3) loss of cervical plasticity, making the fetal membranes more vulnerable to preterm rupture [2]. Site “healing” may require some time, and thus pregnancy soon after conization may be more likely to cause PD.

To our knowledge, three reports on this issue showed contradictory results. The USA data [3] showed that shorter conization to conception interval, i.e., 2.5 vs. 10.5 months, significantly increased the PD rate. Two other nation-register-based studies from Finland [4] and Denmark [5] found no differences, although they only lightly touched on this issue. We re-examined this issue.

We examined patients (n = 30), who 1) had received conization, 2) visited this institute in the first trimester and received regular pregnancy check ups, and 3) gave birth to infants here over a 14-year period. Conizations were performed with various procedures including electric incision (n = 15), LEEP (n = 6), cold knife (n = 4), or others. Ten received cervical cerclage (MacDonald or Shirodkar) and the remaining 20 did not. Procedures and whether to perform cerclage depended on attending doctors’ decision. We examined whether the PD rate (< 37 + 0 weeks) was associated with the interval between conization and conception.

The median time interval between conization and conception was 640 days (range 334-1180 days). Of 30 patients, PD occurred in nine. The rate of PD did not differ between the conization procedures or whether cerclage was performed. Importantly, the rates of PD in patients with intervals < 18 vs. ≥ 18 months was 33% vs. 28%, respectively, showing no significant difference (Fisher’s exact test). This was also true in women with intervals < 12 vs. ≥ 12 months.

Although our study population was small, our strength was that the present data was obtained in a single center, in which treatment including monitoring or tocolysis has been the same during study period. In our population, women became pregnant much later after conization than those in the USA study [3]. Thus, we do not know whether a much shorter interval (i.e., 2.5 months) increases the PD risk. Japanese women may be more cautious about PD and/or recurrence of the cervical diseases, and thus they may postpone pregnancy: we did not recommend the use of contraceptions after conization.

Conization increases the PD rate. It depends on the procedure as shown by Guo et al. [1]. More study is needed to determine whether the interval between conization and pregnancy also affects the rate of PD. This issue should be more widely discussed by both gynecologic oncologists and obstetricians.

References

Reply from the Editor-in-Chief

The pregnancy consequences of conization and electrosurgical excision of the uterine cervix for high grade cervical lesions and rarely for early stage cervical cancer are major considerations, as affected women are mostly young desiring further childbearing. The literature data are still controversial in terms of the rate of post-treatment pregnancy complications and whether electrosurgical excision is preferable to conization. Most data however suggest correlation between the size and depth of the excised cervix and the obstetrical outcome. The point Dr. Shigeki Matsubara and colleagues raised is valid, and it is highly recommended to re-evaluate the available data accordingly.

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The prognostic significance of lymphovascular space invasion in laparoscopic versus abdominal hysterectomy for endometrioid endometrial cancer

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Summary

Introduction: Recent reports have suggested that uterine manipulators can induce lymphovascular space involvement (LVSI) by endometrial cancer in laparoscopic hysterectomy specimens. The prognostic significance of this phenomenon known as “vascular pseudo invasion” remains elusive. Materials and Methods: The authors conducted a retrospective, single institution study of patients who underwent initial surgery for grade 1 and grade 2 endometrioid endometrial cancers with LVSI. Cases were stratified by surgical approach (laparoscopy vs laparotomy). Clinicopathologic and procedure characteristics as well as outcome data were analyzed. Univariate and multivariate analyses were performed. Disease-free survival (DFS) was analyzed using the Kaplan-Meier product limit method. Results: A total of 104 cases (20 laparoscopic, 84 laparotomy) were analyzed. Mean age (65 vs 64 years, respectively), stage distribution, mean number of lymph nodes sampled (18 vs 21, respectively) and use of adjuvant therapy was similar for both groups (p > 0.05). Mean body mass index (BMI) was 30 vs 35 kg/m², respectively (p = 0.002). Mean follow up was 24 months (range 0.1–102). Univariate analysis demonstrated that LVSI in the laparoscopic setting was associated with worse DFS (p = 0.002). After adjusting for grade the risk of recurrence remained higher for laparoscopic cases (HR: 15.7, 95% CI 1.7–140.0, p = 0.014). Conclusions: Adjusted risk of recurrence associated with LVSI is higher in cases approached laparoscopically arguing against the concept of “vascular pseudo invasion” associated with the use of uterine manipulators and balloons. LVSI should be regarded as a serious risk factor and taken into account for triage to adjuvant therapies, even in laparoscopically treated early-stage endometrial cancer.

Key words: Endometrial cancer; Lymphovascular space invasion; Pseudo invasion.

Introduction

A laparoscopic approach to the staging of endometrial cancer has become an accepted alternative to the traditional exploratory laparotomy[1-4]. Benefits of laparoscopy include fewer short term complications and shorter hospital stays. The laparoscopic approach as originally described and whether performed with or without robotic assistance, usually involves the use of a uterine manipulator. These devices have a shaft and balloon that is inserted into the uterine cavity and then used throughout the procedure to manipulate the uterus in order to facilitate exposure and complete removal of the surgical specimen. Logani et al. [5] and Kitahara et al. [6] have recently suggested a phenomenon of “vascular pseudo invasion”. It has been postulated that closed positive pressure or mechanical manipulation of the specimen can cause cancer cells to be mechanically forced into the lymphovascular spaces, creating the artificial appearance of cancer cells in capillary and lymphatic spaces without inherent risk for regional or distant metastasis [5, 6]. Several pathologic characteristics have been proposed in an attempt to distinguish true from artifactual lymphovascular space invasion (LVSI). Unfortunately, despite best efforts at sub-classification of true versus “pseudo-invasion” such pathologic differentiation remains inaccurate [7, 8].

Triage to adjuvant therapies for patients with endometrioid endometrial cancer is currently based not only on stage but also on the presence of certain clinicopathologic risk factors. These include the age of the patient, depth of myometrial invasion, and importantly LVSI as described in Gynecologic Oncology Group (GOG 99). The prognostic significance of “vascular pseudo invasion” and thus the implications of such diagnosis on therapeutic decisions have not been defined. Initial studies, described and focused on the specific features of this phenomenon in cases of grades 1 and 2 endometrioid endometrial cancer [5, 6].

The authors sought to determine the prognostic significance of LVSI associated with the use of uterine manipulators at the time of minimally invasive surgery for endometrial cancer in the above described patient population.

Materials and Methods

The authors conducted a retrospective, single institution study of patients with grades 1 or 2 endometrial cancer with docu-
mented LVS in hysterectomy specimens. All cases underwent primary surgery for management of newly diagnosed endometrioid endometrial cancer. Surgeries were performed by members of the Division of Gynecologic Oncology at Washington University School of Medicine at Barnes Jewish Hospital / Siteman Cancer Center. Patients treated at the institution between January 2000 and March 2010 were identified from the Department of Pathology database. All cases were reviewed by experienced gynecologic pathologists at the institution and had histologically confirmed FIGO grade 1 or grade 2 endometrioid adenocarcinoma of the endometrium with documented LVS. For the purposes of this study, LVS was broadly defined as presence of tumor cells inside vascular spaces lined by endothelium. FIGO 1988 surgical staging was used. Clinicopathologic characteristics and outcome data were obtained from outpatient and inpatient medical records. Cases without LVS as well as those with non-endometrioid histologies, grade 3 tumor, Stage IV disease, and laparoscopic surgeries converted to laparotomy were excluded. Cases were stratified by surgical approach (laparoscopy – including laparoscopically assisted vaginal, total laparoscopic, and robotic hysterectomies – vs laparotomy).

Descriptive statistics were used to characterize the study cohort. The primary outcome was disease free survival (DFS), defined as the time from surgery to the date of recurrence or progression. Recurrence free subjects were censored at the date of last contact. Associations between categorical variables and DFS were described using the Kaplan-Meier product limit method and compared by log-rank test. The effects of continuous variables on survival were assessed using univariate Cox proportional hazard model. Multivariate Cox model was also fit to assess the confounding effects of other demographic and clinical characteristics. Given the small number of events, however, a variable-by-variable approach was employed in the multivariate analysis including those variables of interest that approached significance in the univariate analysis. All analyses were two-sided, and significance was set at a \( p \)-value of 0.05. Statistical analyses were performed using SAS.

This study was approved by the Human Research Protection Office at Washington University in St. Louis (HRPO#10-0451).

**Results**

A total of 113 grades 1 and 2 endometrioid endometrial cancer cases with documented LVS in their final histologic specimen were identified during the study period. Nine patients were excluded, eight for Stage IV disease and one for conversion to laparotomy from a laparoscopic attempt. Of the remaining 104 patients, 84 underwent a laparotomy and 20 underwent laparoscopic surgery.

Demographic and disease characteristics are presented in Table 1. Mean age was 65 years (range 56–73) for the laparoscopic cases and 64 years old (range 53–76) for the laparotomy group. Mean body mass index (BMI) was 29.9 ± 4.3 for the laparoscopy group and 34.9 ± 11 for the laparotomy group. Pelvic and para-aortic lymphadenectomy was not performed systematically in 20 out of 104 cases (three in the laparoscopy group and 17 in the laparotomy group). Of the three incompletely staged laparoscopic cases, one did undergo pelvic lymphadenectomy without para-aortic dissection. Similarly, six of the 17 incompletely staged cases in the laparotomy group underwent pelvic lymphadenectomy without para-aortic dissection. The mean number of lymph nodes was similar for laparotomy and laparoscopy cases (21 and 18 respectively). In the laparoscopy group, a variety of uterine manipulators were used.

Less than half (n = 45, 43%) of the patients received some sort of adjuvant therapy. Of those, 20 received radiotherapy (either vaginal brachytherapy and/or external beam), 16 received both chemotherapy and radiotherapy, and four received chemotherapy only. In five cases the type of adjuvant therapy used was not documented.

Mean follow up was 24.1 months (range 0.1–102.4). There was a significant difference in recurrence rate between cases in the laparoscopy and the laparotomy group. Four patients (20%) in the laparoscopy group recurred and two patients (2.4%) had a recurrence in the laparotomy group (\( p = 0.002 \), Figure 1). The authors did not find differences in frequency of use of adjuvant therapies between the study groups (\( p = 0.45 \)). Of the four patients with recurrence in the laparoscopy group, one received adjuvant vaginal brachytherapy and three did not

### Table 1. — Demographics and disease characteristics.

<table>
<thead>
<tr>
<th>Stage (FIGO)</th>
<th>Laparoscopy (n = 20)</th>
<th>Laparotomy (n = 84)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.1 ± 8.3</td>
<td>64.5 ± 11.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>29.9 ± 4.3</td>
<td>34.9 ± 11</td>
<td>0.002</td>
</tr>
<tr>
<td>Stage (FIGO 1988)</td>
<td></td>
<td></td>
<td>N.S.</td>
</tr>
<tr>
<td>IA</td>
<td>2 (10)</td>
<td>2 (2.4)</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>13 (65)</td>
<td>32 (38.1)</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>1 (5)</td>
<td>23 (27.4)</td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>0 (0)</td>
<td>1 (1.2)</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>2 (10)</td>
<td>2 (2.4)</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>1 (5)</td>
<td>5 (5.9)</td>
<td></td>
</tr>
<tr>
<td>IIIB</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>IIIC</td>
<td>1 (5)</td>
<td>19 (22.6)</td>
<td></td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
<td>N.S.</td>
</tr>
<tr>
<td>Grade 1</td>
<td>6 (30)</td>
<td>45 (53.4)</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>14 (70)</td>
<td>39 (46.4)</td>
<td></td>
</tr>
<tr>
<td>Lymph node count (mean)</td>
<td>18</td>
<td>21</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* BMI: body mass index; Mean ± standard deviation: FIGO: International Federation of Gynecology and Obstetrics; N.S.: Not significant.

### Table 2. — Characteristics of recurrent cases.

<table>
<thead>
<tr>
<th>Case</th>
<th>Surgical approach</th>
<th>Stage</th>
<th>Adjuvant therapy</th>
<th>Location of recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LSC</td>
<td>IB</td>
<td>VBT</td>
<td>Regional</td>
</tr>
<tr>
<td>2</td>
<td>LSC</td>
<td>IB</td>
<td>None</td>
<td>Vaginal</td>
</tr>
<tr>
<td>3</td>
<td>LSC</td>
<td>IB</td>
<td>None</td>
<td>Distant</td>
</tr>
<tr>
<td>4</td>
<td>LSC</td>
<td>IB</td>
<td>None</td>
<td>Vaginal</td>
</tr>
<tr>
<td>5</td>
<td>LAP</td>
<td>IIIC</td>
<td>RT</td>
<td>Distant</td>
</tr>
<tr>
<td>6</td>
<td>LAP</td>
<td>IIIC</td>
<td>Chemo/RT</td>
<td>Distant</td>
</tr>
</tbody>
</table>

LSC: Laparoscopy; LAP: Laparotomy; VBT: vaginal cuff brachytherapy; RT: radiotherapy; Chemo/RT: combined chemotherapy and radiotherapy.
receive adjuvant therapy. In the laparotomy group, two patients had a recurrence. Of those, one had adjuvant radiation and the other had both adjuvant radiation and chemotherapy (Table 2).

On univariate analysis age, BMI, stage, and nodal count were not associated with DFS. Histologic grade was the only factor that appeared marginally associated with DFS ($p = 0.06$). After adjusting for grade, the risk of recurrence remained higher for laparoscopic cases (HR: 15.7, 95% CI 1.7–140.0, $p = 0.014$).

**Discussion**

Considerable effort has gone into developing approaches to better identify endometrial cancer patients at risk for disease progression and recurrence. In the 1980s, the GOG undertook a systematic approach to surgical staging demonstrating that clinical staging missed metastatic disease in ~25% of cases [10]. Given the inadequacy of clinical staging, the International Federation of Gynecologists and Obstetricians (FIGO) approved a revised surgical staging classification for uterine cancer in 1988. Despite complete surgical staging, many women with “early stage” endometrial cancer still experience progression or recurrence and ultimately die from their disease. Multiple algorithms for better prediction of recurrence and progression have been proposed. Those include other prognostic features (such as LVSI, grade, and age) that were not part of the 1988 staging system [9]. Furthermore, recent debate regarding the therapeutic effect of lymphadenectomy for patients with endometrial cancer has renewed interest in recognition of pathologic factors for risk stratification [11, 12]. To this regard, the present group has recently reported on the independent prognostic value of LVSI. Lymphovascular invasion in patients with endometrioid endometrial cancer is associated with significantly worse DFS (HR 2.19, 95% CI: 1.62–2.96, $p < 0.0001$) and overall survival (HR 2.04, 95% CI: 1.49–2.79, $p < 0.0001$) [11].

Minimally invasive approaches have become well-accepted for the management of patients with endometrial cancer. Initial concern led to extensive debate and became the leitmotif for LAP2, a randomized controlled trial designed to compare outcomes after laparotomy versus laparoscopy for the initial management of patients with endometrial cancer [4]. While primary outcome data from this study is not yet mature, initial reports indicate that the laparoscopic approach appears to be feasible and safe [4].

Uterine manipulators are often used to improve exposure and surgical access and to potentially prevent genitourinary injuries during laparoscopic and robotically assisted hysterectomies. These instruments consist of an intracavitary shaft with or without an intrauterine balloon and cervico-vaginal ring. As a result, it is possible that such instrumentation could result in mechanical disruption of the tumor leading to artifacts at the time of pathologic evaluation. Logani et al. coined the term “pseudo invasion” in 2008 to refer to presumed artifactual presence of tumor in capillary and lymphatic spaces [5]. After review of 37 laparoscopic hysterectomy specimens (seven for endometrial carcinoma/ hyperplasia and 30 for benign disease) these authors were able to identify intravascular tumor in 71% of pre-malignant/malignant cases and benign endometrial glands in 13% of cases with benign pathology. They postulated that the creation of a closed pressure system was responsible for the newly reported phenomenon [5]. Kitahara et al. reported on 21 cases of laparoscopically treated low risk endometrial cancer and 28 cases of low risk endometrial cancer treated by laparotomy [6]. The incidence of vascular space invasion appeared significantly higher in the laparoscopic when compared to the cases approached by laparotomy (33% vs none, $p = 0.001$). This group attempted to characterize these cases of vascular invasion and noted occasional lack of perivascular inflammatory infiltrate and detachment from the vessel wall as some of the most notorious characteristics. These authors proposed mechanical transport of tumor to lymphatic spaces during pathologic processing of the specimen as the potential causative factor in those cases [6]. Two subsequent studies have confirmed a higher incidence of vascular space involvement associated with laparoscopic procedures [7, 8].

![Figure 1](image.png)

**Figure 1.** — Kaplan-Meier disease-free survival plot stratified according to surgical approach. Numerals indicate the number of survivors in each group (a. laparotomy, b. LSC: laparoscopy) at each censor point.
incidence of LVSI in cases managed laparoscopically, recent studies acknowledged the lack of consistent findings to allow for accurate pathologic sub-classification of true as opposed to pseudo-invasion. More importantly the uncertainty regarding the clinical implications of such phenomenon is well recognized [7, 8].

In this study the authors sought to evaluate for the first time the outcome differences related to LVSI occurring in endometrioid endometrial cancer cases managed laparoscopically when compared to those managed via laparotomy. In keeping with the potential artifactual occurrence of “pseudo invasion”, the authors originally hypothesized that after controlling for confounding variables, LVSI in cases managed laparoscopically would be associated with better DFS. Contrary to what had been proposed, the present authors found that the adjusted risk for recurrence was significantly higher in cases managed laparoscopically when compared to those managed via laparotomy. In keeping with the potential artifactual occurrence of “pseudo invasion”, the authors originally hypothesized that after controlling for confounding variables, LVSI in cases approached laparoscopically (adjusted HR: 15.7, 95% CI 1.7–140.0, p = 0.014).

The present study is limited by its retrospective nature and the inherent inability to determine whether tumor identified in lymphatic vessels and capillaries was present before the actual surgical procedure. However, its findings suggests that LVSI, as traditionally defined and as described by previous studies, represents a poor prognostic factor whether found after laparoscopic or open surgery. As such, LVSI should be regarded as a serious risk factor even when identified in laparoscopically treated early stage and apparently “indolent otherwise” endometrioid endometrial cancer. Until further understood, the authors strongly advise clinicians against considering “pseudo invasion” as a causative entity of pathologic LVSI at the time of making treatment recommendations for patients with endometrial cancer.

References

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Outcome of fertility-sparing treatment with medroxyprogesterone acetate for atypical hyperplasia and endometrial carcinoma in young Japanese women

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Summary

Purpose: To review the outcome in patients with atypical endometrial hyperplasia (AEH) and endometrial cancer (EC) who received MPA treatment in the present hospital. Materials and Methods: Patients with AEH or EC were administered MPA for 12 weeks followed by curettage. The rates of effect, recurrence, pregnancy, and complications were evaluated. The changes in progesterone receptors and FOXO-1, known as a target of MPA treatment, were examined by immunostaining. Results: Four of seven patients with endometrial cancer and three of three patients with AH had complete response. Four of seven patients had recurred within one year after the treatment and had to undergo hysterectomy. None of the patients showed changes in progesterone receptors. Although six of seven patients were negative for FOXO-1 before and after treatment, all the patients showed increased developments of FOXO-1 during MPA treatment. Conclusion: Progestin as a fertility-preserving treatment is expected to be effective for endometrial cancer, but judicious use might be required because it shows high rate of recurrence. Further studies regarding the mechanism may be necessary to achieve high efficacy.

Key words: Medroxyprogesterone acetate; Endometrial carcinoma; FOXO-1; Progesterone receptor; Fertility-preserving treatment.

Introduction

In recent years, the prevalence of endometrial cancer (EC) has continued to increase. There are 142,000 new cases and 42,000 deaths per year all over the world [1]. Most of these endometrial cancer cases occur in postmenopausal women; 25% of these occur in premenopausal women, and 2.5%–14.4% occur in young women of ages less than 40 years. The number of patients with endometrial cancer who desire fertility is predicted to further increase in the future, considering the social trends such as tendency toward late marriage and childbirth in Japan. Progestin, including medroxyprogesterone acetate (MPA), is traditionally administered as therapy for juvenile endometrial cancer to preserve fertility. However, according to the report by Ushijima K et al., the recent rate of effect of MPA therapy is unexpectedly as low as 64%, and the recent rate of recurrence is as high as 57% [2], which indicates that there are many issues to be improved. It is considered that the mechanism of progesterin must be clearly understood to improve the rate of effect and to decrease the rate of recurrence. The authors retrospectively reviewed the treatment outcome in ten patients with atypical endometrial hyperplasia and endometrial cancer who received MPA therapy in the present hospital to preserve fertility.

appeared. PR was defined as observation of atrophy or regression of the secretory epithelium but no residual atypical cells. NC was defined as no atrophy or regression but presence of residual atypical cells. PD was defined as the presence of lesion (grade 2 or 3 and higher) was observed. The side-effect was evaluated on the basis of the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 2. The efficacy rate was defined as a ratio of CR and PR accounting for the percentage of all cases. The authors examined this treatment in terms of the rates of effect, recurrence, pregnancy, and complication.

**Table 1. — Patient characteristics.**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>AEH</td>
<td>EC</td>
</tr>
<tr>
<td>BMI</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Gravida</td>
<td>0</td>
<td>1, 2</td>
</tr>
<tr>
<td>Para</td>
<td>0</td>
<td>1, 2</td>
</tr>
<tr>
<td>PCOS</td>
<td>3 (30.0%)</td>
<td>3 (30.0%)</td>
</tr>
</tbody>
</table>

AEH: atypical endometrial hyperplasia; EC: endometrial carcinoma; PCOS: polycystic ovary syndrome.

**Table 2. — Response to MPA.**

<table>
<thead>
<tr>
<th>Response</th>
<th>AEH (n = 3)</th>
<th>EC (n = 7)</th>
<th>Total (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>3 (100%)</td>
<td>4 (57.1%)</td>
<td>7 (70.0%)</td>
</tr>
<tr>
<td>Not CR</td>
<td>0 (0.0%)</td>
<td>3 (33.3%)</td>
<td>3 (30.0%)</td>
</tr>
</tbody>
</table>

**Table 3. — All cases of MPA therapy in the present hospital.**

<table>
<thead>
<tr>
<th>No</th>
<th>Age</th>
<th>Histology</th>
<th>Response</th>
<th>Ovulation induction</th>
<th>Outcome</th>
<th>Time to recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>AEH</td>
<td>CR</td>
<td>Free</td>
<td>–</td>
<td>No recurrence</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>AEH</td>
<td>CR</td>
<td>IVF-ET</td>
<td>–</td>
<td>No recurrence</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>AEH</td>
<td>CR</td>
<td>Clomiphene citrate</td>
<td>NVD (39wd)</td>
<td>No recurrence</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>EC G1</td>
<td>CR</td>
<td>Clomiphene citrate</td>
<td>TAH+BSO</td>
<td>3 months, with ovarian cancer</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>EC G1</td>
<td>PD</td>
<td>–</td>
<td>TAH+BSO</td>
<td>PD</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>EC G1</td>
<td>CR</td>
<td>Free</td>
<td>TAH+BSO</td>
<td>6 months</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>EC G1</td>
<td>CR</td>
<td>Clomiphene citrate</td>
<td>TAH+BSO</td>
<td>12 months</td>
</tr>
<tr>
<td>8</td>
<td>38</td>
<td>EC G1</td>
<td>CR</td>
<td>Clomiphene citrate</td>
<td>TAH+BSO</td>
<td>12 months</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>EC G1</td>
<td>PD</td>
<td>–</td>
<td>TAH+BSO</td>
<td>PD</td>
</tr>
<tr>
<td>10</td>
<td>37</td>
<td>EC G1</td>
<td>PD</td>
<td>–</td>
<td>TAH+BSO</td>
<td>PD</td>
</tr>
</tbody>
</table>

Discussion

In the present study, the efficacy rate of MPA for all the cases of AEH were classified into CR, whereas the efficacy rate for EC was 57.1%, which indicated a result that was not good as that shown in previous studies [3–8]. All the four patients with endometrial cancer who were classified into CR showed relapse (100%) and had to undergo total abdominal hysterectomy. The results of the prospective study by Ushijima K et al. were also disappointing in that the efficacy rate of MPA was 64% and the rate of recurrence was 57% [2]. However, the present study results showed an approximately similar rate of effect to the study by Ushijima K et al. To improve the rate of pregnancy, it is important to prevent recurrence. Ushijima K et al. reported that patients who did not receive any treatment after successful study treatment showed higher rate

Figure 1A. — Immunohistological analysis was performed according to Saito’s protocol using primary antibody hPRa7 for PR-A and hPR2 for PR-B. All patients were positive for PR-A and PR-B. None of the patients showed changes in development of PR-A and PR-B and in the PR-B/PR-A ratio (A vs. C, B vs. D).

Figure 1B. — Immunohistological analysis was performed using primary monoclonal antibody FOXO-1 (C29H4). Six of seven patients were negative for FOXO-1 immunostaining before and after treatment (A and C). All the patients showed increased development of FOXO-1 in the nucleus and cytoplasm during MPA therapy (B). Of these, the results of Case 10 are shown.
of recurrence (69%) than patients who received periodical EP (estrogen + progestin) therapy [2]. Similar findings were obtained in the present study, which suggested that no periodic hormone administration after the study treatment might cause a high rate of recurrence. In addition, all patients showed disease relapse within 12 months after the study treatment, and therefore, patients who desired fertility should receive infertility treatment as early as possible. Regarding infertility treatment, Elizur SE et al. reported that 64% of patients who received successful fertility-preserving treatment had to also receive infertility treatment; further, their study showed that six of eight patients (75.0%) became pregnant by aggressive in-vitro fertilization (IVF) and four of eight patients (50.0%) had babies [9]. Han AR et al. reported that ten of 11 patients treated with progestin as primary fertility-preserving therapies had received assisted reproductive technology (ART) and six patients became pregnant [10]. It is believed that more aggressive intervention than timing the treatment is required for successful pregnancy and delivery. It might be important to inform patients of the usefulness of aggressive infertility treatments such as in vitro fertilization (IVF) after successful fertility-preserving treatment. A study demonstrated that levonorgestrel intrauterine device (IUD) leads to regression of endometrial hyperplasia and reduce cancer incidence [11]. This might be considered prior to levonorgestrel IUD use in patients who do not desire immediate pregnancy.

In the present study, treatment was performed by using MPA alone, without a combination drug such as aspirin for thrombosis prophylaxis. However, no patient had serious complications such as thrombosis, significant obesity, and liver function abnormalities. One patient presented complications of ovarian cancer during the course of study (10.0%). Evans-Metcalf ER et al. reported that 11% of young patients with EC show complications of ovarian cancer [12], and the present study results were similar to that shown in their study.

Recently, MPA was shown to be associated with cytostatic effects mainly via PR-B [13]. Jongen V et al. reported that patients with PR-B/PR-A < 1 had a poor prognosis factor [14]. In the present study, pretreatment development of PR-B and PR-A was examined, but the relationship between PR-B/PR-A ratio and prognosis remains unclear. Conversely, there is a report of decreased development of PR-B in progestin-resistant EC cell line [15]. None of the patients who were classified into recurrence or PD showed decreased development of PR-B before and after treatment, which suggested that development of PR-B was not effective in predicting successful MPA treatment (Figure 1A). MPA increases development of FOXO-1 through PR-B and leads to apoptosis in vitro experiment [16]. In the present study, immunostaining showed increased development of FOXO-1 in the nucleus and cytoplasm during MPA treatment, which suggested that FOXO-1 was a target of MPA treatment. However, increased development of FOXO-1 was found in all cases, including the failure-free and aggravated cases; therefore, further studies are required to confirm if FOXO-1 is a biomarker of successful treatment.

Although TVUS was used for monitoring the endometrial thickness, it is not confirmed whether it is useful as one of indexes to judge the effect of treatment, because no significant difference in endometrial thickness was observed between the CR and Not CR group. Thus, the authors considered that monitoring endometrial thickness was not useful to determine the effect of MPA treatment.

Progestin as a fertility-preserving treatment is expected to be effective for endometrial cancer, but judicious use might be necessary because it shows high rate of recurrence as well as the risk of concurrence of ovarian cancer. Once confirmed that the lesions have disappeared, the patients’ therapy should be shifted to an aggressive infertility treatment immediately to help improve the pregnancy rate. Further studies regarding the mechanism may be necessary to achieve high and effective treatment because administering progestin alone for EC has limitations.

References

Outcome of fertility-sparing treatment with medroxyprogesterone acetate for atypical hyperplasia and endometrial carcinoma etc.


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The correspondence between abnormal transformation zone Grade 1 and Grade 2 colposcopic parameters and histology.
Clinical implications.

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Summary
Objective: To analyse the correlation between the colposcopic parameters of Grade 1 and Grade 2 abnormal transformation zone (ANTZ G1 – ANTZ G2) and histological examination of the cone. Materials and Methods: A retrospective analysis of medical records of 600 women who underwent colposcopy and conisation (large loop excision of the transformation zone - LLETZ) between January 1, 2009 and July 31, 2012. The correlation between colposcopic and histological parameters was analysed using the Spearman non-parametric test. Results: In ANTZG1 there was no correlation (r= - 0.03; p= 0.55); in ANTZG2 however, a low degree of correlation (r = 0.21; p = 0.03) was found. Sensitivity, specificity, and positive and negative predictive values of an ANTZ G2 colposcopic picture were 33.45% (confidence interval [CI] 95% 28.0% to 39.2%), 95.48% (CI 95% 92.5% to 97.5%), 87.4% (CI 95% 79.7% to 92.9%), and 60.5% (CI 95% 56% to 64.9%), respectively. Conclusions: The decisive factor in the diagnosis of the cervical oncologic pathologies is the histological examination of the cone, and not the colposcopy which should be seen as a “guiding” investigation in predicting conisation and application of the most appropriate treatment.

Key words: Colposcopy; CIN; Biopsy; Conisation; LLETZ; HPV.

Introduction
Every year about ten million high-grade squamous intraepithelial lesions (HSIL) and over 500,000 cases of cervical carcinomas are diagnosed, of which about 80% occur in developing countries [1, 2]. About 3,500 new cases of cervical carcinoma and 1,500 deaths occur in Italy every year [3]. Cervical cancer is the third most common cancer affecting women worldwide [4]. Infection with human papillomavirus (HPV) is an inevitable cause of cervical cancer [5].

Over the last 50 years, screening has reduced the mortality rate for cervical cancer from 50% to 70% in industrialised countries and is, thus one of the most important methods for the secondary prevention of this disease [6, 7].

More specifically, the prevention and early detection of the cervical carcinoma are based on the interpretation of three analyses: cytology, colposcopy, and the histological results of a possible biopsy or cone biopsy.

Where a smear is abnormal, a colposcopy must be performed before starting therapy, to locate the exact spot from which the abnormal cells originate, to evaluate its extension and, above all, to ascertain its relation with the squamocolumnar (SJ).

Colposcopy can detect alterations not only on the surface and deep below it but also, in the composition of the epithelium and, in the vascularisation of the connective tissue, thus giving images of aceto-white and iodonegative areas, both isolated or associated, like more or less irregular epithelium, fine or coarse punctation, glandular openings inspissation and atypical blood vessels. Other features contributing to the evaluation of the abnormal colposcopic picture are the characteristics of the peripheral edges of the lesions, both clear and blurred, and the absence or presence of glandular openings [8-10], particularly if inspissated [11].

In addition, a good colposcopic test is meant to allow targeted biopsy of the lesions and proper post-therapeutic follow-up as well as to ascertain the horizontal and vertical extension of the lesion in line with preventive criteria.

Colposcopy nonetheless has some diagnostic limitations. The main limitation is that it is impossible to evaluate the characteristics of the endocervical lining, either when the endocervical lesion reaches up through the cervical canal or when the lesion is exclusively localised in the endocervical seat.

The reliability of a good biopsy sample from four mm of chorion cannot be overlooked; samples must also have an overall surface dimension of at least five to seven mm.

In the literature, the comparison between the histological results of the biopsy and the histological results of a larger surgical sample (cone), show variable underestimate (5.8% to 47%) and overestimate (4.6% to 42.4%) values of the lesion that can probably ascribed to the lack of homogeneity of the protocols adopted in various centres [12-14],

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to a sampling physician’s less than perfect professional background, to less than perfect or incomplete sampling of the lesion, or to all of these variables at the same time.

The accuracy of colposcopy has been increasingly questioned. Studies of loop excision after colposcopy have identified women with CIN2+ and cancer who were not detected by colposcopy [14]. Biopsy of colposcopic normal areas may reveal unsuspected CIN2+ [15]. Colposcopic lesion grade may predict histology poorly [16, 17].

Women with negative colposcopy remain at substantial risk for subsequent detection of CIN2+, suggesting that lesions were missed [18].

In the Atypical Squamous Cells of Undetermined Significance/Low Grade Squamous Intraepithelial Lesion Triage Study (ALTS), only 53% of women found to have CIN3 over two years of follow-up were identified at colposcopy intake, though most missed lesions were small and presumably early in their natural history and so at low risk of imminent progression to invasive cancer [19].

Unfortunately, colposcopy does not have optimal sensitivity for CIN2+. The National Health Service Cervical Screening Programme (NHSCSP) Guidelines for Colposcopy and Programme Management, which guides British practice, require evidence of 65% colposcopic accuracy [20].

Zuchna et al. reported 66.2% sensitivity of CIN2+ when up to three directed cervical biopsies were taken as a diagnostic test with the cone specimen as the reference standard [21].

Using digitised cervical images from 919 women referred for equivocal or minor cytological abnormalities to the ASCUS-LSIL Triage Study, Massad et al. reported 39% sensitivity for CIN2+ [22].

The present study aims to analyse the correlation – if there is any correlation – between the component colposcopic parameters of abnormal transformation zone grade 1 and grade 2 (ANTZ G1 – ANTZ G2) and histological examination of the cone.

In the abnormal colposcopic picture, two grades can be identified: ANTZ G1 that is characterised by a thin white epithelium, fine mosaic and fine punctation, and ANTZ G2 that is characterised by a thick white epithelium, coarse mosaic, coarse punctuation, thickened glandular openings, and atypical blood vessels leading to the suspected invasive neoplasia.

Materials and Methods

The present study took into consideration a sample of 600 patients who were attending two Prevention Centres and taking part in the Abruzzi Region Screening Program: these were the Colposcopy and Cervicovaginal Center of L’Aquila, in collaboration with the Colposcopy and Cervicovaginal Center of the Avezzano Hospital. The study covered the period spanning from January 1, 2009 to July 31, 2012.

The first phase of the study was a meticulous case history survey to gather information about the presence of preexisting cytological and/or colposcopic alterations and to check whether any biopsies and/or conisations had already been carried out on the patients.

Each patient underwent a new colposcopy and conisation procedure on the basis of the previous positive colposcopic tests carried out in both the above mentioned centres and in other centres outside the region.

For reporting and colposcopic terminology, the authors used the model approved by the International Federation for Cervical Pathology and Colposcopy (IFCPC) International Congress of Barcelona (2002).

To compensate for underestimates or overestimates in targeted biopsies, the authors performed the conisations directly, avoiding “aggressive” behavior as much as possible and always taking account of the SJ, the lesion, any bending inward of the lesion, and the constitutive parameters of the transformation zone (thick white epithelium, mosaic, punctuation, glandular openings, atypical blood vessels, etc.).

The conisations were carried out in an outpatient regimen through large loop excision of the transformation zone (LLETZ). The patients were put under local anaesthesia with paracervical block, with about 15 ml of two percent mepivacaina injected into the vaginal fornices through a 20 G curved tip needle.

The size of the cones varied according to the topography of the lesion, the morphology of the cervix, and the ecto-endocervical extension of the lesion.

After immediate fixation with 10% buffered formalin, the cone samples were sent to the anatomopathologist with the insertion of a marker wire which allowed the correct orientation of the surgical samples.

The excisional treatment caused no relevant painful symptomatology; in some instances, it caused light bleeding at most.

The authors adopted the WHO classification and used the SNOMED code (Systematized Nomenclature in Medicine) for the histological specimens.

The patients were properly informed by the gynecologist about the modality, characteristics, and purposes of the colposcopic test and conisation was dealt with by means of informed consent.

To begin with the descriptive statistical analysis of the main anamnestic and clinical variables (age, parity, ethnicity, biopsies, and/or conisations) was carried out; subsequently, the analysis of the Spearman non-parametric correlation was performed to assess the correlation between colposcopic parameters and histological examination.

The authors also found the colposcopic index cut-off value with higher degrees of sensitivity and specificity.

The statistical analysis was carried out using the statistical software SAS Version 9.2 (2002-2008) and the statistical software MedCalc Version 12.0.4 (1993-2011).

Results

The characteristics of the women (n = 600) who took part to the study are listed in Table 1. The average age is 37.2 years (range 18-75) and the standard deviation was 11.23 years. It turned out that the patients who underwent a previous conisation for CIN 2+ were 2.3% (n = 14). The remaining population under examination stated they had never undergone colposcopy, even if positive to colposcopy.

The colposcopic test carried out in the population under examination showed an ANTZ G1 clinical picture in 489 patients (81.5%) and an ANTZ G2 picture in 111 patients (18.5%) (Table 2). The conisation results are listed in Table 3. The excisional therapy caused light bleeding in 20 patients.
The correspondence between abnormal transformation zone Grade 1 and Grade 2 colposcopic parameters and histology etc.

The analysis of the correlation between colposcopy and histological examination was carried out separately in ANTZ G1 and ANTZ G2 colposcopic grading. In the former case it did not show any correlation between colposcopic picture and histological examination ($r = -0.03; p = 0.55$); in the latter case it showed a low degree of correlation ($r = 0.21; p = 0.03$).

An ANTZ G2 colposcopic picture had a sensitivity of 33.45% (CI 95% 28.0% to 39.2%) and a specificity of 95.48% (CI 95% 92.5% to 97.5%). These results suggested high probabilities of false negative results. The high rate of specificity instead implied a low probability of false positive results.

Concerning the prevalence ratio of positive results in histological examination (disease prevalence: 48.3%), the positive predictive value (PPV) of an ANTZ G2 colposcopic picture was 87.4% (CI 95% 79.7% to 92.9%). This meant that the probabilities for an ANTZ G2 colposcopic picture to produce a cone positive to histological examination were 87.4%, while only 12.6% turned out as false positives. Instead, the negative predictive value (NPV) of an ANTZ G2 colposcopic picture was 60.5% (CI 95% 56% to 64.9%), thus implying high probabilities of false negatives (39.5%).

**Discussion**

Given the results obtained both clinically and statistically, some conclusions can be drawn:

- The analysis of ANTZ G1 colposcopic parameters and histological examination of the cone did not show any correlations ($r = -0.03; p = 0.55$). More precisely, the correlation between punctuate and histological examination and, to a greater extent, the correlation between thin epithelium and fine mosaic did not correspond perfectly to the degree of the histological lesion supposed by colposcopy (CIN 1-LOW SIL).
- ANTZ G2 constitutive parameters (thick white epithelium, coarse mosaic, coarse punctation, thickened glandular openings, atypical blood vessels) seem to be more indicative in the study of the correlation between colposcopy and histology ($r = 0.21; p = 0.03$). Colposcopically, the most reliable parameters proved to be the atypical blood vessel and the thick white epithelium.
- The present study clearly shows that in clinical practice, the colposcopic report cannot and should not be considered fundamental as concerns the prediction of the degree of gravity of cervical lesions. The correlation between colposcopy and histological grading, also studied by other authors [17] even taking a different course, is not always a certainty.

In conclusion, the decisive factor in the diagnosis of the cervical oncologic pathologies is the histological examination of the cone, and not the colposcopy which should be considered as a “guiding” investigation in prediction of the conisation and of the application of the most appropriate treatment.
References


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An evaluation of immune system cell infiltrate in the cervical stroma of patients with grade III cervical intraepithelial neoplasia after treatment with intralosalonal alpha-2B interferon

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Summary
The aim of this study was to characterize infiltrating immune cells in cervical stroma biopsy samples from patients diagnosed with cervical intraepithelial neoplasias (CINs) who were treated with IFN-α 2b. The authors studied 13 volunteers who were diagnosed with Cervical intraepithelial neoplasia CIN II or III and who received intra-lesional treatment with IFN-α 2b. They collected pre- and post-treatment biopsies from each patient. They also examined the slides under a common optical microscope with a X400 lens for biopsy sample sections that were labeled with immunohistochemistry for T lymphocyte, B lymphocyte, natural killer cell, macrophage, iNOS, and perforin markers. The presence of immune response cells in the lesion was observed after treatment with intralosalonal IFN-α 2b in patients with CIN II/III changes, a reduction in CD4+ and CD8+ T lymphocyte infiltration in the women who responded well to treatment. However, there was a significant increase in these markers in samples from women who did not respond to treatment. Nonetheless, immunotherapy with IFN-α 2b administered intralasonally in patients with CIN II/III yields favorable results in patients who do not smoke.

Key words: Cervical intraepithelial neoplasia; Immunotherapy; Immune response.
The lympho-proliferative response of CD4+ T cells specific to the E2 antigen is associated with HPV elimination. Conversely, CD8+ T cells specific to the E6 and E7 antigens are found in patients with large lesions or cervical tumors [12]. Antigen presentation is compromised by the presence of viral capsids, which impede the activation of Langerhans cells [13].

Understanding the mechanisms and cells involved in the immune response that leads to CIN regression is fundamental to the ability to develop new therapies. Therefore, the objective of this study was to elucidate the composition of infiltrating cells (i.e., proportions of total T lymphocytes, T helper, T cytotoxic, B lymphocytes, natural killer (NK) cells, and macrophages) and the presence of the immune response mediators perforin and nitric oxide in CINs of patients treated with IFN-α 2B. Observations were compared between treatment responders and non-responders.

Materials and Methods

Setting and patients

A prospective study was carried out at the Maria da Glória outpatient clinic of the Hospital School of the Federal University of the Triângulo Mineiro in the discipline of Gynecology and Obstetrics from 2007 through 2009. The group studied consisted of 13 patients, 23–50 years of age, with diagnoses of CIN II or III who had not yet received any treatment. Patients provided information about their age, habits, lifestyles (e.g., smoking, use of drugs, number of sexual partners), contraceptive methods used, history of sexually transmitted diseases, and use of hormone replacement therapy.

All procedures were performed in accordance with the criteria developed by the Ethics Committee (CEP/UFTM Nos. 759 and 1525). The inclusion criteria were: absence of bleeding during the examination; no use of oral antibiotics, vaginal fungicides or creams in the previous 30 days; no sexual activity for two days preceding sample collection; no previous history of treatment for HPV. Colposcopic change is greater than one cm. The exclusion criteria were: immunosuppressive diseases, serious cardiopathies, changes in liver or kidney function, pregnancy, a reported intolerance to IFN, or an absence of a visible lesion at colposcopy.

Application of IFN

Human recombinant IFN-α 2b was used for the therapy. The IFN-α 2b was applied intraleesionally at a dose of 3,000,000 IU (flask-ampoule with lyophilic powder diluted in 1.0 ml of diluent before each application). The applications were performed using a 1.0-ml syringe with a 13 × 0.45 needle and administered three times per week on alternate days until a total of 18 applications were delivered.

A vaginal speculum was used to expose the cervix. The medication was applied following antisepsis of the cervix and the vaginal walls with gauze soaked in topivdine using Sheron forceps. After the first, sixth, 12th, and 18th application of IFN-α 2b, peripheral blood was collected from a vein in the right fore-arm of each patient.

Evaluation of clinical response

Patients were grouped according to treatment response based on colposcopic examination and histological observation of biopsies. If colposcopy showed disappearance or regression of the lesion and this observation was corroborated by histological examination of the patient’s biopsy demonstrating regression to CIN I or no CIN, the treatment was considered successful, and the patient was assigned to the good response group (GR). If no regression of the lesion was observed at colposcopic examination, and the persistence of CIN II/III was confirmed by histological examination of the patient’s biopsy, then the treatment was considered to have failed, and the patient was assigned to the bad response group (BR). All patients with persistent CIN II and III were submitted immediately to cold knife conization. The patients were submitted to follow-up with cytology and colposcopy every six months.

Immunohistochemical methods

The biopsy specimens were paraffinized and then four-µm-thick histological slices were cut, mounted on silanized slides, and then subjected to immunohistochemical staining with a polymer detection kit. To characterize immune cell infiltrates and identify cervical stroma markers in biopsy samples, the authors used antibodies specific to the following proteins: CD3, CD8, CD4, CD20, CD68, CD16, perforin, and induced nitric oxide synthase (iNOS).

The slide-mounted slices were held in an incubator at 56 ºC for 24 hours and then deparaffinized in three five-minute washes in xylol. The tissues were then hydrated in three baths of absolute alcohol and one bath of 80% alcohol, for about ten seconds each. The slides were then hydrated in phosphate buffered saline solution (PBS, pH 7.2) for five minutes. After removal of excess PBS from the slides, drops of 3% oxygenated water were placed on each slide for ten minutes to block endogenous peroxidase activity. After blocking, the slides were washed again in PBS.

The authors performed antigen retrieval using a Pascal pressurized chamber. Briefly, they added 45 ml of buffer (for CD4 TRIS/EDTA, pH 9.0, for all others citrate, pH 6.0) to the chamber and subjected the slides to three minutes of boiling followed by at least 30 minutes of recovery to allow the chamber’s pressure and temperature to decrease. After antigen retrieval, they again washed the slides in PBS buffer three times, five minutes per wash before incubating the slices with primary antibody. Each primary antibody was diluted in buffer with 10% bovine serum albumin to the concentration recommended in the manufacturer’s specifications as follows: CD3 [1:3.200], CD8 [1:200], CD4 [1:300], CD20 [1:6.000], CD68 [1:3.000], CD16 [1:50], perforin [1:100], and iNOS [1:500]. The slides were incubated with primary antibody for about 18 hours in a wet chamber at 3–4 ºC.

Following the primary antibody incubation period, the slides were allowed to warm to room temperature for about 15 minutes. The slides were then washed in PBS and dried. The authors applied post-primary penetration enhancing reagent from the polymer detection kit to each slide, and maintained the slices at room temperature for 30 minutes in a moist chamber. They washed the slides three times, five minutes per wash, in PBS and then added the polymer reagent (also from the kit) and allowed the slices to incubate with the polymer reagent at room temperature for 30 minutes in a moist chamber. After three five-minute washes in PBS, labeling was visualized by exposing the slides to a chromogenic solution for five minutes. The authors then rinsed the slides in tap water and dipped them in Harris’s hematoxylin for two seconds to add a nuclear counterstain. Finally, they immersed the slides in tap water, dipped them in Harris’s hematoxylin for four seconds each to remove excess water, one bath of phenolicated xylol to remove excess alcohol, and three baths of xylol for five minutes each to clarify the slides (diaphanization). The stained specimens were covered with coverslips and viewed under a light microscope.
An evaluation of immune system cell infiltrate in the cervical stroma of patients with grade III cervical intraepithelial neoplasia etc.

Immunohistochemical analysis

The authors evaluated pre- and post-treatment cervical stroma biopsy specimens for total population of T lymphocytes (CD3+), T helper lymphocytes (CD4+), T cytotoxic lymphocytes (CD8+), B lymphocytes (CD20+), NK cells (CD16+), macrophages (CD68+), and the presence of iNOS-expressing cells and perforin. Initially, they observed the cells at low magnification (×100) to evaluate their general distribution. Next, they examined them more closely (×400 magnification); for pre-treatment biopsies, they examined the subjacent stroma below the CIN II or CIN III lesion and for post-treatment biopsies, they examined the CIN or HPV infection site to obtain a final score. Lymphoid cell quantity was scored on a 0–3 scale as follows: 0, absence of inflammatory cells; 1, sparse inflammatory cells; 2, a moderate number of inflammatory cells; and 3, numerous inflammatory cells [14].

Statistical analysis

An electronic database was developed for the statistical analysis. The variables were analyzed using GraphPad Prism 5.0 software. The values were submitted to Student’s t test. The differences were considered statistically significant at p < 0.05.

Results

The mean age of the 13 patient participants was 33.9 ± 9.1 years (range, 23–50). The age, parity, smoking status, initial and final diagnoses, and clinical response to treatment of each patient are summarized in Table 1. Overall, about half (6/13; 46.15%) of the patients were multiparous (≥ two births) and about half (7/13; 53.85%) were smokers. At initial diagnosis, 7/13 patients (53.85%) had CIN II and 6/13 (46.15%) had CIN III.

The authors found that 6/13 patients (46.15%) responded well to treatment with IFNα-2b; conversely, 7/13 patients (53.85%) had therapeutic failure. Of the six patients who responded well to the treatment, four (66.67%) were multiparous, and five (83.3%) were non-smokers (Table 1). Of the seven patients whose therapy failed, four (57.14%) were multiparous and only one (14.28%) was a non-smoker.

Relative to their pre-treatment assessments, the GR patients showed shifts toward greater expression (from 0–1 to 2–3 on the Georgiannos protocol) of the CD3 and CD68 markers following treatment with IFN-α 2b (Table 2). Expression of iNOS was stable from pre- to post-treatment in the GR patients, but two BR patients’ Georgiannos biopsy scores went from 2–3 pretreatment to 0–1 posttreatment (Table 2). The GR and BR patients showed opposite pre- to post-treatment patterns in CD4 (Figure 1) and CD8 (Figure 2) positivity. BR patients showed a strong trend (p = 0.05) toward greater expression of the CD4 marker post-treatment, relative to pre-treatment. And post-treatment, we observed a weak trend (p = 0.09) of BR patients having greater CD4 marker expression than GR patients. Meanwhile, GR patients showed a strong trend (p = 0.056) toward a reduction in expression of the CD8 marker from pre- to post-treatment (Figure 2). In post-treatment, the authors observed significantly lower CD8 expression in GR patients than in BR patients (p = 0.03). They did not observe evidence of treatment effects on expression of CD16, CD20, iNOs, and perforin (Table 2).

Discussion

In this study, the authors observed that 6/13 patients (46.15%) had responded to treatment with IFN-α 2b as evidenced by a disappearance of the high-grade lesion. The remaining seven patients (53.85%) experienced therapeutic failure, meaning that they observed high grade squamous intraepithelial lesion (HSIL) in the initial and final diagnoses.

Since the early 1980s, and particularly in the last decade, numerous studies examining IFN therapies for treatment of gynecological cancers have achieved varying responses [15, 16]. IFN’s actions, both as an antiproliferative and as an immunoregulatory, are now a focus of interest for many investigators. In studies with IFN-α, CIN remission rates vary from 30% to 80% [17-19]. However, Byrne et al. [20], using a topically applied IFN-α gel on CINs, and Frost et al. [21], applying IFN-α 2b intralesionally, obtained results...
similar to a placebo. Dunham et al. [18] performed a study on 14 patients with CIN, seven of which were controls and seven of which were treated twice a week for a month with intralesional injections of IFN-α 2b. They observed an improvement in six out of seven patients in the study group, including two complete cures. Only 3/7 patients in the control group showed spontaneous improvement, but there were no complete cures. Koilocytosis, a cellular characteristic of HPV infection, disappeared in all of the study group cases treated, but two cases of the control cases. Both

<table>
<thead>
<tr>
<th>Marker</th>
<th>Score</th>
<th>Good response</th>
<th>Poor response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before x/n (%)</td>
<td>After x/n (%)</td>
<td>Before x/n (%)</td>
</tr>
<tr>
<td>CD3</td>
<td>0 - 1</td>
<td>4/6 (66.7)</td>
<td>2/6 (33.3)</td>
</tr>
<tr>
<td></td>
<td>2 - 3</td>
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<td></td>
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<tr>
<td>CD8</td>
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<td>4/6 (66.7)</td>
</tr>
<tr>
<td></td>
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<td>2/6 (33.3)</td>
</tr>
<tr>
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<td>4/6 (66.7)</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
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</tr>
<tr>
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<td></td>
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<td></td>
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<td>0/6 (0.0)</td>
<td>0/6 (0.0)</td>
</tr>
</tbody>
</table>

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**Table 2. — Analysis of distribution of expression of cellular markers and products in GR and BR patients.**

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**Figure 1.** — Representation of the distribution of the T helper lymphocyte marker CD4+ in GR and BR patients, before and after treatment with IFN-α 2b, based on immunohistochemistry, scored according to Georgiannos’ classification rubric.

**Figure 2.** — Representation of the distribution of the T helper lymphocyte marker CD8+ in GR and BR patients, before and after treatment with IFN-α 2b, based on immunohistochemistry, scored according to Georgiannos’ classification rubric.
groups were monitored by colposcopy, cervical smears, and biopsies for histology.

There is evidence indicating that both systemic and local immune responses play an important role in progression of CIN [22, 23]. However, local immunity may be more important and efficient than systemic immunity in controlling HPV infection and the development of CINs given that patients with a cellular immune deficiency develop cervical lesions more frequently than the general population [22, 23]. Arany and Tiryng [24] analyzed the local immune response of patients with HPV who were treated with IFN. The biopsies of patients who did not respond to IFN treatment had markedly lower levels of Langerhans cells, leading to reduced expression of major histocompatibility complex (MHC) class II molecules and, therefore, reduced activation of CD4+ T cells. There was a drop in the expression of MHCI as well, with a decrease in levels of CD8+ T cells. However, the biopsies of the patients who responded well to treatment were verified to have NK cells (CD16), macrophages, and activated CD4 T cells present, with recruitment of these T cells against HPV-infected cells, demonstrating that a cellular immune response was occurring.

Mardegan et al. [25] found that 62.5% of patients treated with intralesional IFNα-2b responded to the treatment as evidenced by a complete histological disappearance of the high-grade lesion. Flow cytometry experiments indicated that concentrations of IL-6 and TNF-α in patients’ vaginal secretions during treatment (at sixth application) were significantly higher in patients with failed therapy than in therapy responders [25]. Conversely, using the hybrid capture technique, they found a significant drop in viral load, before versus after treatment, in treatment responders. In a larger study of the same therapy, Ramos and collaborators (2010) [10] obtained a satisfactory clinical response in 60% of patients with high-grade CINs. They found that the Th1 (IFN-γ, TNF-α, IL-2) immune response was related to reduction in CIN grade after treatment with IFNα-2b in patients who responded well. There was also a significant drop in the viral load of high-risk HPV (measured using the hybrid capture technique) in patients who responded to treatment [10].

Based on quantification of cytokines in the blood of patients with high-grade CINs treated with intralesional IFN-α 2b, Misson and colleagues (2011) [11] observed a 50% therapeutic response rate. Only 16.6% of those who responded well were smokers, while 66.6% of those with therapy failure were smokers. They therefore associated the use of tobacco with the induction of failure. In the group of patients with successful therapy, they observed a significant increase in Th1-profile cytokines, which stimulated a drop in Treg-profile cytokines. Their analysis of the cytokines, via enzyme-linked immunosorbent assay (ELISA), showed that the average concentration of IL-12 in the blood of patients with successful therapy was significantly elevated on the 12th day, relative to baseline, and that this increase was associated with an agglomeration of the immune cells that produce a mixture of pro-inflammatory and regulatory cytokines.

Despite the various aforementioned studies cited, which evaluated the production of cytokines both systemically and locally in lesions of patients treated with IFN-α, few studies have determined which immune cells might participate in the regression or persistence of a high-grade lesion. Therefore, knowing the intensity and the different local infiltrates of the immune cells is fundamental to understanding the mechanisms involved in cervical tumorigenesis. More specifically, to the authors’ knowledge, there are no published studies that have revealed the relationship between local immune response before and after treatment with IFN.

Maluf et al. [26] have reported that there are strong indicators of the presence of T lymphocytes (CD3+) in CIN III lesions in patients with a recurrence who had received conization; based on immunohistochemistry studies using Georgiannos’ criteria, they found that 100% of the women with recurrent CIN III had a high level of CD3+ lymphocytes. Using the same technique and the same evaluation criteria, Silva et al. [27] analyzed 60 histological samples (20 control, 20 CIN III, and 20 invasive carcinoma) and identified the inflammatory infiltrate of CD3+ and CD8+ lymphocytes in all three groups, with the invasive carcinoma patients showing the highest levels. They were unable to affirm anything with respect to the cytotoxic capacity of these lymphocytes.

In this study, the authors demonstrated weak perforin labeling in all 13 cases, independent of clinical response, which may confirm the inactivity of these lymphocytes. Perforin is present in T (CD8+) and NK cells, where the formation of pores in the targeted cell may facilitate the entry of toxic enzymes (granymes) into carcinogenic cells or into cells infected with certain viruses; or they may make the cell unable to eliminate ions and water, causing it to die [28].

Understanding the molecular mechanisms that regulate signal transduction as mediated by IFN-α, the escape mechanisms that are active in cancer cells, and the tumor’s resistance mechanisms against IFN-α are of great value to developing new therapeutic strategies based on IFN-α, in order to expand this cytokine’s therapeutic effects. Therefore, the authors conclude that the population of immune response cells in lesions can be changed by treatment with intralesional IFN-α 2b in patients with CIN II/III. There is a general reduction in infiltration of CD4+ and CD8+ T lymphocytes in women who respond well to treatment, whereas women who do not respond to treatment show increases in the infiltration of these lymphocytes. The presents findings indicate that immunotherapy with intralesional IFN-α 2b in patients with CIN II/III yields favorable results in patients who do not smoke.
Acknowledgments

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References

Clinical analysis of sentinel lymph node identification in patients with cervical cancer

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Summary

Objective: To study the accuracy and feasibility of identifying sentinel lymph nodes (SLN) using methylene blue dye in patients with cervical cancer. Materials and Methods: Fifty-six cases with early-stage cervical cancer patients were studied using methylene blue injection into the cervix 90–120 minutes before abdominal pelvic lymph node dissection and extensive hysterectomy. The lymph nodes that resulted from staining were removed and pathohistology was performed. Results: A total of 106 SLN were identified in 49 patients (49/56). The detection rate of SLN was 87.5%. Sensitivity of the SLN was 90.91%, and specificity of the sentinel lymph nodes was 86.67%. Eleven patients (19.64%) were diagnosed with lymph node metastases and ten of them were in the group of SLN. Eight patients had positive SLN only. Two patients had both positive SLN and pelvic lymph nodes. None of the patients had positive pelvic lymph nodes and negative SLN. Conclusion: The use of methylene blue injection for cervical cancer SLN biopsy has a higher detection rate of SLN. SLN detection can accurately predict the pathological status of pelvic lymph nodes in patients with cervical cancer.

Key words: Endometrial cancer; Brachytherapy; Radiotherapy; Toxicity.

Introduction

Cervical cancer is the most common gynecologic malignant tumor. The incidence is second in women with a malignant tumor, that is, after breast cancer [1]. There are about 500,000 new cases in the world each year, approximately 85% concentrated in developing countries [2]. Cervical cancer metastasis occurs through the lymph nodes. Since abdominal radical hysterectomy was first carried out by Wertheim in 1898, pelvic lymph node dissection has been an indispensable part of the surgical treatment of cervical cancer. Pelvic lymph node metastasis is an important factor affecting the cervical cancer prognosis, which is also an important basis for follow-up treatment after surgery. The pelvic lymph node metastasis rate of cervical cancer is 0–4.8% in Stage IA, 0–17% in Stage IB, 12–27% in Stage IIA, and 25–39% in Stage II B [3-5]. If detection and evaluation of pelvic lymph node metastasis state can be achieved, pelvic lymph node negative patients do not require a wide range of pelvic lymph node dissection to reduce the chance of trauma and postoperative complications. It is extremely necessary to improve the quality of life of patients, that is, to retain reproductive function in women of childbearing age. Personalized treatment programs for cervical cancer enable unnecessary overtreatment. It is also the purpose of this study: to improve the quality of life of patients.

Sentinel lymph node (SLN) is the first lymph node which is affected by primary tumor metastasis. SLN is an effective screening method for cancer metastasis prevention [6]. In theory, if the lymph node is negative, this signifies that other lymph nodes will also have no metastasis. Cervical cancer metastasis pathway is based in lymph node, and it is suitable for the introduction of the SLN detection method because of pelvic lymph drainage with certain regularity. SLN biopsy was first used in cervical cancer research by Echt et al. in 1999 [7]. It is expected to improve the reproductive function and hence the quality of life of the patients because it more accurately predicts the pelvic lymph node involvement [8]. SLN is the first to most likely be affected by metastasis. It has a higher grade of precision and low false negative rate and can accurately predict early cervical cancer patients with pelvic lymph node involvement. Therefore, lymph node dissection can be prevented. This research uses methylene blue dye as tracer and the present authors analyzed the pathological result of non-sentinel lymph node (NSLN) and SLN. To study the accuracy and feasibility for SLN treatment for cervical cancer, the authors needed to achieve a base for individualized treatment for cervical cancer.

Materials and Methods

Patients

Between June 2009 and December 2010, 56 patients with FIGO Stage IA2-IIA cervical cancer were scheduled to undergo fertility-sparing surgery at the Affiliated Hospital in Ningxia Medical University and Institute participated in this study. All patients underwent radical hysterectomy and total pelvic lymphadenectomy. This study was conducted in accordance with the Declaration of Helsinki. This study was also conducted with approval from the Ethics Committee of Affiliated Hospital of Ningxia Medical University. Written informed consent was obtained from all participants. The median age was 45.5 years (range 23 to 67). All patients in this study had no surgical contraindication, without severe disease, and no obstetric complica-
All patients had previous biopsy and histology with no neoadjuvant radio-chemotherapy. Iconographical examinations and physical examinations before surgery showed no lymph node intumescence and the patients had no cancer histories in other system and organs. The criteria for staging included FIGO 2008 classification; of the 56 patients, six were in Stage IA2, 24 in Stage IB, and 26 in Stage IIA. Histological assessment confirmed that 50 patients had squamous cell carcinoma, five had adenocarcinoma, and one had other type. Fourteen patients were well-differentiated, 18 were moderately-differentiated, and 16 were poorly-differentiated.

**SLN injection**

SLNs were detected with an isotope injection technique into the uterine cervix. On surgery day 90–120 minutes postoperatively, the authors injected fluid containing two ml (20 mg) of methylene blue into four quadrants (three, six, nine, and 12 o’clock positions) of the cervix, each quadrants one ml, and total was four ml. The diameter of particle ranged from five mm. The SLNs were identified by methylene blue staining during surgery. The authors then sent all of the lymph nodes and other tissues to biopsy after the surgery to confirm whether there was cancer metastasis or not (Figure 1). Through scanning the pelvic sidewall, presacral area, and para-aortic lymph node area, hypercapitive nodes were detected on the basis of counts that were more than ten-fold above background level, and were defined as SLNs. Then the SLNs were excised with safety margins and submitted to fast frozen section. The radioactivity of the tissue was measured in vivo and after excision; as well, the radioactivity of the surgical bed was also analyzed to confirm whether the marked lesion had been fully excised. After removal of the SLNs, bilateral pelvic lymphadenectomy was routinely performed.

**Criteria standard**

According to the SLN criteria standard [9], the state of the SLN was judged with the naked eye by the surgeons. SLN showing blue staining was defined as successful and SLN that remained unstained was considered as failure.

Relevance ratio indicated SLN positive cases/total cases *100%. Sensitive ratio indicated SLN positive cases/pelvic lymph nodes positive cases *100%. Accurate ratio indicated [(SLN positive cases + SLN negative ratios) / SLN identification cases] *100%. Specificity ratio indicated SLN negative cases/non lymph nodes metastasis cases *100%. False negative ratio indicated: false negative cases/pelvic lymph nodes metastatic cases *100%. Predictive value of negative indicated: SLN negative patients had non-pelvic lymph nodes metastasis cases / SLN negative cases *100%.

**Statistical analysis**

Multiplicity was compared with unconditioned logistic regression analysis. Statistical significance was defined at a level of $p < 0.05$. All analyses were performed using SPSS version 13.0.

**Results**

**Detected ratio of SLN**

A total of 1,052 lymph nodes were detected as SLNs in 49 of 56 patients (Figure 2) and the relevance rate was 87.5% (49/56). A total of 106 SLN were cut (10.08% in total: 106 / 1,052). The number of SLNs identified per patient was one in 15 cases (30.61%), two in 19 cases (38.78%), three in eight cases (16.32%), and four in seven cases (14.29%). The number of SLNs identified per patient was six in six Stage IA2 cases (100%), 22 in 24 Stage IB cases (91.67%), and 21 in 26 Stage IIA cases (80.77%).

**Pathological result of detected SLNs**

Eleven cases had pelvic lymph node metastasis in total 56 cases: the transfer ratio was 19.64% (11/56), 15 SLN had cancer cells metastasis in a total of 28 (53.57%), and NSLN in 13 cases in total 28 (46.43%). Ten had cancer cell metastasis in 49 SLN identified as successful cases, which is...
Clinical analysis of sentinel lymph node identification in patients with cervical cancer

Figure 3. — SLN (HE negative x100).

Figure 4. — SLN (HE negative x200).

Figure 5. — SLE (HE positive x100).

Figure 6. — (SLN HE positive x200).

shown in Figures 3 and 4. The metastasis ratio was 20.41%. One cancer cell metastatic case failed in SLN identification. No cases of SLN negative and NSLN positive (false negative cases) resulted, as shown in Figures 5 and 6. Both SLN and NSLN were negative in 39 cases. According to the aforementioned criteria standard, sensitive ratio was 90.91 (10/11), specificity ratio was 86.67% (39/45), accurate ratio was 100% (49/49), false ratio was 0, and predictive value of negativity was 100% (39/39).

Distribution of SLN

Eighteen unilateral SLNs were detected in 49 SLN detected successful cases (36.73%) and 31 bilateral SLNs were detected (63.27%). The distribution of 106 SLNs is shown in Table 1. The distribution of 15 positive SLNs is shown in Table 2. As shown in Table 1, SLN was concentrated on obturator, external iliac, and internal iliac; the detected rate was 92.45% in total (98/106).

As shown in Table 2, positive SLNs were concentrated on the obturator and occupied more than 50%.

The influential factor of SLN detected ratio

The study took into consideration the age of the patients, the diameter of the tumor, the clinical stage, and the detected ratio to formulate an unconditioned logistic regression analysis. The results showed that the ratio was
Table 1. — The distribution of 106 SLNs.

<table>
<thead>
<tr>
<th>The distribution of SLN</th>
<th>No. of patients</th>
<th>No. of cases with SLNs detected (%)</th>
</tr>
</thead>
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<tr>
<td>Obturator</td>
<td>42</td>
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<tr>
<td>External iliac</td>
<td>30</td>
<td>28.30</td>
</tr>
<tr>
<td>Internal iliac</td>
<td>26</td>
<td>24.53</td>
</tr>
<tr>
<td>Deep inguinal lymph nodes</td>
<td>5</td>
<td>4.72</td>
</tr>
<tr>
<td>Common iliac</td>
<td>3</td>
<td>2.83</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
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</tr>
</tbody>
</table>

Table 2. — The distribution of 15 positive SLNs.

<table>
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<th>No. of cases with positive SLNs detected (%)</th>
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</thead>
<tbody>
<tr>
<td>Obturator</td>
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<td>53.33</td>
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<tr>
<td>External iliac</td>
<td>4</td>
<td>26.67</td>
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<tr>
<td>Internal iliac</td>
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<tr>
<td>Common iliac</td>
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<td>6.67</td>
</tr>
<tr>
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<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Table 3. — An unconditioned logistic regression analysis of SLN detected ration.

<table>
<thead>
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<th>Character</th>
<th>β</th>
<th>SE</th>
<th>Wald</th>
<th>Sig</th>
<th>OR</th>
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<td>Age</td>
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<td>0.681</td>
<td>0.701</td>
<td>0.403</td>
<td>1.768</td>
</tr>
<tr>
<td>The diameter of tumor</td>
<td>1.882</td>
<td>1.229</td>
<td>2.345</td>
<td>0.126</td>
<td>6.565</td>
</tr>
<tr>
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<td>1.122</td>
<td>3.101</td>
<td>0.078</td>
<td>7.210</td>
</tr>
</tbody>
</table>

independent from the age of the patients, from the diameter of the tumor, from the clinical stage, and from the detected ratio; the difference had no statistical significance ($p > 0.05$, Table 3).

Discussion

Cervical cancer is the most common gynecologic malignant tumor and it is the first female malignant tumor in China [10]. It tends to occur at a younger age and is a serious threat to women's health and lives in recent years. The main route of metastasis is via the pelvic lymph node, and lymph node metastasis is also the key factor that influences treatment and cure. Therefore, it is very important to assess the accuracy of treatment in order to determine whether the lymph nodes are metastasizing or not. Currently, the standard treatment of cervical cancer is generally still hysterectomy and bilateral pelvic lymphadenectomy. There is no tumor metastasis of the conventional resection of pelvic lymph nodes in the vast majority during surgery. Excessive pelvic lymph node dissection has not only failed to be beneficial to patients, but will also cause them unnecessary vice damage, such as urinary retention, lymph cyst, peripheral vascular nerve damage, pelvic adhesion, infections, immunity problems, etc. Therefore the present authors adopted an individual treatment that prevented over-treatment, which is an area of great concern when studying cervical cancer. SLN detection can assist to further study the treatment of cervical cancer.

SLN detection rate

As early as the 1990s, the study of SLN detection began, and the ratio was only 15% [7]. Along with the developed skills, the ratio was improved. The studies in recent days showed that the ratio was nearly 100% [11]. There is a varying detection rate according to different testing methods. Cervical cancer SLN detection rate is 62.5% ~ 87% with the dye method [12], the detection rate is 70% ~ 100% with the nuclide method [13], and the detection rate is 88.8% ~ 100% with the joint method [11, 14]. Different detection methods with detection rate differences may be associated with the surgeon’s technological experience. Dargent et al. used methylene blue as a tracer, completed the laparoscopic lymphadenectomy, and achieved 89% SLN detection ratio [15]. Di Stefano et al. used methylene blue as a tracer to study 50 cases, and achieved a 90% SLN detection ratio [16]. In their study, 49 cases were successfully detected and the ratio was 87.5% (49/56). One hundred six SLNs were detected in total and 2.16 on average; this is in accordance with the present report. Therefore, the present authors believe that methylene blue used as a tracer in SLN detection is reliable.

Accuracy and false-negative rate of SLN

The accurate ratio and the false negative ratio of SLN are the important index to predict whether the tumor has metastasized or not and false negative ratio is the key to accurately predict SLN. False negative results can lead to mistaken results and therefore lead to incorrect treatments. Cervical cancer SLN biopsy accuracy and false-negative rate reported in the literature are not consistent; the false-negative rate is between 0% to 25%. There are five summaries of the reason [17]: 1) technical proficiency of the operator, including the surgeon, cannot adequately identify and remove all of the SLNs; 2) the later tumor metastatic tumor thrombus blocking nor diverting lymphatic drainage which does not recognize the true SLN; 3) there is about one percent of the lymph node metastasis jumping; 4) there is error and omission micrometastases in SLN biopsy; 5) detecting the number of cases is too small. Malur et al. [18] reported 50 cases SLN predictive value of negative was 97.1%, and sensitive ratio was 83.3%. Niikura et al. [19] reported 295 cases; the detected ratio of SLN was 85%, sensitive ratio was 93%, and predictive value of negative ratio was 99%. This study showed the sensitive ratio was 90.91 (10/11), specificity ratio was 86.67% (39/45), accurate ratio was 100% (49/49), false ratio was 0, and predictive value of negative was 100% (39/39). The results suggest that SLNs and pelvic lymph node metastasis are consistent.
The distribution of the sentinel lymph node

In 1971, Plentl, Friendman [20] formulated the cervical cancer of the lymphatic drainage system description. It is interstitium → subserosal lymphatic → parametrical lymph nodes in the cervical → obturator → internal iliac and common iliac lymph nodes outside → perirectal lymph nodes → abdominal aortic lymph node. In this lymphatic drainage pathway, it is not difficult to see that parametrical lymph nodes should be the first to arrive at the lymph nodes of the cervical lymph circulation. The transfer rate reached up to 30% in some reports [21]; however most of the local literature at home and abroad is not found parametrical the SLN or very low recognition rate. The reason may be due that cervical cancer SLN identification method is injection of tracer from the cervical part of parametrical lymph nodes which is the closest to the cervical tissue, while parametrical lymph nodes smaller tracer injection cause the entire cervical blue dye. This will affect the recognition of parametrical SLN. In addition, during surgery, the parametrical lymph nodes are removed with extensive hysterectomy. This is easily ignored by both pathologists and clinicians. No parametrical SLN was found in this study.

Foreign literature reports that the most cervical cancer SLN identification occurs in iliac external lymph nodes. SLN is identified in methylene blue tracer method by Di Stefano et al. [16], 50 cases of patients with cervical cancer early detection SLN of the communist party of 86, which accounts for 55% of them located in the iliac outer area and the obturator foramen area in 38%. In this study, 106 SLNs were detected in a total of 56 patients. The most common site for SLN detection was the obturator (42SLNs), detected in 39.62% (42/106); followed by the external iliac (30SLNs), detected in 28.30% (30/106); the internal iliac (26SLNs) in 24.53% (26/106); inguinal deep five, accounted for 4.72%, and the common iliac (3SLNs) in 2.83% (3/106). There were 15 positive SLNs in total, eight were in the obturator fossa, accounting for 53.33%, four were in iliac external region, accounting for 26.67%, two were in iliac internal region, accounting for 13.33%, one was in iliac area, accounting for 6.67%; obturator, external iliac, and internal iliac together were 92.45, and this is in accordance with Kushner et al. [22], who reported that these three areas can evaluate more than 80% SLNs. This result shows that recognition of SLN can indicate a certain area for lymph drainage, avoiding excessive lymphadenectomy.

The influencing factors of sentinel lymph node

Whether the SLN could be detected successfully depended on the next step. According to O’Boyle et al. [23], SLN detection is in relation to the diameter of the tumor. Some studies showed the stage could influence SLN detection. According to Darai et al. and Coutant et al. [24, 25], early-stage SLN detection ratio was higher than in a later one. When tracer was injected, osmosis was not good or deleted due to osmosis. In this study, the authors found except the factors mentioned above, that the depth and the angle when they injected the trace, the distance from the tumor, and the doctor’s skill are also the factors. This study showed that 49 cases of SLN detection was successful in all 56 cases, whereas seven cases failed. In these latter seven cases, two were in Stage IB and five were in Stage IIA; the detected ratio was lower with the increasing of the stage, but the difference has no statistical significance. In these seven failed cases, three with a diameter > four cm, the left obturator lymph node became enlarged (3 x 4 x 3cm) firm and fixed in one case, the pathological result after the surgery confirmed lymph node metastasis. This indicates that the SLN detection in the diameter of tumor > four cm is lower than it ≤ four cm, (p > 0.05); the difference has no statistical significance. At the beginning of this study, due to blue dye effluence after cervical injection of tracer, the recognition of SLN failed in two cases.

Problems and prospects

In conclusion, the current study indicated that SLN procedure using methylene blue, as a tracer is minimally invasive, and an accurate technique to assess pelvic lymph node status in patients with cervical cancer. Lymph node mapping using SLN biopsy may help predict the metastatic status of cervical cancer patients. It can provide a new perspective in the treatment for cervical cancer, but still the following questions remain: 1) How to improve the sentinel lymph node with to predict pelvic lymph node detection rate, sensitivity, and accuracy? 2) How to reduce the SLN identification of false negative rate? 3) SLN detection method for patients whether relapse, follow-up treatment, and survival rate influence existence? Cervical cancer SLN research is still in the primary stage whether to use SLN biopsy replace traditional pelvic lymph node dissections remains to be further researched. The sample quantity of this study is less, if to want to this technique is applied to clinical guidance operation scope, it still needs to large sample, multicenter, prospective case study.

References

The impact of epithelial ovarian cancer diagnosis on women’s life: a qualitative study

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Summary
Purpose of investigation: To describe the experience of ovarian cancer patients from symptoms complained before diagnosis until the impact of ovarian cancer diagnosis perceived by women. Materials and Methods: The authors used the Psychological General Well being Index (PGWBI) and a semi-structured interview to measure the overall well being of 39 women diagnosed with ovarian cancer in the period 2005-2010 at a secondary care hospital in northern Italy. Results: The PGWBI showed that the majority of the women reported general stress. On the semi-structured interview, 95% of women reported having symptoms and 69% reported a stressful event prior to diagnosis. More than 50% of women reported changes concerning life course. Almost all reported that their primary concerns had to do with the surgical scar, weight gain, and hair loss. Conclusions: Ovarian cancer diagnosis has a very stressful effect on the quality of life. Early assessment of psychological problems must be an integral part of the therapeutic pathway. Gynaecologists must provide clear and useful information regarding the disease itself as well as regarding correlated symptom relief.

Key words: Ovarian cancer; Survival; Quality of life; Patterns of care.

Introduction
Epithelial ovarian cancer (EOC) is the most lethal gynaecological tumour, with 224,747 new cases yearly worldwide and an estimated 140,163 disease-related deaths [1]. In Europe, approximately 66,700 new ovarian cancer cases are diagnosed yearly, with the highest incidence in northern European countries and the UK [2,3]. In the Emilia-Romagna Region, a region of Northern Italy, there is a yearly average of 17.6 new EOC per 100,000 females (about 401 new cases) [4]. The majority of women (about 65%) are diagnosed at advanced International Federation of Gynecology and Obstetrics (FIGO) Stage III-IV [5]. The most recent report shows overall European age-standardized five-year relative survival at 36.1% [6]. Survival in Italy is also below 40%, with a marked age gradient: in older women, the higher frequency of advanced stage disease and the presence of comorbidities compromise the benefits of radical interventions and chemotherapy [7].

Due to the advanced stage at diagnosis, side-effects, radical surgery, and chemotherapy, physical, and psychological sequelae emerge which can radically change a patient’s life.

The aim of this study was to describe the experience of EOC patients in order to raise the awareness of general practitioners and specialists regarding these patients’ emotional and psychological needs. EOC patients were asked to describe their experiences with diagnosis and treatment, as well as the relationships with their general practitioner, gynaecologist, oncologist, and nurses.

Materials and Methods
The study was approved by the provincial Ethical Committee. The study design was observational in that it describes the characteristics of the women with ovarian cancer who underwent surgery at the Department of Gynaecologic Oncology and Surgery of the IRCCS-Arcispedale Santa Maria Nuova in Reggio Emilia, Italy during the period 2005-2010.

The Department Chief contacted 77 women to explain the details of the study and to ascertain whether they would be willing to participate in a psychological interview. Of these 77 patients, 20 refused, ten had had recurrence, and one had died. A psychologist then contacted the remaining 46 women, of whom seven refused to participate in the interview and 39 accepted; these latter completed the questionnaire.

Two tools were used to collect data: the Psychological General Well being Index (PGWBI) and a semi-structured interview.

The PGWBI, a self-administered questionnaire that measures the level of well being or distress related to the emotional/affective sphere, was developed in the United States in the 1970s. The present study used the Italian version of this tool [8]. Chosen for its simplicity and for its reliability, the PGWBI is made up of 22 items covering the six dimensions of anxiety, depressed mood, positive well being, self-control, general health, and vitality. The items referred to the four weeks prior to completing the questionnaire and is self-administered at the beginning of the meeting with the psychologist.

The semi-structured interview was developed ad hoc to investigate the patient’s experiences with reference to:
1) symptoms and experiences prior to diagnosis;
2) experiences during clinical pathway
3) experiences after treatment
4) plans and expectations for the future.
5) psychological well being

Both tools are included in the Annex to this study.
The meetings with the psychologist
A psychologist met with each patient participating in the study; meetings lasted between 35 minutes and two hours and included a detailed explanation of the research study. The participants were given forms pertaining to the protection of privacy. Also, they signed a release authorising the recording of the conversation in its entirety and the use of the data for purposes strictly related to the study. Each meeting began with a self-administered PGWBI, followed by a semi-structured interview.

Results
Characteristics of the study participants are illustrated in Table 1. The mean age was 55.8 years (range 39-74), 82% of the women were married, 59% had a secondary school education, and 41% were housewives. While the study period was from 2005 to 2010, most of the participants were operated after 2007. Interviews were conducted in 2009-2010.

1) Symptoms and experiences prior to diagnosis
The presence, type, and timing of symptoms are reported in Figure 1. A mean of three symptoms was reported (range 0-9 symptoms), with 65% of the patients reporting symptoms of high intensity. The most common symptoms were bloating, pelvic pain, lower abdominal distension, intestinal disorders, frequent urination, severe fatigue, weight loss, and stomach disorders. Less common symptoms included groin pain, lower back pain, menstrual disorders, “sensation of a ball in the lower abdomen”, and pain/heaviness in the legs. Symptoms appeared more than four months before diagnosis in 38% of women. Of the 39 participants, 29 reported symptoms to their general practitioner. In 41% of these, the attending GP did not request further diagnostic testing. “My doctor played it down and told me that it was nothing, that it was menopause. I felt abandoned”. Regarding any stressful events in the months prior to diagnosis, 69% reported at least one (Figure 2).

Table 1. — Characteristics of ovarian cancer patients in the Reggio Emilia province, during the period 2005-2010.

<table>
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<tr>
<td>Age in years</td>
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<tr>
<td>25-49</td>
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<td>50-69</td>
<td>22</td>
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<td>70-79</td>
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<td>Marital status</td>
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<td>Married</td>
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<td>Separated/ divorced</td>
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<tr>
<td>Single</td>
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<td>5.1%</td>
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<tr>
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<tr>
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<td>17.9%</td>
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<tr>
<td>Other</td>
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<tr>
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<tr>
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<tr>
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Figure 1. — Ovarian cancer patients in the Reggio Emilia province, 2005-2010: a) presence; b) type; c) timeline of symptoms.
The impact of epithelial ovarian cancer diagnosis on women’s life: a qualitative study

2) Experiences during clinical pathway
All the women interviewed reported that the most critical moment was at the communication of the diagnosis. “At first I couldn’t grasp what was happening to me. It was a tremendous shock. – It was a storm. A typhoon – It was like crashing into a wall. – It was the greatest blow I could ever imagine receiving in my life”. After the communication of the diagnosis, the most common mood reported was fear (79.5%), the most common fears being of death, recurrence, physical suffering, and chemotherapy. However, as the women’s moods did fluctuate between negative and positive states, they also reported feeling tranquillity, inner strength, and optimism.

3) Experiences after treatment:
The main physical changes after diagnosis are reported in Figure 3. “I put on weight because I ate more desserts. I used to be slimmer; I’ve put on eight kg (17 lbs) and can’t seem to lose them. – I started eating more. I put on seven to eight kg (15-17 lbs). – When I look at myself I look completely different, ruined, unrecognizable. I’ve put on 12 kg (26 lbs)”. Fifty-one percent of the women reported changes in their relationships with their partner, especially in terms of sexual activity. “Sexually, I was totally unprepared. They could have prepared us a bit more for this. – Stupidly, I thought it would be like it was before. Instead, I can no longer have intercourse. – Over time we stopped having sex. – My partner and I don’t have sex anymore. The first few months, it was because of physical problems; now, it’s probably a psychological block”.

The women reported other changes as well post-treatment in terms of their life course (e.g., job loss), their daily lives (e.g. greater difficulty doing housework), and interpersonal relationships (e.g. becoming more selective in friendships).

4) Plans and expectations for the future:
Sixty-two percent of women reported having plans for the future, the most common being travelling, buying a house, and returning to work. Some, however, especially the older women, preferred focusing on the present. “I plan to continue doing what I did before. – I don’t make plans anymore. I want to learn how to live day to day”.

5) Psychological well being
The PGWBI provides self-reported status in terms of well being or distress related to the emotional and affective spheres and whether this status is higher or lower than the “normal” level, i.e., a stress-free state. The higher the score obtained in each of the six dimensions measured, the greater the degree of well being. Scores are reported in Figures 4 and 5.

Most women did not show any particular psychological distress: for each dimension examined, results were average, or even above average when compared to a group of healthy women the same age, except for the last scale – General Health. In this case only, almost half of the women interviewed (44%) scored below average but this was due to the fact that 23 women out of 39 (59%) reported having physical problems unrelated to their cancer.

Despite these positive results, it must be noted that 39% of cases reported stress, and in 23% of these, stress was severe, meaning with psychopathological features. These
cases were exclusively among those women who participated in the interview less than one year after the end of treatment; for those women, instead, who had finished treatment two to three years before the interview, the psychological situation was definitely better. In any case it would be opportune to evaluate the need for psychological support in those cases where distress appears to be severe, where a social network is lacking or is inadequate, and/or when there other concomitant critical events.

Discussion

Ovarian cancer survival has risen from 32.4% in the 1990s to 36.3% in the 2000s: only younger women had higher survival rates [6]. The fact that these survival rates do not describe the psychophysical well being of these women has been widely reported in the literature [9-11]. Thus, healthcare must include both the tumour and the person.

The quality of life of women with ovarian cancer has been widely studied. Early assessment of psychological problems must be an integral part of the therapeutic pathway [12-15], which begins with the general practitioner’s examination; assessment results must be useful to the GP as well [15]. Many instruments have been used to assess the problems of ovarian cancer patients [17, 18], some of which are applicable to all cancers while others are more specific [19]. There is not yet, however, an ideal instrument [20].

Physicians must quickly understand whether the patient is in physical or psychological distress, and whether the woman is bearing the burden of her distress alone or has someone with whom she can share it [21]: In the present study, 80% of the women shared their moods and fears with others. However, the quality of this sharing was not always such that the women could bear their anxieties and fears. Nevertheless, being able to talk about one’s moods and feelings positively affects the women’s ability to cope with their disease.

Age was also a strong determinant of the general health of these patients: younger women had more family- and work-related problems [22, 23]. Although younger women may have a wider social network than older women, and as in the present study, may receive more support from their partner, the disease is more likely to impact on their everyday life. Further, the possibility of recurrence or death upsets the patient and those around her, such as her partner and/or her children [24]. Although
61% of the women in this study did not report stress after diagnosis, their PGWBI scores would suggest otherwise. On the one hand, the women reported feeling vitality, positive well being, and self-control, while on the other, they also reported anxiety, depressed mood, and poor general health. However, what appears to be a contradiction has been reported in the literature [21] and is partially explained by the frequent psychophysical fluctuations these women are subject to.

Although symptoms are vague and non-specific, recognizing them as such and giving them due consideration appear to be crucial to early diagnosis. Only five percent of the women in the present study did not report having any symptom prior to diagnosis; of the remaining 95%, 49% reported having one to three symptoms, 38% four to six symptoms, and eight percent more than six symptoms. The association between frequency and severity of symptoms must not be underestimated [25].

As far as clinical pathway satisfaction is concerned, 88% of the women reported positively: “I was very pleased with everything, and with the gynaecology department, in particular: the care, the professional competence, their kindness, attention, and consideration – everything someone in my position could possibly want. – I was pleased with both the gynaecology and the oncology departments and the hospital. The doctors and nurses were all very kind.”

Instead, their reports on the kind of information they received from the gynaecologists is less positive: “They told me everything about the operation but in technical terms. What I wanted to know was what it was going to be like afterwards, so as to be more psychologically prepared”.

After the operation, the women perceived their bodies as being irreparably damaged, especially if the operation was very invasive: “I’ve got this huge tear up to here. Physically, I make myself sick. – I have a scar as big as a house. I see it every time I look at myself. – Every time I get undressed I remember what happened”.

Chemotherapy also contributes to exacerbating this experience, and thus negatively affected the psychological condition of these women. Chemotherapy often contributes to lowering self-esteem and to making sexual activity more stressful: “When they told me that I would have to do chemo, I felt as if I had a weight on my chest, that I couldn’t breathe. I cried for three days. You’ve got to experience it yourself to know what it’s like. And … your hair. That’s your first thought. At least for a woman. – The worst moment for me was the six cycles of chemo I did. Crossing that threshold to be injected with poison. For me, losing my hair was terrible, even though I was prepared for it. – Thinking about chemotherapy made me lose it. I was afraid of feeling sick physically, of feeling bad psychologically, of losing my hair. – The most traumatic thing for me was losing all my hair.”

In a study on the psychosocial distress of EOC patients, participants clearly expressed the need for more information and emotional support right from the moment of diagnosis, and throughout the treatment process. Telephone interventions rather than face-to-face contact were proposed as the most likely to be effective for providing psychosocial counselling specifically but also for specific disease-related information. It is strongly recommended that health care organizations explore setting up a telephone-based service for EOC patients which includes EOC survivors as counselors, ideally with the collaboration of patient groups and advocates [26].

Gynaecologists/surgeons should explain how the surgical procedure will be performed (preferably using visual aids such as drawings or diagrams) and should describe what the surgical scar will look like, i.e., that it will be about 30 cm long, more or less from the pelvic bone to the sternum. If the scar is particularly unsightly, the GP or specialist could provide some practical suggestions on how best to deal with it.

Before chemotherapy is commenced, the oncologist should inform the patient of the possible side effects (e.g., nausea, vomiting, nail disorders, and so on) and must absolutely deal with the side effect that most concerns women: hair loss. Hair loss is inevitable but temporary, lasting about six to eight months from the beginning of chemotherapy. Specialists should suggest some options and practical solutions here as well, such as cutting your hair very short before the start of chemotherapy and buying a wig that is as close to your natural colour and style as possible (ideally also providing information about costs and where to buy a wig in the local area). As hair loss also includes eyebrows and eyelashes, practical suggestions could include getting eyebrow tattoos and using black eyeliner to “cover” the lack of eyelashes, and/or wearing eyeglasses with frames that “accessorize”.

Another important issue concerns weight gain: women with cancer often react to the stress by eating more, and in particular by eating more sweets and desserts. Associated with this is the fact that cortisone and other drugs induce fluid retention, which alone leads to an immediate weight gain of two to three kg. Weight gain results in changes in the woman’s appearance and can thus negatively affect her self-image and her mood. Specialists must therefore not only tell women to be very careful about weight gain, they could also provide patients with a list of suggested low-calorie and/or sugar-free snacks to have on hand to relieve hunger pangs. Further, specialists and GPs must explain the importance of getting regular exercise, providing practical information concerning kinds of appropriate exercise and how much is necessary (e.g. “walk 30 minutes a day, every day, at a brisk pace”) so that patients can realistically implement a program as soon as possible. This will improve both the patient’s health and her mood [27]. Involving family members throughout the entire clinical pathway could be helpful [28] in dealing with all aspects of this disease.
Monitoring the entire pathway is essential, especially for those women undergoing chemotherapy. [29]. Today, it should be mandatory to carefully assess all EOC patients for symptoms of anxiety and depression, to ensure social and, if necessary, specialist support. It is reported that social support is associated with survival advantage for EOC patients [30].

Finally, GPs and specialists should encourage and facilitate help and support provided by “former” patients who are willing to talk about their experiences with patients newly diagnosed with the same kind of cancer. Hearing other women talking about their personal experiences may be more convincing and thus more helpful than any scientific information from a specialist would be: “You change because afterwards you don’t feel the same, and whoever has had this kind of experience has a different perspective on things, on their importance. – I feel more fragile, but I’m also more attentive to respecting myself. I am more in tune with myself. – I feel I’ve become a better person, much more understanding – My illness has helped me change. It sounds strange, but it helped me calm down and enjoy every moment. – My illness helped me become wiser and has built up my self-esteem. – It’s been positive; I never would have believed I could be so strong, that I could stand the pain without complaining, that I could deal with my anxieties without unloading them on others. – I feel I’ve changed for the better. Now I care only about what’s really important. I feel more confident and more serene”. It would benefit these women if they formed support EOC groups or associations like those that have become more and more common among breast cancer patients in order to help each other throughout their illness.

It would benefit these women if they formed support EOC groups or associations like those that have become more and more common among breast cancer patients in order to help each other throughout their illness. It has been seen that positive stimuli can be found in other women’s experiences. While each woman’s experience may be longer or shorter, the important thing is that not one day is wasted and that each is a special day.

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Assessment of the tumor-associated trypsin inhibitor (TATI) marker in patients with carcinoma of the uterine body 17 years after treatment

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Summary

On the basis of literature review, the structure of the tumor-associated trypsin inhibitor (TATI) marker and its usefulness in diagnosing and monitoring of various malignant neoplasms has been described. The authors’ own experiences are presented stemming from evaluation of TATI levels in a group of 305 patients suffering from carcinoma of the uterine body, who were primarily operated and then subjected to supplementary therapy in the Center of Oncology in Warsaw, classified in accordance with the FIGO 1988 protocol in the years 1994-1995, and who were observed for 17 years after discontinuation of treatment. A statistical analysis of the level of the TATI marker was carried out in the group of patients with unfavorable prognostic factors, that is the presence of cancerous infiltration in the uterine body, also found in the parametrium, ovaries, as well as diagnosed metastases to the lymphatic nodes found on the basis of postoperative histopathological protocol. The marker was determined three to seven times in serum after each stage of supplementary treatment, and at the beginning of the follow-up. Strong significance and elevation of the TATI marker were affirmed for the mean of four initial collections in patients, who had a relapse or metastases within one month to 11 years after termination of therapy.

Key words: Neoplastic markers; TATI; PSTI; Prognostic factors; Endometrial carcinoma.

Introduction

Diagnostic process of neoplastic diseases relies on various diagnostic methods, which aim at proper end effective treatment. Since the mid-1960s markers have also been used for that purpose, since they are supposed to be highly sensitive and specific. As a reliable examination tool markers might facilitate diagnosis of disease, its recurrence, and would help determine prognosis.

The marker whose prognostic role in monitoring and early diagnosis of neoplasms is still being investigated is tumor-associated trypsin inhibitor (TATI) First reports of the marker TATI determination appeared about 30 years ago [1]. In 1982 Stenman et al. described structural and functional similarity of the TATI marker to the earlier identified pancreatic secretory trypsin inhibitor (PSTI) [1]. Increased concentration of PSTI is observed in inflammatory states of the pancreas. TATI is a protein produced in high amounts by the cells of ovarian tumor, and this inhibitor undergoes expression in cells of other solid tumors. The TATI marker was first isolated in urine from patients suffering from ovarian cancer. The elevated TATI level in serum or urine is usually caused by an inflammatory state of tissue damage. The primary function of TATI is protection of pancreatic cells against damages resulting from trypsinogen activation [1, 2]. TATI and PSTI are coded by the same single gene, and the cDNA sequences of both inhibitors are identical. The inhibitor is coded by the SPINK1 gene whose name originates from another peptide synonym – serine protease inhibitor kazal-type 1. It is acknowledged in the literature that the PSTI term refers to the pancreatic inhibitor, while TATI refers to the inhibitor, which undergoes expression in neoplastic cells [3, 4].

Characteristics of the neoplastic TATI marker

TATI is a protein whose mass is 6 kDa, produced in high concentrations filled with mucus ovarian cysts and by the mucous membrane of the digestive tract. A rise in its level accompanies many types of various neoplasms [2, 5].

The concentration of TATI as determined by immunofluorometric methods with the use of monoclonic and polyclonic antibodies is 6.9 μg/l in healthy individuals, the referential range being 3.1 to 16 μg/l. TATI serum levels between three and 21 μg/l, mean value 11.3 μg/l, in healthy persons are considered normal, while levels in urine considered normal are 5-50 μg/l, mean 25 μg/l. Higher concentrations are determined in the case of some malignant neoplasms of the ovary, the uterine cervix, as well as of the pancreas, stomach, liver, gall bladder, rectum, lungs, and breasts. Elevated values are also observed in pregnant females in their amniotic fluid between the 14 and 16 week of pregnancy [6, 7]. This inhibitor is also present in the human colostrum, the highest PSTI concentration, 150 ng/ml, is found during lactation [8]. The PSTI/TATI proteins and EGF (epidermal growth factor) are characterized
Table 1. — Mean TATI values in patients with negative prognostic features on consecutive seven collections (T1 - T7).

<table>
<thead>
<tr>
<th>Cancer beyond the uterine body</th>
<th>Number of patients</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine cervix</td>
<td>76</td>
<td>27.5394</td>
<td>27.1081</td>
<td>44.6379</td>
<td>41.0526</td>
<td>31.3333</td>
<td>33.0000</td>
<td>17.5000</td>
</tr>
<tr>
<td>Parametrium</td>
<td>40</td>
<td>27.6875</td>
<td>30.2500</td>
<td>46.1750</td>
<td>26.8750</td>
<td>7.2750</td>
<td>3.0750</td>
<td>1.2750</td>
</tr>
<tr>
<td>Ovaries</td>
<td>38</td>
<td>32.2105</td>
<td>38.9736</td>
<td>44.7105</td>
<td>32.2702</td>
<td>7.2702</td>
<td>2.2432</td>
<td>0.5675</td>
</tr>
<tr>
<td>Int. iliac lymphatic nodes</td>
<td>12</td>
<td>29.4100</td>
<td>29.2500</td>
<td>23.2500</td>
<td>32.0833</td>
<td>14.4166</td>
<td>5.2500</td>
<td>2.4166</td>
</tr>
<tr>
<td>Ext. iliac lymphatic nodes</td>
<td>10</td>
<td>33.5000</td>
<td>331.800</td>
<td>23.1000</td>
<td>27.4000</td>
<td>15.2000</td>
<td>5.6000</td>
<td>2.9000</td>
</tr>
<tr>
<td>Obturatory lymphatic nodes</td>
<td>8</td>
<td>35.0000</td>
<td>28.2500</td>
<td>20.7500</td>
<td>27.3750</td>
<td>16.5000</td>
<td>3.12500</td>
<td>1.25000</td>
</tr>
<tr>
<td>Vessel</td>
<td>71</td>
<td>31.8169</td>
<td>31.5197</td>
<td>40.2957</td>
<td>25.1875</td>
<td>4.7329</td>
<td>2.0704</td>
<td>0.8450</td>
</tr>
<tr>
<td>Number of negative features</td>
<td>255</td>
<td>30.0055</td>
<td>30.9181</td>
<td>41.0803</td>
<td>31.7160</td>
<td>14.6731</td>
<td>11.7931</td>
<td>6.0022</td>
</tr>
</tbody>
</table>

by similar size and manifest great homology of amino acid sequences, which is connected with having similar biological effects such as stimulation of fibroblasts and epithelial cells growth [9].

The TATI concentration in serum within referential ranges given above is detected not only in healthy individuals, but also in patients after total pancreatectomy (pancreatectomia totalis). This effect means the majority of the inhibitor present in serum is not produced by the pancreatic gland, but derives from other organs [6, 9].

The chief function of the TATI inhibitor undergoing expression in neoplasms is protection of cancerous cells against trypsin. Trypsinogen undergoes hyper-expression in several types of cancerous cells, and after conversion into trypsin stimulates its growth and contributes to malignant transformation of tumors. Excessive production of proteinases also acts destructively on the proteins of the extracellular matrix, which may increase the migration of neoplastic cells and generate metastases [10-12].

The goal of the work was to assess the changes of the TATI marker level in patients affected by carcinoma of the uterine body who underwent surgical treatment in the case of poor prognostic signs having been found, and to determine the prognostic role of TATI in evaluation of the therapeutic result in patients with endometrial carcinoma who were primarily surgically treated.

Materials and Methods

The levels of TATI marker in 305 patients suffering from carcinoma of the uterine body, who were surgically treated in the years 1994-1995 and followed up for 17 years at the Center of Oncology in Warsaw are presented. All patients were referred to therapy at an oncological facility after removal of the reproductive organ, and in some cases after removal of lymphatic nodes in certain hospitals. The first TATI examination was performed at an oncological center on first patient’s report, consecutive collections were carried out after each therapeutic stage, e.g. teletherapy, brachytherapy, and on the initial observation period after treatment. Three to seven blood collections from each examinee were recorded. Mean levels of the marker were analyzed only in patients with confirmed unfavorable prognostic factors (Table 1.) found on the basis of histopathological protocol, i.e. cancerous infiltration in the cervical canal of the uterine cervix present in 76 patients (25%), in the parametrium in 40 patients (13%), confirmed metastases to the ovaries in 38 patients (12%), metastases to the internal iliac lymph nodes in 12 patients (4%), external iliac lymph nodes in ten (3%), obturatory lymph nodes in eight patients (3%), and cancer cells present in blood vessels in 71 examined patients (23%).

It has been agreed on that the referential range of concentration determined in serum of healthy individuals is up to 21 μg/l of the value recently assessed as elevated or abnormal.

Results

The yielded measurements of the levels of the TATI marker showed general tendency to rise on second and third collection (T2 and T3). The maximum was reached on the third collection (T3), and then the marker level progressively decreased to zero (Figure 1). The concentration observed in serum was 25-1,125 μg /l. In all patients with present poor prognostic features in comparison to patients free from such features the TATI marker levels were higher, especially on first four collections (Table 1.)

Therefore, as a new indicator of the TATI marker was assumed the mean of first four determinations of the marker level (T1-T4) in patients that histopathologically manifested all negative cancerous features. Values of that indicator were compared in groups of patients with present negative prognostic features with groups free of those features.

Significant differences of the mean TATI marker level were not found in the first four collections between groups with present neoplastic cells in the cervix, but neither in the parametrium nor in the ovaries and lymphatic nodes, compared to patients without these features. However, the occurrence of relapse and metastases highly-differentiated the levels of the TATI marker. Tables 2 and 3 show a comparison of the mean of the first four TATI marker collections in groups with relapse and metastases, respectively.

Only in the group of patients with confirmed follow-up relapse and without metastases or other neoplastic disease in comparison to patients without relapse and additional negative features differed significantly by mean TATI marker level in the first four determinations (Mann-Whitney U = 2806.000; Z = 5.20811; p = 0.00000). Similar re-
Assessment of the tumor-associated trypsin inhibitor (TATI) marker in patients with carcinoma of the uterine body 17 years after treatment

Table 2. — Assessment of the influence of the 4 TATI collections mean in the relapse group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mann-Whitney U Test (Data T without zero) relative variable relapse</th>
<th>Highlighted results are significant, ( p &lt; 0.05000 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 1-4</td>
<td>41,032.00</td>
<td>9,371.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relapse</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>270</td>
<td>18.61</td>
<td>32.40</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>44.32</td>
<td>51.94</td>
</tr>
<tr>
<td>Total</td>
<td>305</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. — Assessment of the influence of the mean of four TATI collections in the metastases group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mann-Whitney U Test (Data T without zero) relative variable Metastases 1 Y/N</th>
<th>Highlighted values are significant, ( p &lt; 0.05000 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 1-4</td>
<td>36,082.00</td>
<td>14,321.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>META1</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>242</td>
<td>19.58</td>
<td>30.33</td>
</tr>
<tr>
<td>1</td>
<td>63</td>
<td>37.61</td>
<td>56.38</td>
</tr>
<tr>
<td>Total</td>
<td>305</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. — Graphic representation of mean TATI levels for consecutive collections (T1-T7) of the marker in the tested group.
lations were found in a group of patients with existing metastases and sans metastases, in whom the TATI levels differ greatly by significant mean marker level in the first four determinations (Mann-Whitney U = 5206,000; Z = - 4,97475; p = 0.000001). Thus an assessment of marker level after termination of therapy is quite essential. Significant elevation of TATI after treatment should be associated with negative prognosis due to relapse and metastases. Failures, that is metastases, were found in 63 (21%) patients, relapses in 35 (11%) patients, diagnosed one month to 11 years after treatment.

Discussion

There are several reports in the accessible literature regarding uses of TATI in prognosis of the courses of various neoplasms. Most numerous reports refer to the role of this marker in diagnosis of ovarian mucosa carcinoma. It was found that the level of TATI concentration rose evidently in the first stages of disease, later the tempo decreased and was correlated with the progress of disease. In the case of neoplasms of the reproductive organ, high concentration of TATI was observed both in urine and in serum. Austrian scientists conducted a study assessing the sensitivity of the TATI marker in women with endometriosis [13]. The results indicate that in the early stages the marker demonstrated little sensitivity, which gradually increased at the higher stages of disease. Some authors recommend the increased diagnostic sensitivity of the TATI marker connect its assessment with assessment of other marker, for example CA 125 [14]. In the case of pulmonary carcinoma, apart from TATI a determination of carcinoembryonic antigen (CEA) is recommended – the sensitivity of determination of both markers increases to about 74 per cent [15].

It was found that in the case of breast neoplasm the TATI level rises along with the stage of disease progress, but determination of TATI alone is not a sufficient prognostic indicator, and connecting it with other markers is clinically useless [15, 16].

Paju et al. in their study of patients suffering from prostate carcinoma observed that TATI expression increases along with the stage of cancerous progression and malignancy. It was demonstrated that high concentrations similarly to other types of tumor occur most frequently in the cases of malignant neoplasms, and sustained high concentrations may contribute to invasion and metastasizing [17]. In diagnosis of urine bladder carcinoma, TATI resulted out to be more useful than other commonly determined in serum markers, that is tissue polypeptide antigen (TPA), CEA, or the antigen of squamous cell carcinoma (SCC-AG). Depending on the progression stage, increased concentrations occur in 20% to 70% of patients suffering from urine bladder carcinoma, which indicates an essential role of TATI in monitoring the therapeutic process and in the assessment of its efficacy [18].

Studies indicating a significant prognostic value of the TATI marker in the diagnosis of ovarian carcinoma, of neoplasms of the urine bladder, and in postoperative observation of patients with renal carcinoma prompted researchers to analyze the clinical effectiveness of this marker [19-21]. Solakidi et al. found that TATI may have great clinical utility as supplementation of biomarkers in diagnosis and monitoring of malignant neoplasms of the digestive tract indicating its higher sensitivity than s-CEA [22].

The present study demonstrated that determination of the TATI level in patients with carcinoma of the uterine body accompanied by negative prognostic factors, defined on the basis of histopathological evaluation, does not show characteristic fluctuation of levels in relation to patients without those negative features. It has been found however, that the values of TATI in the group of patients with present negative factors are higher in relation to patients without negative features. It has been observed in the course of 17-year long follow-up that the elevations of the TATI marker level, which were determined on first check-up after finished therapy, correlated in a statistically highly significant manner (p = 0.000000) with cancer relapse and distant metastases (p = 0.000001), even in the cases where therapeutic failure occurred many years before its termination. Therefore, the authors suggest that assessment of the TATI marker immediately after termination of treatment is in prognostic terms the most important feature, which defines the probability of therapeutic failure in the cases of patients treated for carcinoma of the uterine body.

Acknowledgment

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References

Assessment of the tumor-associated trypsin inhibitor (TATI) marker in patients with carcinoma of the uterine body 17 years after treatment


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Expression of budding uninhibited by benzimidazoles - 1 and mitotic arrest deficient - 2 in endometrial carcinoma and its significance

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Summary

Objective: The aim of this study was to explore the expression of budding uninhibited by benzimidazoles-1 (Bub1) and mitotic arrest deficient-2 (Mad2) in endometrial carcinoma and its significance. Materials and Methods: The expression of Bub1 and Mad2 in 30 human normal endometrial tissues (group A), 30 complexly-hyperplastic endometrial tissues (group B), and 63 endometrial carcinoma tissues (group C) was observed using immunohistochemistry (the streptavidin-peroxidase method). Results: The positive expression rates of Bub1 in groups A, B, and C were 86.67%, 56.67%, and 28.57%, respectively. The positive rate of Bub1 protein was correlated with the differentiation degree and clinical stage of endometrial carcinoma (p < 0.05): A higher differentiation degree and a more advanced stage of endometrial carcinoma indicated a higher positive rate of Bub1 protein. The positive rates of Mad2 protein in groups A, B, and C were 23.33%, 56.67%, and 85.71%, respectively. The positive rate of Mad2 protein was correlated with the differentiation degree of endometrial carcinoma (p < 0.05) other than its clinical stage and lymph node metastasis (p > 0.05): A lower differentiation degree indicated a higher positive rate of Mad2 protein. Bub1 and Mad2 proteins were negatively correlated in the endometrial carcinoma tissues (r = -0.719, p < 0.001). Conclusion: Bub1 and Mad2 proteins interact with each other. They may play an important role in the initiation and development of endometrial carcinoma.

Key words: Endometrial carcinoma; Budding uninhibited by benzimidazoles-1 (Bub1); Mitotic arrest deficient 2-like protein 1; Spindle checkpoint.

Introduction

Endometrial carcinoma is one of the three major gynecological malignant tumors with an increasing incidence in recent years. It greatly threatens the life quality of patients and their families. Its early diagnosis and timely treatment, particularly its precancerous diagnosis and prevention, have become a widespread concern of patients as well as of gynecologists. Budding uninhibited by benzimidazoles-1 (Bub1) and mitotic arrest deficient-2 (Mad2) are important components of spindle checkpoints [1]. They monitor and ensure the fidelity of cell mitosis and have a predictive value for the prognosis of some tumors [1, 2].

To explore the roles by Bub1 and Mad2 in the initiation and development of endometrial carcinoma and their clinical significance, the authors observed their expression in human normal endometrial, complexly-hyperplastic endometrial, and endometrial carcinoma tissues.

Materials and Methods

General data

A total of 63 paraffin-embedded specimens of endometrial carcinoma resected between January 2007 and January 2011 (group A) were collected. Meanwhile, 30 paraffin-embedded specimens of complex-hyperplastic endometrial tissues during the same period (group B), as well as of normal endometrial tissues (group C), were taken. The averages ages of the involved patients in the three groups were 49.43 ± 3.50, 49.27 ± 5.47, and 50.77 ± 4.82 years, respectively, showing no significant differences. All the patients did not receive hormonal therapy, radiotherapy, or chemotherapy before surgery. According to classification and staging of endometrial carcinoma by the International Federation of Gynecology and Obstetrics (FIGO) in 1980, 39 tissues were in Stage I, 17 in Stage II, 5 in Stage III, and 2 in Stage IV. Thirty-eight tissues were well differentiated, 17 were moderately differentiated, and eight were poorly differentiated. Eight patients had lymph node metastasis. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was also obtained from all participants.

Methods and result judgment

The expression of Bub1 and Mad2 in different groups was observed using immunohistochemistry (the streptavidin-peroxidase method). Endometrial glandular epithelial cells with yellow- or buffy-stained granules in cytoplasm and/or nucleus were considered positive. Results were observed using the semi-quantitative, two-observer double-blind method. Ten high power fields (10 × 40) were selected for each section. According to the percentage of the number of positive cells taken in that of the total cells in a field, four levels were assigned: (-) for no positive staining or no positive cells, (+) for 1% – 30%, (++) for 31% – 50%, and (+++) for ≥ 51%, in which (-) indicates negative expression, whereas (+)–(+++) indicate positive expression [2]. If a difference of ten
percent occurred between the observed results of the two observers, re-evaluation was performed.

**Statistical analysis**

Data were processed by SPSS13.0 software using Chi-square (χ²) test. Spearman rank correlation coefficient analysis was performed for the correlation between Bub1 and Mad2. A $p < 0.05$ was considered statistically significant.

**Results**

**The expression of Bub1 and Mad2 proteins**

The results of Bub1 and Mad2 proteins by immunohistochemistry were summarized in Table 1. The positive expression rates of Bub1 in groups A, B, and C were 86.67%, 56.67%, and 28.57%, respectively, with the expression of group A significantly lower than that of any other group ($p < 0.01$). Furthermore, the less differentiated endometrial carcinoma and the more advanced the clinical stage, the lower the positive rate ($p < 0.05$). However, the positive rate was not associated with lymph node metastasis ($p > 0.05$, Table 3). The positive rates of Mad2 proteins in groups A, B, and C were 23.33%, 56.67%, and 85.71%, respectively, with the expression of group A significantly higher than that of any other group ($p < 0.01$). Furthermore, the less differentiated endometrial carcinoma, the higher the positive rate ($p < 0.05$). However, the positive rate was not associated with the clinical stage or lymph node metastasis ($p > 0.05$, Table 3).

**The correlation between the expression of Bub1 and Mad2 proteins**

The results of the correlation analysis of Bub1 and Mad2 in endometrial carcinoma are summarized in Table 4. Positive Bub1 and Mad2 existed concurrently in 11 patients and their negative concurrence in two. The result of the Spearman rank correlation coefficient analysis based on the percentage of the number of the positive cells in the number of the total cells in the same field showed a negative correlation between Bub1 and Mad2 ($r = -0.719, p < 0.001$).

**Discussion**

Endometrial carcinoma is one of the three major malignant tumors commonly occurring in female reproductive system. In recent years, its onset age shows a younger tendency and its incidence is on the increase. Disorders in cell cycle regulation greatly contribute to the initiation and development of tumors [3]. Bub1 and Mad2 are critical components of spindle checkpoints. During the process of cell mitosis, they monitor the morphology of the spindles, the connection between kinetochores and spindle microtubules, as well as the location and arrangement of chromosomes. Whenever abnormalities in the expression and/or function of Bub1 and Mad2 occur, spindle checkpoints will neglect chromosome damage and continue with their function in

### Table 1. — The positive expression rates of Bub1 and Mad2 proteins in different groups (n%).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mad2 (+)</th>
<th>Bub1 (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30</td>
<td>7 (23.33)</td>
<td>26 (86.67)</td>
</tr>
<tr>
<td>B</td>
<td>30</td>
<td>17 (56.67)</td>
<td>17 (56.67)</td>
</tr>
<tr>
<td>C</td>
<td>63</td>
<td>54 (85.71)</td>
<td>18 (28.57)</td>
</tr>
</tbody>
</table>

$\chi^2 = 6.9441, 35.0402, 9.4953$  
$x^2 = 6.6481, 27.5152, 6.8353$  
$p = 0.0002, 0.0081, 0.0023$  

Note: 1group A vs. group B; 2group A vs. group C; 3group B vs. group C.

### Table 2. — Correlations between Bub1 protein expression and endometrial carcinoma parameters.

<table>
<thead>
<tr>
<th>Group</th>
<th>Clinical Stage</th>
<th>Differentiation degree</th>
<th>Lymph node metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>n</td>
<td>39</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>The positive rate of Bub1 (n%)</td>
<td>(41.03)</td>
<td>(11.76)</td>
<td>(39.47)</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>8.125</td>
<td>6.409</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>$p$</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

### Table 3. — Correlations between Mad2 protein expression and endometrial carcinoma parameters.

<table>
<thead>
<tr>
<th>Group</th>
<th>Clinical Stage</th>
<th>Pathological grade</th>
<th>Lymph node metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>n</td>
<td>39</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>The positive rate of Mad2 (n%)</td>
<td>(82.05)</td>
<td>(88.24)</td>
<td>(100.00)</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>1.684</td>
<td>6.903</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>$p$</td>
<td>&gt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

### Table 4. — Correlation between Mad2 and Bub1 in endometrial carcinoma tissues.

<table>
<thead>
<tr>
<th>Mad2 protein</th>
<th>Bub1 protein</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>11</td>
<td>43</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Sum</td>
<td>18</td>
<td>45</td>
</tr>
</tbody>
</table>

Note: $r = -0.719, p < 0.001$. 

Q. Zhao, A.P. Bian, Y. Zhang, L. Qin, H.R. Shi, K. Su
Expression of budding uninhibited by benzimidazoles - 1 and mitotic arrest deficient - 2 in endometrial carcinoma and its significance

cell mitosis. This phenomenon is very likely to lead to errors in chromosome distribution, causing chromosome instability [4]. Bub1 is principally located in cytoplasm and closely relates with the proliferation and differentiation of cells [5]. Bub1 spindle checkpoint is a critical regulatory factor for chromosome mitosis and meiosis [6]. Its numerous functions in chromosome division are of great significance for predicting tumorigenesis and abnormal development of cells [7]. In this study, the positive expression rates of Bub1 in groups A, B, and C were 86.67%, 56.67%, and 28.57%, respectively, which is consistent with the result reported by Jeganathan K et al [8]. According to these authors, loss of Bub1 may lead to tumorigenesis. The positive rate of Bub1 protein in group C was significantly lower than that of any other group \((p < 0.01)\). Furthermore, a lower positive rate was always indicated by a lower differentiation degree of endometrial carcinoma \((p < 0.05)\). Bub1 has a very low probability of gene mutation. A decrease in Bub1 protein in endometrial carcinoma tissues may be caused by the mutation of the upstream genes of Bub1 in carcinoma tissues. Deficiency of Bub1 expression can lead to spindle checkpoint dysfunction in the early apoptosis pathway of damaged cells mediated by PS3, causing the formation and accumulation of aneuscopic chromosomes [9]. The rate of cells with aneuscopic chromosomes in endometrial carcinoma tissues is noticeably higher than those in complex-hyperplastic and normal endometrial tissues [10]. Aneuploids can lead to carcinogenesis independently of gene mutation [11]. In addition, Bub1 overexpression leads to the formation of non-diploids and tumors, although the mechanism underlying its overexpression remains to be explored [12].

Mad2 is mainly expressed in cytoplasm. In this study, the positive rates of Mad2 in groups A, B, and C were 23.33%, 56.67%, and 85.71%, respectively, with the positive rate of group C higher than that of any other group \((p < 0.01)\). Furthermore, a lower positive rate of Mad2 protein was always indicated by a lower differentiation level of endometrial carcinoma \((p < 0.05)\). Mad2 regulates cyclins to make cellular chromosomes to separate [13]. In spindle checkpoints, it monitors and allows mitotic midanaphase transformation and normal fission [14]. Mad2 also has a very low probability of gene mutation. The overexpression of Mad2 in endometrial carcinoma may be caused by a certain or more than one factor. Such a phenomenon can damage the function of spindle checkpoints to cause their control functional abnormalities, thus increasing the instability of chromosomes and the probability of chromosome deletion [15]. Mad2 overexpression stimulates the onset and development of various types of tumors in mice [16] and has a predictive value for the prognosis of primary lung carcinoma [2].

Bub1 is located in the upstream of signal cascades, which is necessary for it to detect tension kinetochores. When chromosomes are damaged, the spindle checkpoint will be activated. In such a situation, Mad2 blocks cellular anaphase transformation by inhibiting the activity of anaphase promoting compounds [17]. A decrease in Bub1 decreases the kinetochore-localizing capacity of Mad2; in such a situation, abnormalities in chromosomes are neglected and the process of mitosis is accelerated [18]. Accordingly, errors may occur to the distribution process of chromosomes, leading to their instability. Abnormalities in the expression of both Bub1 and Mad2 proteins may further aggravate such instability. Cells with instable chromosomes are prone to chromosomal aberrations, ultimately resulting in tumorigenesis. The blockage of the subaqueous increase in Mad2 during mitosis is an important signal component of mitotic checkpoints, but whether Mad2 serves as an important component of mitotic effect remains controversial [19].

In conclusion, abnormalities in the expression of Bub1 and Mad2 cause unstable chromosomes and aneuploid cells, ultimately leading to the proliferation and differentiation of cells [20] and tumorigenesis. Their interaction plays an important role in the initiation and development of endometrial carcinoma. The more advanced the clinical stage of endometrial carcinoma is, the lower the positive rate of Bub1 will be, which indicates Bub1 protein expression may be correlated with the prognosis of endometrial carcinoma. Further exploration of the functions of spindle checkpoint proteins is expected to provide a new idea for the treatment of endometrial carcinoma in clinical practice.

References


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Evaluation of primary prophylaxis with granulocyte colony-stimulating factor for epithelial ovarian cancer

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Summary

Purpose: Primary prophylaxis with G-CSF has been used to minimize myelosuppression caused by anticancer agents and to avoid severe neutropenia. The authors retrospectively examined the value of primary prophylaxis using granulocyte colony-stimulating factor (G-CSF) for epithelial ovarian cancer. Materials and Methods: From 2001 to 2010, 105 patients with ovarian cancer receiving chemotherapy in the present hospital were divided into two groups: one received primary prophylaxis with G-CSF and the other did not receive it in compliance with the guidelines for G-CSF usage. The incidence of febrile neutropenia (FN), degree of neutropenia, frequency of G-CSF administration, number of days of hospitalization, progression-free survival (PFS), and overall survival (OS) were evaluated. Results: Neutrophils decreased almost equally and the length of hospitalization was not significantly lower between the groups. Five-year PFS or OS showed no significant difference either. Conclusions: Primary prophylaxis with G-CSF in chemotherapy for epithelial ovarian cancer could be of low significance.

Key words: Epithelial ovarian cancer; Primary prophylaxis; Granulocyte colony-stimulating factor; Febrile neutropenia.

Introduction

Neutropenia is one aspect of bone marrow toxicity caused by many anticancer agents. In neutropenia patients with fever, a serious bacterial infection is a likely complication and requires close management. The frequency of serious infection in patients with neutrophil counts of 1,000 neutrophils/mm³ or more is around 5%, while those in patients with neutrophil counts of < 500 and < 100 neutrophils/mm³ are 19% and 28%, respectively; the frequency increases in proportion to the severity of neutropenia [1]. Granulocyte colony-stimulating factor (G-CSF) is used as a strong weapon against serious infection associated with neutropenia. Conventionally, primary prophylaxis with G-CSF has been used to minimize myelosuppression caused by anticancer agents and to avoid severe neutropenia. However, in clinical situations, G-CSF is often used even if patients experience only mild neutropenia, and in some cases that require frequent visits to the clinic or hospital for reasons such as examinations of blood samples. More recently, G-CSF is not generally recommended in various guidelines unless chemotherapy associated with frequent febrile neutropenia (FN) is administered [2, 3]. Regarding the validity of G-CSF administration in patients with epithelial ovarian cancer from the perspective of quality of life (QOL) and medical economics, there has so far been no detailed report indicating whether G-CSF can reduce the dosage of antibacterial agents and improve patient prognosis.

In this study, the authors retrospectively examined the value of primary prophylaxis using G-CSF for epithelial ovarian cancer, by comparing two groups in which primary prophylaxis with G-CSF was either used, or not used, in patients with ovarian cancer who received chemotherapy.

Materials and Methods

From 2001 to 2010, 105 patients with ovarian cancer (initial onset or recurrence) received chemotherapy (taxane and platinum-based combination therapy) in the present hospital. They were divided into two groups: one consisting of patients who received primary prophylaxis with G-CSF (primary prophylaxis group) and the other with patients who did not receive G-CSF in compliance with the guidelines for use of G-CSF issued by the Japan Society of Clinical Oncology (compliance group); these two groups were then compared. The items evaluated were the incidence of FN, degree of neutropenia, frequency of G-CSF administration, number of days of hospitalization, progression-free survival (PFS), and overall survival (OS).

FN was defined as a condition of < 500 neutrophils/mm³, or a condition of < 1,000 neutrophils/mm³ with the expectation of a drop to 500 neutrophils/mm³ or less, together with body temperature of 38°C or higher or fever (37.5°C or more) that continued for at least one hour [4].

The degree of neutropenia was evaluated as the lowest number of neutrophils seen during the entire treatment period. For the frequency of G-CSF use, 75 μg of filgrastim and 50 μg of lenograstim/nartograstim were considered to offer equivalent efficacies, and therefore each was counted as one dose.

Statistical analysis was performed by the Student t test and Chi-square (χ²) test for the comparison between groups, and survival analysis was performed by the Kaplan-Meier method; a value of p < 0.05 was considered statistically significant.
Results

Of 105 patients with epithelial ovarian cancer who received chemotherapy, 38 patients were assigned to the primary prophylaxis group, and 67 patients were assigned to the compliance group. Table 1 shows patient characteristics in each group. Age at the time of chemotherapy administration, performance status (PS), progression stage of ovarian cancer, and histological type were examined, but there were no significant differences between the groups.

The incidence of FN was 15.8% (six patients) in the primary prophylaxis group and 9% (six patients) in the compliance group; the primary prophylaxis group showed a tendency to include more cases of FN in comparison to the compliance group (Table 2). The degree of neutropenia was not different between the groups ($p = 0.90$; Figure 1): statistical analysis was performed by Student t test and $p < 0.05$ was considered statistically significant. Neutrophils decreased almost equally in patients who received and did not receive primary prophylaxis. In addition, the length of hospitalization was not significantly lower in either group ($p = 0.20$).

Figures 2 and 3 shows the results of prognosis analysis by the Kaplan-Meier method. Five-year PFS tended to be higher in the primary prophylaxis group than in the compliance group (41.4% vs 31.3%; log rank $p = 0.26$; Figure 2). Five-year OS showed no significant difference between the groups (36.8% vs 50.0%; log-rank $p = 0.64$; Figure 3).

Furthermore, a total of 12 patients with FN from both groups were examined (Table 3). The mean age of onset of FN was 56.6 years (range 39-70), with FN occurring in relatively younger patients. FN was frequently observed in patients with advanced ovarian cancer such as Stage III and IV, patients with poor PS, and patients with recurrent ovarian cancer.

All patients with FN who were treated with a fourth-generation cephem, such as cefepime dihydrochloride (CFPM) or carbapenem antibiotics (imipenem/cilastatin [IPM/CS] or meropenem [MEPM]), showed complete response.

Table 1. — Patient characteristics.

<table>
<thead>
<tr>
<th>Primary prophylaxis group</th>
<th>Compliance group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year) Median</td>
<td>57</td>
<td>59</td>
</tr>
<tr>
<td>Range</td>
<td>38-77</td>
<td>26-74</td>
</tr>
<tr>
<td>EOCG performance</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>I</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>23</td>
<td>33</td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Stage of disease I</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>23</td>
<td>33</td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Histological type Serous</td>
<td>25</td>
<td>35</td>
</tr>
<tr>
<td>Mucinous</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Clear cell</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

The statistical difference was determined by Student’s t and $p$ test.

Table 2. — Incidence of FN between two groups.

<table>
<thead>
<tr>
<th>Primary prophylaxis group</th>
<th>Compliance group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN (+)</td>
<td>6 (15.8%)</td>
<td>6 (9.0%)</td>
</tr>
<tr>
<td>FN (-)</td>
<td>32 (84.2%)</td>
<td>61 (91.0%)</td>
</tr>
</tbody>
</table>

Total 38 67 105 $p = 0.29$

The statistical difference was determined by $p^2$ test.

Table 3. — Cases with FN from two groups.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Stage</th>
<th>FN</th>
<th>Antimicrobial agent used (IV)</th>
<th>Administration period (days)</th>
<th>Intravenous fluid balance</th>
<th>MAR score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>I</td>
<td>Yes</td>
<td>CFPM 2g</td>
<td>5</td>
<td>Balanced</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>I</td>
<td>Yes</td>
<td>IPM/CS 1g</td>
<td>4</td>
<td>Balanced</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>I</td>
<td>Yes</td>
<td>CFPM 2g</td>
<td>5</td>
<td>Balanced</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>IV</td>
<td>Yes</td>
<td>FMOK 2g</td>
<td>4</td>
<td>Balanced</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>IV</td>
<td>Yes</td>
<td>CFPM 2g</td>
<td>3</td>
<td>Balanced</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>IV</td>
<td>Yes</td>
<td>CFPM 2g</td>
<td>3</td>
<td>Balanced</td>
<td>22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Stage</th>
<th>FN</th>
<th>Antimicrobial agent used (IV)</th>
<th>Administration period (days)</th>
<th>Intravenous fluid balance</th>
<th>MAR score</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>59</td>
<td>I</td>
<td>Yes</td>
<td>CDP 1g</td>
<td>10</td>
<td>Balanced</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>62</td>
<td>I</td>
<td>Yes</td>
<td>CFPM 2g</td>
<td>3</td>
<td>Balanced</td>
<td>29</td>
</tr>
<tr>
<td>9</td>
<td>79</td>
<td>I</td>
<td>Yes</td>
<td>IPM/CS 1g</td>
<td>3</td>
<td>Balanced</td>
<td>22</td>
</tr>
<tr>
<td>10</td>
<td>56</td>
<td>I</td>
<td>Yes</td>
<td>MEPM 1g</td>
<td>7</td>
<td>Balanced</td>
<td>22</td>
</tr>
<tr>
<td>11</td>
<td>70</td>
<td>I</td>
<td>Yes</td>
<td>CFPM 2g</td>
<td>6</td>
<td>Balanced</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>53</td>
<td>I</td>
<td>Yes</td>
<td>MEPM 1g</td>
<td>7</td>
<td>Balanced</td>
<td>22</td>
</tr>
</tbody>
</table>

Figure 1. — Degree of neutropenia between two groups. There was no difference in the degree of neutropenia between two groups ($p = 0.90$). Statistical analysis was performed by Student t test and $p < 0.05$ was considered statistically significant.
Pathogenic bacteria causing FN were detected very infrequently by blood cultures (8.3%), but the rate of detection was 23.1% when urine cultures and cultured vaginal discharge were included. All of the detectable pathogenic bacteria were *Escherichia coli*.

**Discussion**

In Japan, the number of deaths from ovarian cancer has been obviously increasing, and this form of cancer has the highest mortality among gynecological malignant tumors [5]. Although the first treatment is conducted by surgical procedure, chemotherapy is often performed for patients at risk of recurrence even in the initial stage. For patients with advanced cancer who cannot undergo debulking surgery as the primary operation, chemotherapy is the main treatment method. Furthermore, it is reported that about 55% of patients with ovarian cancer relapse within two years, and most of them require chemotherapy [6].

FN is one of the severe complications associated with neutropenia caused by chemotherapy, and requires particular care. The present study showed that in patients with initial or recurrent ovarian cancer who were treated with taxane- and platinum-based combination therapy, the incidence of FN was 9% in the group without G-CSF prophylaxis. However, Crawford et al. reported that the incidence of FN was 77% when patients did not receive G-CSF for small cell lung cancer [7]. In addition, Pettengell et al. reported that the incidence of FN was 85% when patients with non-Hodgkin’s lymphoma did not receive G-CSF as a part of the CHOP treatment regimen [8], and they recommended primary prophylaxis with G-CSF. In comparison to these reports, the incidence of FN in patients treated for ovarian cancer was extremely low in the present study. Based on the American Society of Clinical Oncology (ASCO) guidelines proposed in 2006, which recommend primary prophylaxis with G-CSF when the risk of developing FN is over 20% [3], the present results suggest that primary prophylaxis with G-CSF is of low significance in the treatment of ovarian cancer.

A meta-analysis by Kuderer et al. reported that risk of FN, mortality of infection, and mortality during chemotherapy were decreased by 40% or more with primary prophylaxis with G-CSF for solid cancers and malignant lymphoma [9]. However, in the present study, no early deaths caused by chemotherapy-induced infection were seen in either group.

There are many reports that G-CSF administration results in significant shortening of the length of neutropenia and hospitalization, and in decreased mortality due to infection, but it does not extend survival time [10-12]. In this study, the prognosis of the primary prophylaxis group was not improved in comparison to that of the compliance group, as shown in Figure 2. Furthermore, significant shortening of the length of hospitalization was not observed. It can be concluded that primary prophylaxis with G-CSF is of low significance in the treatment of ovarian cancer.

On the other hand, the 2006 ASCO guidelines mention that primary prophylaxis with G-CSF is appropriate for patients with concurrent risk factors, even if the incidence of FN was 20% or less. Concurrent risk factors are defined as age greater than 65 years, poor PS, history of FN, high-level previous treatment (for example, extensive radiation exposure), hematopenia caused by the bone marrow infiltration, poor nutrition, open wound or active infection, chemotherapy with radiotherapy, and advanced cancer. In individual examinations of the 12 patients with ovarian cancer who developed FN in this study, FN was found relatively more frequently in patients with recurrent cancer, poor PS, or advanced cancer. FN was observed even in patients who were relatively young, suggesting that age is not an important risk factor in patients with ovarian cancer.

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(MASCC) score is generally used to determine risk in patients with FN [12]. The symptoms of many patients in this study were mild, and as shown in Table 3, most of the patients showed low risk at evaluation using the MASCC score.

With respect to antibacterial agents, treatment with fourth-generation cephalosporin antibiotics or carbapenem antibiotics showed complete response. No patients required prolonged hospitalization due to poor efficacy. In a prospective study, Klastersky et al. proved that treatment with oral antibacterial agents was possible for patients with low risk of FN [11]. In the future, we can expect to manage FN in patients receiving treatment for ovarian cancer with outpatient care using oral antibacterial agents, resulting in improvements in patients’ QOL and medical economics.

Although it is important to identify the pathogenic bacteria when administering antibiotic therapy, the positive identification rate is as low as 10% [10]. In the present study, the identification rate via blood cultures was 8.3%, approximately equivalent to previous reports. However, the rate increased to a high level of 23.1% when including urine cultures and cultured vaginal discharge. From the viewpoint of infection control, it is important to identify pathogenic bacteria early using urine and vaginal discharge culture, in addition to blood cultures. Bacteria could be detected in three patients in this study, and in all cases, the pathogen was *E. coli*. Houges et al. reported that 60%–70% of pathogenic bacteria causing FN are gram-positive bacteria [13], and therefore infection by gram-negative bacteria such as *E. coli* is relatively rare. At present, there is no report that the pathogenic bacterium of FN associated with ovarian cancer treatment is often *E. coli*. This causal relationship is unclear and should be examined in a larger patient group.

Primary prophylaxis with G-CSF in chemotherapy for epithelial ovarian cancer appears to be of low value in terms of its relationship to the incidence of FN and prognosis, as well as from a medical economic viewpoint. In particular, the occurrence of FN deserves special attention in patients with recurrence, with poor PS, and advanced cancer. However, such patients might be managed with oral antibacterial agents in outpatient care.

References


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Accuracy and diagnostic value of outpatient hysteroscopy for malign and benign disease

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1 Department of Obstetrics, Women’s Diseases and Oncogynecology, Central Clinical Hospital of Ministry of Interior and Administration, Warsaw
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Summary

Background: The aim of this study was to evaluate the accuracy of the outpatient hysteroscopy. Materials and Methods: This was a retrospective cohort study of 494 women who underwent outpatient hysteroscopy after administration of non-steroidal anti-inflammatory agents with the 3.2 mm hysteroscope. Normal saline solution was used as the distension medium. All women were discharged in good general condition afterwards. Results: In cases of abnormal uterine bleeding, there was no apparent pathology found in 112 cases (83.6%). Detection rate (DR) of endometrial polyps was 88.7% with false positive rate (FPR) of 4.6%. Positive predictive value (PPV) was 82.7% with negative predictive value (NPV) of 93.1%. Detection rate (DR) of the submucosal fibroids was 57.7%. Positive predictive value (PPV) was 57.7% with negative predictive value (NPV) of 95.0%. Endometrial cancer was confirmed in ten cases (2.0%) with FPR of 0.4%. PPV was 66.7% with NPV of 99.6%. Conclusions: Outpatient hysteroscopy seems to be an effective and accurate diagnostic tool.

Key words: Office hysteroscopy; Outpatient hysteroscopy; Hysteroscopy.

Introduction

Outpatient hysteroscopy also known as “office hysteroscopy” is an established diagnostic tool [1-4]. The procedure involves miniaturised endoscopic device to visualise and examine the uterine cavity, without the need for operating room facilities or any anaesthesia.

“Office hysteroscopy” is indicated mainly in the assessment of women with abnormal uterine bleeding [1-4]. It has been also employed in the work-up of reproductive problems. As a result of recent improvements in technology, modern hysteroscopy is a completely different technique than operative hysteroscopy. Instruments that combine endoscopes smaller than three mm and 5F forceps with a total external diameter less than five mm made it possible to perform diagnostic and operative procedures in an office setting [1] without the use of local anaesthesia. AlphaScope is a fiberoptic hysteroscope of 1.7 mm calibre that uses plastic distensible external sheath so that it final diameter including the forceps does not exceed 3.5 mm. Common procedures include endometrial polypectomy [5], removal of small submucous fibroids [6], endometrial ablation [7], removal of lost intrauterine devices, and transcervical sterilisation [8]. Outpatient hysteroscopy, whether diagnostic or operative is considered as effective, safe, and well tolerated [9].

The aim for this study was to evaluate the accuracy and predictive value of outpatient hysteroscopy measured by comparison of initial and final diagnosis. Secondary, the study aimed in statistical work-up that might be useful for the patient specific counseling directly after the procedure.

Materials and Methods

The data for this study were derived from a retrospective cohort study. Between June 2011 and June 2012, 494 women had undergone outpatient hysteroscopy in our Department of Obstetrics, Women’s Diseases and Oncogynecology, Central Clinical Hospital of Ministry of Interior, Warsaw. Data on the initial and intra-operative diagnoses were directly recorded in the authors’ database, as well as patients’ demographics. Data on procedure outcomes were obtained from computerised hospital records and were also recorded in their database.

There were 318 women referred to the present Department, due to suspected intrauterine abnormality on the ultrasound examination. Transvaginal sonography (TVS) was performed in an office setting by a various gynecologists. The uterine anatomy and adnexae were visualized using a 7.5 MHz vaginal probe transducer.

The procedure of “office hysteroscopy” was performed according to the Royal College of Obstetricians and Gynecologists Green-Top Guideline Nr 59 [10]. Briefly, the patient was informed about the procedure and signed the consent. Outpatient hysteroscopy was conducted outside of the formal operating theatre in a treatment room with adjoining private changing facilities and toilet. The procedure was performed approximately 30 minutes after administering iv. non-steroidal anti-inflammatory agents (NSAIDs, ketoprofen). The 3.2 mm hysteroscope was used with the normal saline solution as the distension medium. When appropriate, a Versapoint was used to cut the polyps or fibroid, and to facilitate extraction of fragments the 5F forceps were used. For the simple biopsy of myometrium, only the 5F forceps were used. A 300 W xenon lamp and video camera were used. Distension fluid pressure was generated using a simple fall form bag suspended one m above the patient.

All the endometrial samples were immediately fixed in buffered formalin and then wholly embedded in paraffin, cut into sections, mounted on slides and stained with hematoxylin and eosin (H&E). All the histological slices were coded and archived. Microscopic evaluation was than performed on all the

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Patients’ characteristics are presented in Table 1. Abnormal uterine bleeding (AUB) was an indication for the admission and the procedure in 134 cases, abnormal appearance of the endometrium on the ultrasound examination in 318 cases and submucosal fibroids in 42 cases.

Out of 134 women admitted for the outpatient hysteroscopy due to AUB there was no apparent pathology found during the procedure in 112 cases (83.6%). Endometrial cancer was confirmed only in two cases (1.5%) leaving the other 20 women with the diagnosis of benign intrauterine pathology.

There were 318 women referred due to suspected intrauterine abnormality on the ultrasound examination with 162 cases of suspected endometrial polyps and 156 cases of suspected endometrial hyperplasia. During the procedure the initial diagnosis of the endometrial polyp was confirmed in 144 cases (92.3%) and the hypertrophy of the endometrium only in 100 cases (64.1%) suggesting other intrauterine pathology in 54 cases.

During the procedure the diagnosis of endometrial polyp was established in 208 cases being confirmed with pathology studies in 172 cases (82.7%). Endometrial cancer was found in four cases (1.9%). The detection rate (DR) of the “office hysteroscopy” in case of the endometrial polyp was 88.7% with the false positive rate (FPR) of 4.6%. The positive predictive value (PPV) was 82.7% with the negative predictive value (NPV) of 93.1%. The accuracy of “office hysteroscopy” was 88.7%.

During the procedure the diagnosis of submucosal fibroid was established in 52 cases being confirmed with pathology studies in 30 cases (57.7%). The DR of the “office hysteroscopy” in case of the submucosal fibroid was 57.7% with the false positive rate (FPR) of 0%. The PPV was 57.7% with the NPV of 95.0%. The accuracy of “office hysteroscopy” was 91.1.

Endometrial cancer was confirmed in ten cases (2.0%) being suspected in eight cases during the procedure. The DR of the outpatient hysteroscopy in case of the endometrial cancer 80.0% with the FPR of 0.4%. The PPV was 66.7% with the NPV of 99.6%. The accuracy of outpatient hysteroscopy in case of endometrial cancer was 98.8%.

### Discussion

The findings of this study demonstrate that the outpatient hysteroscopy seems to be effective, accurate and reliable diagnostic tool.

Despite the fact that hysteroscopy is now being performed in an ambulatory setting, there is still continuing debate about the value of hysteroscopy in diagnosis of serious endometrial diseases, such as cancer or hyperplasia. For example, as it was mentioned above, AUB is common gynecologic problem. Up to 33% of woman referred to gynecological outpatient clinics have AUB, and it rises to 69% in a perimenopausal or postmenopausal group of patient [11]. The prevalence of benign intracavitary abnormalities, such as submucous myomas and endometrial polyps, is approximately 35% [12] in a group of perimenopausal women with AUB. The prevalence of endometrial polyps and submucous myomas in this patient group without AUB is not known completely, but it supposed too not different statistically [13]. In women with AUB, the reported sensitivity of “office hysteroscopy” for the detection of endometrial abnormalities is 90% and specificity 91% [14].

TVS is increasingly being used as a first-line of investigation of patients with abnormal bleeding [15]. However, reports on the diagnostic accuracy of TVS are conflicting [16, 17]. A sensitivity of TVS in diagnosing intracavitary abnormalities by direct observation varies depends from studies from 56% to 96% and also specificity varies from 72% to 89% [18, 19]. The general consensus of opinion is that an endometrial thickness of less than five to six mm in a patient presenting with postmenopausal bleeding does not warrant an extensive workup, as the risk of endometrial carcinoma or/and endometrial hyperplasia is very small [20, 21]. Results also indicated that in this group of patients, when a double layer of endometrial thickness was greater than five mm, the sensitivity for the detection of any endometrial disease was 92% and specificity was 81% [22].

In the present study endometrial cancer was confirmed in ten cases (2.0%) being suspected in eight cases during the hysteroscopy procedure. The DR of the outpatient hysteroscopy in case of the endometrial cancer was 80.0% with the FPR of 0.4%. The PPV was 66.7% with the NPV of 99.6%. The accuracy of outpatient hysteroscopy in case of endometrial cancer was 98.8%.

Out of 134 women admitted for the outpatient hysteroscopy due to AUB, there was no apparent pathology found during the procedure in 112 cases (83.6%). Endometrial cancer was confirmed only in two cases (1.5%), leaving other 20 women with the diagnosis of benign in-
trouterine pathology. The present findings are in line with results of others [23], where in patient group with AUB prevalence of cancer was 4% but was much higher in postmenopausal group (11%). In this analysis LR of 0.15 (95% CI, 0.13-0.18) for a negative test result was not low enough to negate the need for further diagnostic testing in this patient group, and relates “office hysteroscopy” to diagnosing cancer rather than as a tool to exclude it. In the authors’ opinion this seems to be not entirely correct as results of this study support hypothesis that normal findings during the procedure are of patient assurance with over 99% NPV.

The limitation of the present findings can be that transvaginal ultrasonography (TVUSG) were performed not by one gynecologist, and recognition of uterine pathology in USG can differ by each other, and it can be a reason for difference in USG diagnosis of uterine polypus or fibroid, but if we directly compared “office hysteroscopy” to TVS, we know that in hysteroscopy we have opportunity to direct visualization of endometrial cavity and hence detection of any pathology (for example lesion) which might not be seen in TVS. It also offers an opportunity of obtaining direct biopsy or removing fibroids or polypus from uterine cavity. Studies have also demonstrated a superior or yield of direct biopsies compared to dilatation and curettage in providing representative histological specimens [24, 25]. In the present trial there were 318 women referred due to suspected intrauterine abnormality on the ultrasound examination with 162 cases of suspected endometrial polyps and 156 cases of suspected endometrial hyperplasia. During the procedure, the initial diagnosis of the polyps was confirmed in 144 cases (92.3%) and the hypertrophy of the endometrium only in 100 cases (64.1%), suggesting other intrauterine pathology in 54 cases. During the procedure the diagnosis of endometrial polyposis was established in 208 cases, being confirmed with pathology studies in 172 cases (82.7%). Endometrial cancer was found in four cases (1.9%). The DR of the “office hysteroscopy” in case of the endometrial polyposis was 88.7% with the FPR of 4.6%. The PPV was 82.7% with the NPV 93.1of %. The accuracy of “office hysteroscopy” was 88.7%.

During the procedure, the diagnosis of submucosal fibroid was established in 52 cases being confirmed with pathological studies in 30 cases (57.7%). The DR of the “office hysteroscopy” in case of the submucosal fibroid was 57.7% with the FPR of 0%. The PPV was 57.7% with the NPV of 95.0%. The accuracy of “office hysteroscopy” was 91.1%

The present authors were unable to perform to the end “office hysteroscopy” in 17 cases (3.3%). In 11 cases the reason was pain correlating with procedure and in six women they found atresia of external ostium of cervix, so it can be stated that technical problems were encountered in these cases, but an attempt was made to expand the external ostium of cervix with forceps or bipolar each time, but in these cases it was correlated with patient pain and were withdrawn from the procedure. In any cases of failed hysteroscopy, there were no problems with inadequate visualization of uterus. At the end, the present paper shows, that “office hysteroscopy” is safe procedure with a low incidence of failure rate, which can be used as very good diagnostic and therapeutic tool. In the present era of so-called “cost-effective medicine”, the physicians should not only be interested in the relative informative yield, but also in the cost per investigation, and choosing the best diagnostic approach.

Results of the present study support main idea of professor Betocchi i.e. “See and threat theory”. High accuracy of outpatient hysteroscopy allows medical professionals preforming the procedure to suggest the diagnosis and counsel the patient before final pathological studies.

Conclusions

Our results indicate that “office hysteroscopy” is highly accurate and clinically useful in diagnosing endometrial cancer in women with or without AUB and what is also very important is that the present study support the hypothesis that normal findings during the procedure are of patient assurance with over 99% NPV.

References

[10] RCOG Green-top Guideline No. 59 Best Practice in Outpatient Hysteroscopy?

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Cervical dysplasias 1982–2010 in the Republic of Panama. Diagnosis, treatment, and evolution

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Summary

The aim of this work was to demonstrate the effectiveness of colposcopic diagnosis and conservative local treatments in cases of dysplasia after colpo-cytology and directed biopsy. The main treatments applied were cryosurgery and cauterization. The association of dysplasia and human papillomavirus (HPV) showed an increase of 96% in recent years. The local destructive treatments (TLC), in addition to destroy the virus, have also successfully activated the local immunological system mechanism against HPV. The evidence of such affirmation is based on the obtained results that are: 76% cured, 11% improved, 6.8% persistent, 3.4% recurrence, and only one percent progression.

Key words: Dysplasia; Diagnosis; Management; Evolution.

Introduction

Cervical dysplasia is a precancerous lesion on the surface of the cervix. It is classified as low-, middle- or high-grade. Low-grade cervical dysplasia progresses very slowly and often regresses spontaneously. High-grade cervical can lead to cervical cancer. Without treatment, 30% - 50% of cases of severe cervical dysplasia progress to invasive cancer. The risk of cancer is lower for mild dysplasia.

Cervical dysplasia is associated with human papillomavirus (HPV), a sexually transmitted virus. A vaccine is available to protect against HPV, and regular Pap tests and colposcopy can diagnose cervical dysplasia and treat it in its early stages.

Materials and Methods

The total number of dysplastic cases evaluated from 1982 to 2010 were 1,115 and received by health control with 84%, as already known patients without previous risk in 11%, and only four percent with pre-existing disease, considered as progressive oncogenic risk (OR).

The dysplastic cases were represented in 17% of those patients considered at OR, but experienced an increase in incidence during this decade with significant peaks between 2005 and 2006.

The affinity with HPV has been demonstrated in 96% of dysplasias, which forced the present author to adjust the treatments in order to achieve a personalized immunological response. It was for this reason that conservative local treatments were applied.

In most cases, the present author chose cryotherapy or cryosurgery, based on the outstanding experience of the Europeans, with successful eradication of the lesion with no dysplastic recurrence at short, medium, and long term.

The present author designed follow up with a protocol similar of diagnosis via colposcopy, colpo-cytology, and biopsy, if it was necessary. In addition, polymerase chain reaction (PCR) was performed whose results today are subject to further analysis.

Between 1982 and 2010, the present author attended 11,855 patients in which 25,764 colposcopies, 25,202 colpo-cytologies, and 5,793 biopsies were performed. They were mostly evaluated through health controls (evaluation), that is, without patho-cytological study.

The number of patients that were considered with a lesion with OR reached 6,358 cases, but the present author dedicated this article to the dysplasias which reached 1,155 cases between 1982 and 2010.

The patients were divided into two groups: Group 1, whose diagnosis was made during 1982 and 1999, and Group 2, with 562 cases diagnosed from 2000 to 2010. Both groups had very similar clinical profiles and distribution of degrees of dysplasia [1-3] (Table 1).

Group 2 was selected as 21st century dysplastic cases and corresponded to a total of 565 patients, distributed as 343 mild, 175 moderate, and 47 severe cases (Table 2).

The incidence of dysplasias over the years of study remained constant since its inception, and was broken by an excessive increase during the years 2005 and 2006, and remained constant thereafter.

The incidence of dysplasias corresponded to 17% in the group of patients with OR, which was higher compared to the international cases. The prevailing age in the patients was between 20 and 44 years, with 53.8% represented by younger ages, however, all ages were represented. In these, cytology reached 31% with pathological diagnosis, with the predominance of simple HPV, mild dysplasia with condyloma, and moderate dysplasia with condyloma, in order of frequency, with 27% resulting as false negatives (Table 3).

It should be mentioned that the false negatives of the patients with dysplasia corresponded to 27% and this was in accordance with the risk pathology that corresponded to 73%.

Colposcopy obtained 63% of atypical images, and the atypical transformation zone as predominant picture in all types of dysplasias, while the false negatives found were 32% (Table 4).

Directed biopsies were applied in this group of patients. Ninety-four percent of them showed pathology: mild, moderate, and severe dysplasias were the most frequent. All of them were associated with condylomas. There were only 6.02% of false negatives (Table 5).
Results

During the years of this study, with follow up controls ranging from one to 20 years, the author found that 76% of patients were cured, 11% improved, 6.8% persisted, and 3.4% recurred. In none of the cases did the dysplasia recur, which was the most frequent feature in the group of patients with HPV alone. Progression reached one percent [4] (Table 6).

Of the updated PCR studies, in the patients that had an analytic diagnosis as normal or cured, HPV 18, 31, and 35 were identified in the same order of frequency, represented by 82% and one negative case resulted, which is in contrast to the international studies, in which HPV 16 and 18 had the almost absolute incidence [5-7].

Table 1. — 21st century dysplastic cases.

<table>
<thead>
<tr>
<th>Year</th>
<th>Dysplasias</th>
<th>Cancer</th>
<th>HPV alone</th>
<th>Pure OR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982 - 1999</td>
<td>550</td>
<td>175</td>
<td>3,160</td>
<td>548</td>
<td>4,433</td>
</tr>
<tr>
<td>2000 - 2010</td>
<td>565</td>
<td>35</td>
<td>1,080</td>
<td>235</td>
<td>1,915</td>
</tr>
<tr>
<td>Total</td>
<td>1,115</td>
<td>210</td>
<td>4,240</td>
<td>783</td>
<td>6,348</td>
</tr>
</tbody>
</table>

Table 2. — Dysplastic incidence in the 21st century.

<table>
<thead>
<tr>
<th>Year</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>18</td>
<td>6</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>2001</td>
<td>10</td>
<td>2</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>2002</td>
<td>31</td>
<td>13</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>2003</td>
<td>53</td>
<td>25</td>
<td>6</td>
<td>84</td>
</tr>
<tr>
<td>2004</td>
<td>10</td>
<td>17</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>2005</td>
<td>69</td>
<td>33</td>
<td>5</td>
<td>107</td>
</tr>
<tr>
<td>2006</td>
<td>71</td>
<td>31</td>
<td>10</td>
<td>112</td>
</tr>
<tr>
<td>2007</td>
<td>42</td>
<td>22</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>2008</td>
<td>24</td>
<td>9</td>
<td>5</td>
<td>38</td>
</tr>
<tr>
<td>2009</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>2010</td>
<td>7</td>
<td>11</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>343</td>
<td>175</td>
<td>47</td>
<td>565</td>
</tr>
</tbody>
</table>

Table 3. — Cytologic vs. dysplastic cases in the 21st century.

<table>
<thead>
<tr>
<th>Mild dysplasia</th>
<th>Moderate dysplasia</th>
<th>Severe dysplasia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>24</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Inflammation</td>
<td>8</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Colpitis</td>
<td>51</td>
<td>37</td>
<td>6</td>
</tr>
<tr>
<td>HPV</td>
<td>118</td>
<td>55</td>
<td>14</td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td>9</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td>130</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>HPV</td>
<td>1</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Moderate dysplasia</td>
<td>1</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>7</td>
<td>7</td>
<td>413</td>
</tr>
<tr>
<td>HPV</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>343</td>
<td>175</td>
<td>47</td>
</tr>
</tbody>
</table>

Table 4. — Colposcopy in patients with dysplasia in the 21st century.

<table>
<thead>
<tr>
<th>Mild dysplasia</th>
<th>Moderate dysplasia</th>
<th>Severe dysplasia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO</td>
<td>22</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>ZRT</td>
<td>86</td>
<td>39</td>
<td>7</td>
</tr>
<tr>
<td>CD (dystrophy)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Colpitis</td>
<td>9</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Subtotal</td>
<td>180</td>
<td>31.8</td>
<td></td>
</tr>
<tr>
<td>HPV</td>
<td>31</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Leuko</td>
<td>50</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Base</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>ZRA</td>
<td>108</td>
<td>69</td>
<td>16</td>
</tr>
<tr>
<td>ALT. MET</td>
<td>33</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>ZTA</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Irreg. vasc.</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>343</td>
<td>175</td>
<td>385</td>
</tr>
</tbody>
</table>

Table 5. — Biopsies compared to the dysplasias in the 21st century.

<table>
<thead>
<tr>
<th>Mild dysplasia</th>
<th>Moderate dysplasia</th>
<th>Severe dysplasia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colpitis</td>
<td>17</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>HPV</td>
<td>32</td>
<td>10</td>
<td>42</td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td>87</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Mild dysplasia / HPV</td>
<td>187</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Moderate dysplasia</td>
<td>1</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Moderate dysplasia / HPV</td>
<td>91</td>
<td>3</td>
<td>94</td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe dysplasia / HPV</td>
<td>1</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>Undefined</td>
<td>483</td>
<td>93.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>325</td>
<td>150</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 6. — Evolution of treated patients.

<table>
<thead>
<tr>
<th>Mild dysplasia</th>
<th>Moderate dysplasia</th>
<th>Severe dysplasia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cure</td>
<td>178</td>
<td>80</td>
<td>31</td>
</tr>
<tr>
<td>Improvement</td>
<td>26</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Persistence</td>
<td>20</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Recurrence</td>
<td>9</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Progression</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>236</td>
<td>105</td>
<td>39</td>
</tr>
<tr>
<td>343</td>
<td>175</td>
<td>47</td>
<td>565</td>
</tr>
</tbody>
</table>

When reviewing the PCR reports versus the evolution of dysplasia, it was found that 83% of patients appeared to be cured, as confirmed by both colposcopy and cytology which were both negative, and with positive PCR; the recurring cases were 8.5% and the persisting ones were 1.8%.
It is interesting to note that although the dysplasia had been eradicated, positive PCR persisted. Most striking was the follow up period that reached up to 20 years after treatment.

Discussion

The present study confirms the advantages of applying colposcopy and cytology; furthermore, conservative treatment and the contemporary monitoring of the lesions enables to diagnose the eventual persistence of the same and treatments can then be repeated, if necessary.

Despite the increase of dysplasias in this century, especially during the years 2005 and 2006, treatments have nonetheless achieved the objective of destroying the dysplastic effect caused by HPV, through the activation of specific defense responses, as reported by other authors [8].

The present research maintains the protocol of follow up every three months until healing is achieved; however, in cases that persist, repetitive destructive local conservative treatments: cryotherapy or cauterization are indicated.

The present author can affirm that the cases with persistence of the lesion are the minority, in which dysplasia is not present or only with HPV without isolated white areas after treatments.

In the author’s experience, a good response to the local treatments and a decrease in the incidence of cervical cancer were observed. More attention should be paid to interpersonal and general education and above all, in the application of changes in the preventative medicine policy, which is still based on Pap tests or cytology, and whose false negatives still today range from 30% to 70%, as reported by several authors [9-11].

Conclusion

The real significance of dysplasias is its possible evolution to cancer with the presence of HPV. Both are not present in treated patients. The effect of inflammation caused by the conservative treatment can be able to stimulate both local and systemic immunological response, avoiding recurrence. The PCR value in patients considered as cured is different from those of other countries. HPV infection in various forms, alone or associated, should be treated as an infection and should not be exclusively classified as until now, solely as a sexually transmitted disease.

References


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Evaluation of the effect of GnRH agonist on menstrual reverse in breast cancer cases treated with cyclophosphamide

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Summary
Twenty-five percent of breast cancer cases are detected during premenopausal period and the number of young women suffering from breast cancer is increasing in the world, especially in Iran. Preservation of fertility and ovarian function leads to improved quality of life of these patients. The aim of this study was to evaluate the effect of gonadotropin releasing hormone (GnRH) agonist on menstrual reverse in breast cancer cases treated with cyclophosphamide regimen. Materials and Methods: This randomized clinical trial (RCT) was conducted on 42 adenocarcinoma cases. Mean age of patients was 37 ± 5 years (range 25 to 45). Primary stages to Stage II (T2N1M0) whose histology reports were negative ER/PR were enrolled in this study. All the enrolled patients were candidates for cyclophosphamide (600 mg/m²), Adriamycin (60 mg/m²), and Taxotere (75 mg/m²) chemotherapy regimens. Results: Spontaneous menstrual reverse occurred in 90.5% of patients receiving diphereline at three to six months after treatment which occurred in 33.3% of control cases. In control group, 14.3% (three cases) had oligomenorrhea and hypomenorrhea during chemotherapy and 19% (four cases) had spontaneous menstrual reverse at three to six months. It should be noted that there was a significant difference between controls and cases (p < 0.001). This difference was insignificant in cases younger than 35 years (p < 0.594). In 100% of patients older than 35 years who received diphereline, spontaneous menstrual reverse occurred during six months after chemotherapy, but this occurred in only 20% of controls (p < 0.001). Mean serum level of follicle stimulating hormone (FSH) and luteinizing hormone (LH) during and at three months after therapy was significantly lower in cases in comparison with controls, but serum level of estradiol was significantly more.

Conclusion: GnRH agonists significantly improve ovarian function and fertility. They also lead to spontaneous menstrual reverse in negative ER/PR breast cancer cases.

Key words: Breast cancer; Fertility; Menstrual reverse; Chemotherapy; Cyclophosphamide.

Introduction
Breast cancer is the most prevalent cancer among Iranian women and the second most common cancer in the world following lung cancer. Although less than one percent of breast cancer occurs in women younger than 25 years, however the rate has a significant increase after 30 years. [1]

On the other hand as marrying age has increased, it’s not uncommon for a women older than 40 years to become pregnant [2]. Studies performed in Iran reported breast cancer in women ten years younger than the mean age in the world, which can be in correlation with the young population of Iranians [3, 4].

Probably, the most common side-effect of prolonged chemotherapy is gonad dysfunction which can be transient, but the recovery is often unpredictable and in most cases this side-effect is permanent. Therefore fertility preservation and decrease in long-term effect of chemotherapy is very important in improving quality of life of patients. [4, 5]

Most of studies showed positive effect of gonadotropin-releasing hormone (GnRH) agonists on decreasing gonadotoxic side-effect of chemotherapy in breast cancer and Hodgkin’s lymphoma. These studies also showed that GnRH agonists decrease ovarian damage following chemotherapy [5, 6].

Materials and Methods
This clinical trial study was conducted on 42 adenocarcinoma cases visited in Shahid Sadoughi Hospital during 2010-2011 who were in primary Stage to Stage II (T2N/M0) in the age ranging from 25-45 years. Inclusion criteria were negative ER/PR, primary stages of breast cancer, and candidate for cyclophosphamide (600 mg/m²), Adriamycin (60 mg/m²), and Taxotere (75 mg/m²) chemotherapy regimen.

The patients were randomly divided into two groups. Serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol were checked before chemotherapy through the enzyme-linked immunosorbent assay (ELISA) method. In one group, 3.75 mg/IM diphereline was prescribed every 28 days for six months simultaneous with chemotherapy and the other group just received chemotherapy.

The data were analyzed by SPSS. T-test, Chi-square, S exact and Fisher tests were utilized. Exclusion criteria were advanced stages of the cancer, low complaint cases in receiving diphereline monthly, positive ER/PR, concurrent malignancies, and FSH ≥ 30 at admission.
Results

Mean age of cases was 37±5 years (range 25-45). In the group received diphereline, spontaneous menstrual reverse occurred in 19 of 21 women (90.5%) during three to six months following chemotherapy and amenorrhea continued in only two women until end of month six. However, menstrual bleeding continued during chemotherapy in only three controls (14.3%). In four controls (19%), spontaneous menstrual reverse occurred during three to six months following chemotherapy and in 14 of them (66.7%) menstrual reverse did not occur. This difference was significant ($p < 0.001$, Table 1).

Dividing patients into two groups younger than 35 and older than 35 years of age showed that menstrual reverse rate difference was not significant in group younger than 35 years (14 cases and 14 controls). In other words, menstruation reversed in six out of eight patients (75%) younger than 35 years who received diphereline and in the other two cases it did not occur until the end of six months follow up. In the other group older than 35 years, all women (n=13) received diphereline had spontaneous menstrual reverse, which was only reported in three out of 15 controls (20%). ($p < 0.001$, Table 2)

Comparing serum levels of LH in two groups showed that mean of LH in the group receiving diphereline was lower than the control group during the study and three months following treatment. Mean serum level of LH three months after chemotherapy was 11.70 ± 6.35 and 22.64 ± 14.98 in patients on diphereline and controls, respectively ($p < 0.001$).

### Table 1 – Comparing menstrual reverse in two groups, six months following treatment (Chi-square test).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chemotherapy without diphereline</th>
<th>Diphereline + chemo</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual reverse</td>
<td>7 (33.3%)</td>
<td>19 (90.5%)</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>Amenorrhea</td>
<td>14 (66.7%)</td>
<td>2 (9.5%)</td>
<td></td>
</tr>
</tbody>
</table>

In the other group older than 35 years, all women (n=13) received diphereline had spontaneous menstrual reverse, which was only reported in three out of 15 controls (20%). ($p < 0.001$, Table 2)

### Table 2 – Comparing menstrual reverse in two groups, six months following treatment (Fishers, S. exact test).

<table>
<thead>
<tr>
<th>Age &gt; 35 years</th>
<th>Age &lt; 35 years</th>
<th>Menstruates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemo - diphereline (15)</td>
<td>Chemo + diphereline (13)</td>
<td>Chemo - diphereline (6)</td>
</tr>
<tr>
<td>3 (20%)</td>
<td>13 (100%)</td>
<td>4 (67.7%)</td>
</tr>
<tr>
<td>12 (80%)</td>
<td>0</td>
<td>2 (33.3%)</td>
</tr>
</tbody>
</table>

### Table 3 – Comparing FSH, LH, and estradiol levels with ovarian function test (OFT).

<table>
<thead>
<tr>
<th>p value</th>
<th>3 months after treatment</th>
<th>6 months during treatment</th>
<th>3 months during treatment</th>
<th>Before chemo</th>
<th>Time OFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>48.09 (29.67SD)</td>
<td>29.64 (14.11SD)</td>
<td>50.42 (31.98SD)</td>
<td>12.47 (7.11SD)</td>
<td>35.57 (22.20SD)</td>
</tr>
<tr>
<td>LH</td>
<td>22.64 (14.98SD)</td>
<td>11.70 (6.35SD)</td>
<td>21.56 (13.87SD)</td>
<td>5.87 (3.10SD)</td>
<td>13.11 (8.24SD)</td>
</tr>
<tr>
<td>Estradiol</td>
<td>40.14 (37.35SD)</td>
<td>40.81 (19.35SD)</td>
<td>32.40 (33.62SD)</td>
<td>19.04 (11.10SD)</td>
<td>45.76 (31.65SD)</td>
</tr>
</tbody>
</table>

### Table 4 – Ovarian function preservation during chemotherapy in young cases of breast cancer.

<table>
<thead>
<tr>
<th>Result (reverse of menstruation)</th>
<th>Number of patients</th>
<th>Disease or cancer type</th>
<th>Publication year</th>
<th>Author’s name</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH a: 89.6%</td>
<td>80</td>
<td>Breast cancer</td>
<td>2009</td>
<td>Badawy et al. [5]</td>
</tr>
<tr>
<td>No GnRH a: 33.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt; 40: 94%</td>
<td>29</td>
<td>Breast cancer</td>
<td>2005</td>
<td>Del Mastro et al. [6]</td>
</tr>
<tr>
<td>Age &gt; 40: 42%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GnRH a: 93%</td>
<td>366</td>
<td>Many of cancers in young women</td>
<td>2007</td>
<td>Clowse et al. [8]</td>
</tr>
<tr>
<td>No GnRH a: 48%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GnRH a: 93.7%</td>
<td>36</td>
<td>Hodgkin’s lymphoma</td>
<td>1996</td>
<td>Blumenfeld et al. [9]</td>
</tr>
<tr>
<td>No GnRH a: 39%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GnRH a: 95%</td>
<td>120</td>
<td>Hodgkin’s lymphoma</td>
<td>2002</td>
<td>Blumenfeld et al. [10]</td>
</tr>
<tr>
<td>No GnRH a: 45%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No GnRH a: 63%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GnRH a: 90.5%</td>
<td>42</td>
<td>Breast cancer in young women</td>
<td>2011-2012</td>
<td>This study</td>
</tr>
</tbody>
</table>
Comparing serum levels of LH in two groups showed that mean of LH in the group receiving diphereline was lower than the control group during the study and three months following treatment. Mean serum level of LH three months after chemotherapy was $11.70 \pm 6.35$ vs. $22.64 \pm 14.98$ in patients on diphereline and controls, respectively ($p < 0.001$, Table 3).

FSH serum level at three and six months during and three months after treatment was significantly lower in patients receiving diphereline ($29.64 \pm 14.11$ vs. $48.09 \pm 14.98$ in patients on diphereline and controls, respectively ($p < 0.001$, Table 3).

Mean estradiol serum level at the end of month three following chemotherapy was $81 \pm 19.35$ vs. $40.14 \pm 37.35$ in patients receiving diphereline and control groups, respectively ($p < 0.001$, Table 3).

**Discussion**

The present findings showed that GnRH agonists significantly increase ovarian function and spontaneous menstrual reverse in 90.5% in comparison with 33.3% controls. This result was similar to the Badawy et al. [5] study which was performed on 80 breast cancers (89.6% vs. 33.3% in GnRH agonist and control groups, respectively) (Table 4).

Del Mastro et al. [6, 7] conducted a study on 29 cases of breast cancer which showed the menstrual reverse in GnRH agonist group (Table 4). In this study, in 16 out of 17 cases younger than 40 years (94%) and five out of 12 patients older than 40 years (42%), menstruation reversed, but in the present study 100% of 13 cases older than 35 years who received diphereline and three controls (20%) had menstrual reverse. This difference might be due to effect of age and sensitivity of ovarian tissue to chemotherapy. However, more similar studies with a higher number of cases are needed to confirm this finding.

A meta-analysis performed by Clowse et al. [8] on data collected from nine studies from 1966 to 2000 on 366 different types of breast cancer in young women showed that in 93% of 178 cases who received GnRH agonist and 48% of controls, normal ovarian function was preserved (Table 4).

A study by Blumenfeld et al. [9], performed on 36 cases of lymphoma showed that menstrual reverse occurred in 93.7% of women on GnRH agonist during three to six months following treatment. It should be noted that only in 39% of controls in this study did menstruation reverse. The chemotherapy regimen and findings were similar to the present study.

Blumenfeld et al. [10] showed spontaneous menstrual reverse in 95% in comparison to 45% control.

Blumenfeld et al. [11] performed another survey on 150 cases with Hodgkin’s lymphoma which showed menstrual reverse or spontaneous ovulation in 96.9% and 63% of cases receiving GnRH agonist and controls, respectively.

**References**


Comparison of hematologic toxicity between 3DCRT and IMRT planning in cervical cancer patients after concurrent chemoradiotherapy: a national multi-center study

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5Acibadem Hospital, Department of Radiation Oncology, Istanbul
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Summary

Purpose: To compare the incidence and severity of acute and chronic hematologic toxicity (HT) in patients treated with three-dimensional conformal radiotherapy (3DCRT) and intensity modulated radiotherapy (IMRT) for curative treatment of cervical cancer and to ascertain the dosimetric parameters of two techniques associated with acute and chronic HT. Materials and Methods: A total of 127 patients with cervical cancer receiving concomitant pelvic radiotherapy (RT) and cisplatin were evaluated. Pelvic bone marrow (BM) was contoured for each patient and divided into five sub-regions: lumbosacrum (LS), ilium (IL), lower pelvis (LP), pelvis (P), and whole pelvis (WP). The volume of each BM region receiving 10, 20, 30, and 40 Gy was calculated (V10, -V20, -V30, and -V40). The lowest level of hemoglobin, leukocyte, neutrophil, and platelet counts were obtained during chemoradiotherapy and six months after RT. The nadir values were graded according to Common Terminology Criteria for Adverse Events (version 3.0).

Results: Grade 2 or greater acute anemia, leukopenia, neutropenia, and thrombocytopenia was observed in 2%, 41.5%, 12%, and 0% in 3DCRT group and in 27%, 53%, 24.5%, and 4.5% in IMRT group, respectively. Grade 2 or greater chronic anemia, leukopenia, neutropenia, and thrombocytopenia was observed in 11%, 9%, 4.5%, and 0% in IMRT group and in 11%, 9%, 4.5%, and 0% in IMRT group, respectively. LS-V30,40; IL-V10,20,30,40; LP-V10,20,40; P-V10,20,30,40, and TP-V10,20,30,40 were significantly reduced with IMRT planning compared to 3DCRT planning. Logistic regression analysis of potential predictors showed that none of the dosimetric parameters were significant for predicting acute and chronic HT.

Conclusion: The present findings showed that IMRT planning reduced irradiated BM volumes compared to 3DCRT planning. However, no difference between the two techniques was observed in terms of acute and chronic HT. Further studies are needed to confirm these results.

Key words: Hematologic toxicity; Cervical cancer; Radiotherapy; Chemotherapy.

Introduction

Concomitant chemoradiotherapy is a standard treatment for locally advanced cervical carcinoma. Although the combination of chemotherapy and radiotherapy (RT) improves the outcomes [1, 2], it may cause hematologic toxicity (HT) [2-4]. The presence of high grade HT, particularly leukopenia and neutropenia, increases the risk of infection, which leads to interruption during treatment [5], and it is well known that prolongation of RT decreases the local control of cervical cancer patients [6].

Bone marrow (BM) is one of the most radiosensitive structures of the pelvis and approximately 40% of the total-body BM reserve lies within the pelvic bones [7]. When the two treatment regimes are applied concomitantly, cytotoxic agent may induce stem cells to divide, making these cell populations more radiosensitive [8]. Serious HT, which may negatively affect the course of the treatment, is rare in patients who receive pelvic RT alone, because of increased compensatory hematopoiesis in un-irradiated BM [1]. However, most chemotherapeutic agents used for cervical cancer are myelotoxic [2, 3]. When chemotherapy is concurrently applied with RT, compensatory hematopoiesis is suppressed in un-irradiated BM and this increases the incidence of severe HT [8]. Severe HT may preclude the delivery of chemotherapy and may protract the treatment time.

Recovery of BM depends on RT dose and volume. When larger fields, as 25%-50% of the BM are irradiated, permanent hypoplasia occurs at similar dose levels as for small fields. After greater than 50 Gy, irreversible injury may

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occur due to irreparable damage to the microvasculature manifested by BM fibrosis [8]. Damage to the BM stromal cells due to the irradiation and certain chemotherapeutic agents primarily account for chronic radiation injury by reducing the ability of hematopoietic stem cells to self-renew [9]. Since BM cannot sustain a normal hematopoietic activity, latent damage of BM and chronic HT may become an important issue in gynecological cancer patients who should receive chemotherapy in the setting of recurrence. Several studies showed that higher rates of HT were observed when chemotherapy was delivered to patients who received RT previously. This condition leads clinicians to reduce the drug dose in treatment of recurrent disease thus this may relate to poor outcome [10, 11]. Therefore, preventing HT becomes an important issue in cervical cancer patients to improve tolerance to treatment and enhance outcomes.

The relationship between dose-volume parameters of irradiated BM and acute HT has been reported in patients treated with three-dimensional conformal radiotherapy (3DCRT) or intensity modulated radiotherapy (IMRT) [12-14]. The volume of pelvic BM receiving low doses, such as 10 Gy or 20 Gy, was shown to be associate with acute HT [4, 12, 15]. However, greater doses have not been found as relevant as low doses for acute HT, but they may have a significant effect on chronic HT. The aim of the present study was to compare the incidence and severity of acute and chronic HT in patients treated with 3DCRT and IMRT and to ascertain the dosimetric parameters of two techniques associated with acute and chronic HT.

Materials and Methods

Patient selection
A total of 127 patients with Stage I-IV cervical cancer who received concomitantly cisplatin with pelvic RT between 2004 and 2012 were retrospectively analyzed in this national multi-center study. Five centers contributed to 3DCRT data and one center contributed to IMRT data. Patients who were previously treated with extended-field RT or received chemotherapy or RT for any reason were not included in the study group. The patient and treatment characteristics of the study group are summarized in Table 1.

Radiotherapy
Eighty-two patients (64.5%) received pelvic 3DCRT with a standard four-field ‘box’ technique and 45 (35.5%) patients received pelvic IMRT. Patients underwent contrast-enhanced planning computed tomography with appropriate immobilization. Clinical target volume (CTV) and organs at risk were contoured on axial slices. The CTV included cervical tumor, paracervical, and parametrial tissues, uterus (if present), upper one-half of the vagina, presacral region, and regional lymph nodes at risk (common, external, and internal lymph nodes). Nodal margins were obtained by adding 0.5 to one cm around the vasculature according to the treating physician. Planning target volume (PTV) was defined as the CTV plus a 0.5 to one-cm margin. Normal tissues including bowel, bladder, and rectum were contoured for each patient. All patients received 45 to 50.4 Gy in 1.8 to two Gy daily fractions by use of 6-18 MV photons. The planning goal of all centers was to give 100% of the prescription dose to at least 95% of the PTV while minimizing the dose delivered to the small bowel, bladder, and rectum. Using standard forward planning methods constituted the plans of 3DCRT. A standard four fields (anteroposterior-posteroanterior, and two lateral beams) were designed by using ten to 18 MV photons. The weights of the individual fields were optimized to achieve dose uniformity.

Intensity modulated RT plans were generated with Varian Eclipse planning software version 8.6. Seven co-planar beams with angles 0, 51, 102, 153, 204, 255, and 306 angles. For the IMRT plans, couch rails were located at the outer edges of the couch, and these beam angles likewise avoided. Dose objectives and priorities defined by user and adjusted interactively during

<table>
<thead>
<tr>
<th>Grade 2 or greater hematologic toxicity during treatment and at six months after completion of radiotherapy.</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ Grade 2 HT</td>
</tr>
<tr>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Acute</td>
</tr>
<tr>
<td>Chronic</td>
</tr>
<tr>
<td>WBC</td>
</tr>
<tr>
<td>Acute</td>
</tr>
<tr>
<td>Chronic</td>
</tr>
<tr>
<td>ANC</td>
</tr>
<tr>
<td>Acute</td>
</tr>
<tr>
<td>Chronic</td>
</tr>
<tr>
<td>Platelet</td>
</tr>
<tr>
<td>Acute</td>
</tr>
<tr>
<td>Chronic</td>
</tr>
</tbody>
</table>

3DCRT: three-dimensional conformal radiotherapy, IMRT: intensity modulated radiotherapy. SD: standard deviation, ANC: absolute neutrophil count

Table 1. — Patient characteristics.

| | 3DCRT n = 82 | IMRT n = 45 | p value |
| Age |
| Mean ± SD (range) | 55 ± 11.5 (30-80) | 52 (26-77) | 0.16 |
| Histology (n, %) |
| Squamous cell | 74 (90) | 43 (95.5) | 0.49 |
| Adeno | 8 (10) | 2 (4.5) | |
| Stage n (%) |
| IA-IIA | 13 (16) | 7 (15.5) | 1 |
| IIB-IVA | 69 (84) | 38 (84.5) | |
| Surgery n (%) |
| Yes | 14 (17) | 6 (13) | 0.8 |
| No | 68 (83) | 39 (87) | |
| Baseline blood counts |
| Hemoglobin (g/dl) |
| Mean ± SD (range) | 12 ± 1.3 (10-15.5) | 12 ± 1.5 (10-17) | 0.47 |
| WBC (µg/dL) |
| Mean ± SD (range) | 7.3 ± 2.7 (4-15.5) | 7.8 ± 2 (4-11) | 0.27 |
| ANC |
| Mean ± SD (range) | 4.7 ± 2 (2-10) | 5 ± 2 (1.5-9.5) | 0.57 |
| Platelets (µg/dl) |
| Mean ± SD (range) | 277 ± 87 (130-531) | 300 ± 94.5 (140-610) | 0.51 |

3DCRT: three-dimensional conformal radiotherapy, IMRT: intensity modulated radiotherapy.
effects of hematologic toxicity. The lowest levels of hemoglobin, leukocyte, neutrophil, and platelet count less than 2 x 10^9/L, ANC less than 1 x 10^9/L and platelet count less than 50 x 10^9/L. 

Chemotherapy delivery

Patients were treated with cisplatin (weekly, 40 mg/m²) concurrently with pelvic RT. They were planned to receive four to six cycles of cisplatin during RT. Cisplatin was not given under the following conditions: WBC less than 2 x 10^9/L, ANC less than 1 x 10^9/L and platelet count less than 50 x 10^9/L.

Bone marrow delineation

The external contour of all bones within the pelvis were contoured on the planning computed tomography (CT) scan for each patient according to the method described by Mell et al. [12] and Albuquerque et al. [14]. The entire bony contours were defined as the five following sub-sites: 1) Lumbosacral region (LS): including the region from superior border of L5 vertebra to the inferior border of sacrum, 2) Ilium (IL): including iliac crests extending to the superior border of the femoral heads, 3) Lower pelvis (LP): including pubis, ischium, acetabulum, and proximal femurs, 4) Pelvis (P): including iliac and lower pelvis, 5) Whole pelvis (WP): including lumbosacrum, ilium, lower pelvis, and pelvis. Three-dimensional rendering of the iliac, lumbosacral, and lower pelvic BM was shown in Figure 1. Dose volume histograms were constituted for each contoured BM regions. The volume of each BM region receiving 10, 20, 30, and 40 Gy was calculated. These parameters were defined as follows: 1) LS-V10, -V20, -V30, -V40 2) IL-V10, -V20, -V30, -V40 3) LP-V10, -V20, -V30, -V40 4) P-V10, -V20, -V30, -V40 5) WP-V10, -V20, -V30, -V40.

Hematologic toxicity evaluation

The hemoglobin, leukocyte, neutrophil, and platelet counts were obtained before and during RT and six months after RT. Because any treatment related morbidity that occurs later than six months after the beginning of RT is defined as a late reaction [16], blood counts at six months after completion of RT were collected for evaluation of chronic HT. The lowest levels of hemoglobin, leukocyte, neutrophil, and platelet counts were defined as nadir. The reason for the nadir values were graded according to Common Terminology Criteria for Adverse Events (version 3.0) and grade 2 or greater toxicity was defined as event. Patients with grade 2 or greater HT before chemoradiotherapy were not included in the study.

Table 3. — Descriptive statistics of dosimetric parameters.

<table>
<thead>
<tr>
<th>Bone marrow region</th>
<th>3DCRT (mean ± SD)</th>
<th>IMRT (mean ± SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbosacrum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V10</td>
<td>97 ± 5.8</td>
<td>100 ± 0.4</td>
<td>0.003</td>
</tr>
<tr>
<td>V20</td>
<td>95 ± 7.8</td>
<td>97 ± 3.8</td>
<td>0.1</td>
</tr>
<tr>
<td>V30</td>
<td>83 ± 17</td>
<td>75.5 ± 11</td>
<td>0.01</td>
</tr>
<tr>
<td>V40</td>
<td>70 ± 22.5</td>
<td>50 ± 21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ilium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V10</td>
<td>93.5 ± 7.5</td>
<td>90.5 ± 7.3</td>
<td>0.041</td>
</tr>
<tr>
<td>V20</td>
<td>87.5 ± 10</td>
<td>75 ± 13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V30</td>
<td>58 ± 18</td>
<td>43 ± 15.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V40</td>
<td>36.6 ± 12.5</td>
<td>18 ± 10.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lower pelvis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V10</td>
<td>90 ± 9</td>
<td>83 ± 10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V20</td>
<td>85 ± 11</td>
<td>70 ± 14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V30</td>
<td>50 ± 18.5</td>
<td>45.5 ± 13.5</td>
<td>0.144</td>
</tr>
<tr>
<td>V40</td>
<td>34.5 ± 16</td>
<td>24 ± 13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pelvis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V10</td>
<td>92 ± 5.8</td>
<td>86 ± 8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V20</td>
<td>87 ± 7.5</td>
<td>72.5 ± 11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V30</td>
<td>54 ± 17</td>
<td>45 ± 12.5</td>
<td>0.001</td>
</tr>
<tr>
<td>V40</td>
<td>36.5 ± 14</td>
<td>22 ± 11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Whole pelvis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V10</td>
<td>93 ± 6</td>
<td>89 ± 6.5</td>
<td>0.001</td>
</tr>
<tr>
<td>V20</td>
<td>88.5 ± 7</td>
<td>78.5 ± 8.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V30</td>
<td>61.5 ± 14.5</td>
<td>53.5 ± 11.5</td>
<td>0.002</td>
</tr>
<tr>
<td>V40</td>
<td>44 ± 14.5</td>
<td>28.5 ± 13</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

3DCRT: three-dimensional conformal radiotherapy, IMRT: intensity modulated radiotherapy.

Table 4. — Distribution of number of radiotherapy breaks and chemotherapy cycles missed due to hematologic toxicity and number of transfusions or growth factor administration in treatment groups.

<table>
<thead>
<tr>
<th></th>
<th>3DCRT</th>
<th>IMRT</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT breaks, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13 (16)</td>
<td>10 (22)</td>
<td>0.37</td>
</tr>
<tr>
<td>No</td>
<td>69 (84)</td>
<td>35 (78)</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD Range</td>
<td>5 ± 2.8</td>
<td>4.5 ± 0.7</td>
<td>0.82</td>
</tr>
<tr>
<td>1-4</td>
<td>2-10</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>CHT cycles missed, n (%)</td>
<td>28 (34)</td>
<td>13 (30)</td>
<td>0.34</td>
</tr>
<tr>
<td>Yes</td>
<td>47 (57)</td>
<td>32 (39)</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD Range</td>
<td>1.5 ± 0.8</td>
<td>1.5 ± 0.9</td>
<td>0.63</td>
</tr>
<tr>
<td>1-4</td>
<td>1-4</td>
<td>1-4</td>
<td></td>
</tr>
<tr>
<td>Transfusion received, n (%)</td>
<td>15 (18)</td>
<td>10 (22)</td>
<td>0.64</td>
</tr>
<tr>
<td>Yes</td>
<td>67 (82)</td>
<td>35 (78)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>76 (93)</td>
<td>41 (91)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

The frequency of missed chemotherapy cycles and the fractions of interrupted RT were recorded along with number of using transfusions or growth factors for analyzing the impact of acute HT.

Statistical analyses
Ki-square and Student t-tests were used to test the difference in proportion or continuous variables, respectively. Logistic regression analysis was used to correlate the risk of grade 2 or greater HT with the BM volumes.

Results
Hematologic toxicity
There was no significant difference between treatment groups in baseline counts of hemoglobin, white blood cell (WBC), platelet and absolute neutrophil count (ANC). All patients had complete blood counts before and weekly during RT and 79 (62%) patients had complete blood count at six months after completion of RT.

The most common grade 2 or greater acute toxicity was leukopenia, occurring in 34 (41.5%) patients of 3DCRT group, 24 (53%) patients in IMRT group (p = 0.26). Grade 2 or greater acute anemia was observed in 17 (21%) patients in 3DCRT group and 12 (27%) patients in IMRT group (p = 0.45). Grade 2 or greater acute neutropenia was observed in ten (12%) patients in 3DCRT group and 11 (24.5%) patients in IMRT group (p = 0.09). Although no patient developed grade 2 or greater acute thrombocytopenia in 3DCRT group, two (4.5%) patients in IMRT group developed grade 2 or greater acute thrombocytopenia (p = 0.12). Results of HT during treatment are shown in Table 2.

Grade 2 or greater HT at sixth month after completion of RT was evaluated and no significant difference was observed between the groups. Grade 2 or greater chronic anemia was observed in nine (11%) patients in 3DCRT group and five (11%) patients in IMRT group (p = 0.98). Grade 2 or greater chronic leukopenia occurred in 8 (10%) patients in 3DCRT group and four (9%) patients in IMRT group (p = 0.97). Grade 2 or greater chronic neutropenia occurred in five (6%) patients in 3DCRT group and two (4.5%) patients in IMRT group (p = 0.99). Results of grade 2 or greater HT at six months after completion of RT are shown in Table 2.

Association between dosimetric parameters and hematologic toxicity
The mean volumes of BM regions for different dose levels in the treatment groups were summarized in Table 3. LS volume receiving 30 and 40 Gy; IL volume receiving 10, 20, 30 and 40 Gy; LP volume receiving 10, 20 and 40 Gy; P volume receiving 10, 20, 30 and 40 and TP receiving 10, 20, 30 and 40 Gy were significantly reduced with IMRT planning compared to 3DCRT planning. However, LS volume receiving 10 Gy was 97% in 3DCRT planning and 100% in IMRT planning (p = 0.003). LS volume receiving 20 Gy was 95% in 3DCRT planning and 97% in IMRT planning (p = 0.1). Logistic regression analysis of potential predictors showed that none of the dosimetric parameters were significant for predicting acute and chronic HT.

Radiotherapy and Chemotherapy Delivery
In 3DCRT treatment group, 13 (16%) patients had RT breaks mean 5 (2-10) fractions due to HT. Twenty-eight (34%) patients had chemotherapy breaks mean 1.5 (1-4) cycles due to HT. Six (7%) patients received growth factor, 15 (18%) patients received blood transfusions. In one (1.2%) patient cisplatin dose was reduced. In IMRT treatment group, 10 (22%) patients had RT breaks mean 4.5 (4-5) fractions due to HT. Thirteen (30%) patients had chemotherapy breaks mean 1.5 (1-4) cycles due to HT. Four (9%) patients received growth factor, 10 (22%) patients received blood transfusions. In two (4.5) patients cisplatin dose was reduced.

No significant difference was shown in terms of RT breaks, missed chemotherapy cycles, number of transfusions and growth factor administrations between treatment groups. Data are summarized in Table 4.

Discussion
Therapeutic strategies combining chemotherapy and RT primarily aim to improve tumor control, however potential side effects are much more complicated when these two treatment modalities are given concomitantly. One of potential side effects that should be taken into consideration is the dose limiting BM suppression.

Most of the knowledge about HT that mentioned above depends on the experimental studies. Few clinical studies evaluate the acute HT of concomitant cisplatin and pelvic RT in cervical cancer patients [2, 4, 12, 14]. Moreover, no trials assess the chronic effects of chemoradiotherapy. For this reason, the impact of 3DCRT and IMRT on acute and chronic HT in cervical cancer patients who received concomitantly cisplatin with pelvic RT were evaluated in the study.

To explain the impact of RT techniques on HT, we assessed the volume of irradiated pelvic BM with 3DCRT and IMRT planning. We found IMRT planning reduced the irradiated volume of BM compared to 3DCRT planning. Our findings are consistent with the results of Brixey et al. [4] and Mell LK et al. [13] studies; the volume of iliac, lumbar, sacral and pelvic BM irradiation was reduced with IMRT compared to four-field box technique. Although less BM volume was irradiated in IMRT planning, grade 2 or greater acute anemia, leukopenia, neutropenia and thrombocytopenia were higher in IMRT group compared to 3DCRT group. This is possibly because the areas of low dose regions of LS are larger with IMRT in contrast to 3DCRT; low doses such as 10, 20 Gy may cause acute HT because of BM radiosensitivity [12]. Using IMRT did not provide any benefit on reducing RT breaks and missed chemotherapy cycles with requirement of blood transfusions and growth factor. In addition, we evaluated the BM effects of
3DCRT and IMRT at six months after completion of RT. Chronic effects of chemoradiotherapy was observed in few patients and no significant difference was seen between 3DCRT and IMRT and none of the dosimetric parameters were significant for predicting acute and chronic HT.

In contrast to the present findings, Brixey et al. reported a non-significant decrease in grade 2 and 3 HT for patients treated with IMRT compared to a conventional whole pelvic RT. IMRT patients were also less likely to miss chemotherapy [4]. Mell et al. showed an association between the volume of whole pelvis BM receiving low-dose radiation (V10 and V20) and acute HT in patients receiving concomitant cisplatin and whole pelvis IMRT [12]. Similarly, Albuquerque et al. showed a correlation between whole pelvis BM volume received 20 Gy and acute HT in patients treated with concomitant chemotheraphy and 3DCRT [14]. As reported in experimental and clinical studies, the present authors could not find any dose predictors for acute and chronic HT. However, the present results need to be interpreted with caution because of retrospective nature of the study. Due to multi-centric nature of the study, there is inevitable heterogeneity in treatment protocols and data of 3DCRT. It should also be considered that dose-volume parameters, which were obtained in this study, were based on a planning protocol from multi-centers using different commercial planning systems. Furthermore, using entire bones as a proxy for BM is another limitation of this study. Active and inactive BM regions cannot be distinguished with CT imaging [17]. Recently functional imaging with 18F-FDG-PET was shown to be one method to identify active BM sub-regions. Irradiation of sub-regions with higher 18F-FDG-PET activity is associated with HT [18]. In the future, optimal SUV thresholds may be introduced to identify active BM sub-regions and new techniques can be developed to spare these regions for reducing HT.

Despite the limitations of the study, the present findings showed that IMRT planning reduced irradiated BM volumes compared to 3DCRT planning. However, no difference between two techniques was observed in terms of acute and chronic HT. A prospective study designed to measure blood counts during treatment and after treatment to evaluate the acute and chronic HT is warranted to compare toxicity across treatment techniques and confirm the present results.

References

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Evaluation of serum CA 125 level and its relation to surgical, histopathologic and ultrasonographic findings in patients with pelvic mass

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Summary

Objective: The aim of this study was to determine the relationship between the levels of tumour marker CA 125 antigen and pelvic tumour size, histopathological type, stage, bilateral status, ascites, type of surgery, and postoperative complications. Materials and Methods: A retrospective cross-sectional descriptive study was conducted on 203 patients with a pelvic mass who were visited in the Shahid Sadoughi hospital in Yazd, Iran from 2007 to 2010. Data were analyzed by software SPSS v.14. Results: Statistical analysis, based on Fisher’s exact test, showed that patients with pelvic mass who presented with either of bilateral involvement/ascites (p = 0.000), higher stage (p = 0.001), inability for complete resection (p = 0.000), or postoperative complications (p = 0.001) had significantly higher serum concentrations of CA 125 antigen. There was no relationship between serum level of CA 125 and such variables as tumor size (p = 0.883) and abdominal ultrasound findings (p = 0.297). Conclusion: Using CA 125 as a diagnostic and prognostic tool in patients with newly-discovered pelvic mass can be helpful in some aspects, but cannot estimate size of the tumor and its solid/cystic status. It also cannot predict post-surgical complications of malignant pelvic masses.

Key words: CA 125 antigen; Pelvic neoplasms; Tumour marker; Histopathology; Survival; Surgical complication.
cations to assist in deciding how to deal with patients with pelvic mass and optimum use of paraclinical studies, based on comprehensive review of the performance of these tests.

Materials and Methods

This study was a retrospective cross-sectional descriptive study. Information, using questionnaires, was collected from patients with a pelvic mass who were examined in Shahid Sadoughi Hospital in Yazd, Iran from 2007 to 2010.

Of 251 patients evaluated with respect to the inclusion criteria, 203 patients were finally included. An informed written consent form was signed by all patients before entering the study. In patients who were hospitalized due to a pelvic mass, blood sample was taken before surgery. All blood samples were sent to a single accredited laboratory for evaluation of tumour marker levels. The utilized method for measuring the CA 125 was electrochemiluminescence.

According to the manufacturer’s kit, the normal level of CA 125 was less than 35 units/ml [12, 13]. The effect of smoking, because of its influence on some tumour markers [14], was studied and corrected according to self report by the patients. Based on patients’ ultrasonographic reports, the size of their mass (less than seven cm or seven cm and more) [2, 10] and the abdominal ultrasound characteristics of tumour (cystic, solid or heterogenous) [15] were also recorded.

All these patients underwent comprehensive staging, hysterectomy, oophorectomy, omentectomy, para-aortic biopsy, and pelvic and peritoneal biopsies performed by a gynaecologist. During surgery, a sample of the mass was sent for pathologic assessment. All patients underwent supervision or secondary treatment according to their histopathology report. Results of serum CA 125 level and histopathologic types of tumour were assessed and analyzed. Surgical complications were defined as fever (measured or orally) more than 39°C, lasting for more than three days, urethral, bowel, and/or bladder injuries.

To calculate the survival rate in patients with malignancy, the beginning time was considered from cancer diagnosis (according to the pathology report) until August 2010. Survival status of patients was monitored by following-up the patients (by phone) and was recorded per month and displayed by the Kaplan-Meier curves. All data was analyzed using the software SPSS v.14. For statistical analysis, Fisher’s exact test was used. A p value less than 0.05 was considered significant difference.

Results

The youngest patient was two-years-old and the oldest was 80-years-old. Based on pathology reports, patients were classified into eight groups (Table 1). Benign uterine neoplasms and benign ovarian/para-ovarian masses were the most common tumours, each comprising 71 patients. In 49 patients there was a primary malignancy involving the ovary.

Table 1.—Histopathologic types of tumours in patients (N = 203) and their serum concentration of CA 125 [number (percentage)].

<table>
<thead>
<tr>
<th>Serum CA 125 (Units/ml)</th>
<th>Invasive epithelial ovarian tumor</th>
<th>Borderline epithelial ovarian tumor</th>
<th>Sex cord stromal ovarian cancer</th>
<th>Benign uterine tumour</th>
<th>Malignant uterine tumour</th>
<th>Benign ovarian/para-ovarian tumour</th>
<th>Metastatic to ovary</th>
<th>Mixed germ cell tumor</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>9 (23.7)</td>
<td>4 (44.4)</td>
<td>1 (100)</td>
<td>68 (95.8)</td>
<td>6 (85.7)</td>
<td>40 (56.3)</td>
<td>2 (40)</td>
<td>0 (0)</td>
<td>130 (64)</td>
</tr>
<tr>
<td>≥35</td>
<td>29 (76.3)</td>
<td>5 (55.6)</td>
<td>0 (0)</td>
<td>3 (4.2)</td>
<td>1 (14.3)</td>
<td>31 (43.7)</td>
<td>3 (60)</td>
<td>1 (100)</td>
<td>73 (36)</td>
</tr>
</tbody>
</table>

Malignant tumours comprised 61 of these tumours, in whom 22 (36.1%) had CA 125 < 35 units/ml and the remaining 39 (63.9%) patients had CA 125 > 35 Units/ml. One hundred and twenty patients suffered from ovarian tumours, in whom 54 (45%) had CA 125 < 35 Units/ml (including 40 benign and 14 malignant tumours), and 66 (55%) had CA 125 > 35 Units/ml (including 31 benign and 35 malignant tumours). Fisher exact test showed that p value of differences between these two types of ovarian tumours regarding the serum level of CA 125 is 0.03 (valid).

Table 2.—Frequencies of different variables in all patients (N = 203) and their serum concentration of CA 125 [number (percentage)].

<table>
<thead>
<tr>
<th>Serum CA 125 (Units/ml)</th>
<th>Ultrasound finding</th>
<th>Ultrasound evidence of bilateralism or ascites</th>
<th>Type of surgery</th>
<th>Postoperative complications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cystic</td>
<td>Solid</td>
<td>Heterogenous</td>
<td>No</td>
</tr>
<tr>
<td>&lt; 35</td>
<td>43</td>
<td>8</td>
<td>79</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>(33.1)</td>
<td>(6.2)</td>
<td>(60.8)</td>
<td>(99.2)</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>21</td>
<td>9</td>
<td>43</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>(28.8)</td>
<td>(12.3)</td>
<td>(58.9)</td>
<td>(83.6)</td>
</tr>
<tr>
<td>p value (Fisher exact test)</td>
<td>0.297 (invalid)</td>
<td>0.000 (Valid)</td>
<td>0.000 (Valid)</td>
<td>0.001 (valid)</td>
</tr>
</tbody>
</table>

Results of serum CA 125 level and histopathologic types of tumour were assessed and analyzed. Surgical complications were defined as fever (measured orally) more than 39°C, lasting for more than three days, urethral, bowel, and/or bladder injuries.

To calculate the survival rate in patients with malignancy, the beginning time was considered from cancer diagnosis (according to the pathology report) until August 2010. Survival status of patients was monitored by following-up the patients (by phone) and was recorded per month and displayed by the Kaplan-Meier curves. All data was analyzed using the software SPSS v.14. For statistical analysis, Fisher’s exact test was used. A p value less than 0.05 was considered significant difference.
Evaluation of serum CA 125 level and its relation to surgical, histopathologic and ultrasonographic findings in patients with pelvic mass

Table 3. — Frequencies of different variables in patients with malignancy (N = 61) and their serum concentration of CA 125 [number (percentage)].

<table>
<thead>
<tr>
<th>Serum CA 125 (Units/ml)</th>
<th>Ultrasound finding</th>
<th>Tumour size (cm)</th>
<th>Tumour stage</th>
<th>Postoperative complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic</td>
<td>Solid</td>
<td>Heterogenous</td>
<td>&lt; 7</td>
<td>&gt; 7</td>
</tr>
<tr>
<td>&lt;35</td>
<td>1</td>
<td>4</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>(4.55)</td>
<td>(18.2)</td>
<td>(77.25)</td>
<td>(29.6)</td>
<td>(41.2)</td>
</tr>
<tr>
<td>&gt;35</td>
<td>21</td>
<td>9</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>(28.8)</td>
<td>(12.3)</td>
<td>(58.9)</td>
<td>(70.4)</td>
<td>(58.8)</td>
</tr>
</tbody>
</table>

*p value*  
(Fisher exact test) 0.435 (invalid) 0.883 (valid) 0.001 (invalid) 0.173 (valid)

Figure 1. — The association of tumor markers (CA 125) and survival in patients with malignancy.

To assist in deciding how to deal with these patients, a comprehensive study examining the relationship between serum CA 125 and tumor size, histology, survival, complications, stage, and ultrasound features was designed on 203 patients with pelvic masses who were admitted to that center during four years.

Early diagnosis of cancer can increase survival chance of the patients. There are many tools to detect cases with pelvic masses, and the ovarian tumour marker CA 125 as a simple and non-invasive method, can help to improve treatment, early diagnosis, and follow up of these patients [10].

As high as 90% of these patients can be detected at an early stage by using this test [11]. While in the early stages most of the cases are asymptomatic, unfortunately they are usually discovered at higher stages in which they have only 20% chance of treatment and recovery.

Therefore, introducing an easy and accessible way that can quickly identify more of these patients and in a more economical way can certainly be useful. The relationship between tumour marker CA 125 levels and malignancy in this study was not statistically valid, due to the small number of samples that were divided into two groups, and therefore, the authors only relied on the description of these values. Based on abdominal ultrasound reports, masses were divided into cystic, solid or heterogeneous groups. About 31% of patients had cystic masses, 8.4% solid masses, and 60.1% had heterogeneous masses. This result is in accordance with the ultrasound data reported by McDonald et al. from Cancer Center in Kentucky Hospital, USA [13].

In this study, no significant relationship was observed between cystic, solid, or heterogeneous masses and tumour marker CA 125 levels. In reviewing the literature, no previous study that examined the level of tumour markers and ultrasound characteristics (cystic, solid or heterogeneous pelvic neoplasms) was found. There are only a few studies that have examined the relationship between cystic, solid, and heterogeneous masses [13, 14]. Therefore, further studies to investigate this relationship in more patients are recommended.

Postoperative complications were defined as injury to the urethra, bladder, intestines, and fever over 39°C for more significantly more in patients with high serum CA 125 levels (*p* = 0.001). Bilateralism of tumour and ascites were also significantly more in those patients who had high serum CA 125 (*p* = 0.000). Inability to complete resection of tumour was also more likely when CA 125 was high (*p* = 0.000).

Table 3 shows frequencies of different variables in patients with malignancy (N = 61) and their serum concentration of CA 125.

Until the end of survival time, eight patients had died. No significant relationship was observed between the tumour marker level (CA 125) and the survival rate of patients (*p* = 0.18, Figure 1).

Discussion

In many cases referring to the Shahid Sadoughi hospital, as a referral center in the central and southern provinces of Iran, tumour markers had been found to be under-used. On the other hand, no obvious relationship was found between tumor markers and other clinical findings in patients with pelvic mass.
than three days. Given the low number of complications in patients and also simultaneous injury to multiple organs in some patients, we decided to divide the cases into two groups as complicated and uncomplicated groups. A significant association (p = 0.001) was found between the tumour marker CA 125 and complications after surgery. Only a few papers have addressed the levels of tumour markers, complications after surgery, and survival of patients with ovarian cancer, so more studies are needed in this area.

A study conducted by Gadducci et al. showed that a continuous increase in tumour marker CA 125 level in people with residual disease after initial treatment was present, especially in patients whose disease recurred within three to six months. They believe that CA 125 can be considered as a biomarker for patients’ management instead of using it for early detection of pelvic masses. Nevertheless, in the present study, serum level of CA 125 was significantly related to the benign or malignant status and also to the stage of the disease [15, 16].

In this study, survival rate of the patients was analyzed using the Log Rank, which showed no significant association with CA 125 levels. A study by Cooper et al. for assessment of the prognostic impact of CA 125 level on survival rate of patients with epithelial ovarian cancer, they found that the higher levels of this tumour marker are associated with decreased survival rate in these patients [17].

Durdević et al. also evaluated the role of CA 125 in survival rate of the patients with ovarian cancer, and showed that in cases where there is no residual tissue, levels of > 35 U/ml of this tumour marker, and in cases where there is residual tissue, levels of > 65 U/ml of this tumour marker are correlated with decreased survival rate of the patients [18]. The relationship between other tumour markers and patients’ survival was not assessed. In the two aforementioned studies, the same authors checked the level of tumour marker in epithelial ovarian cancers. As it is difficult to compare the present study with theirs, further studies using greater numbers of patients are highly recommended.

Despite the advantages of CA 125, false-positive results in benign cases such as endometriosis and fibroids are still seen, and various studies have shown that CA 125 values are more reliable in post-menopausal women [19].

Oltmann et al. conducted a study on 424 cases in 2009, to risk classify malignant ovarian masses prior to surgery, and reported that the most common indicators of ovarian cancer before surgery were complaints of precocious puberty, ovarian tumours larger than seven cm or solid tumour in imaging reports. They believe that tumour markers (CEA, CA 125, βHCG, and AFP) - both positive and negative - might be useful just for follow-up [20].

Ayhan et al. in 2007 reviewed 60 cases with borderline ovarian tumours to determine the relationship between tumour marker panels (CA 125, CA 19-9, CEA, and CA 15-3) with tumour size and tumour histopathology. They concluded that high levels of tumour markers, especially CA 125 and CA 19-9 might be suggestive of larger size of tumour. They also suggested that increased CA 125 might indicate the likelihood of serous tumour while high levels of CA 19-9 and CEA implied a greater likelihood of borderline ovarian tumours [21].

To investigate the clinical value of tumour markers (CEA, CA 125), Jun-qing in 2007 studied 208 patients with pelvic pathology and found that increased CA 125, CA 19-9, and AFP are good markers in predicting the likelihood of malignancy of pelvic masses, while CEA is of limited clinical value [22].

Schutter et al. in 2002 reviewed 412 cases to assess the value of tumour markers (CA 125, CA 15-3, and CA 42-4) in differential diagnosis of the pelvic lesions, and concluded that simultaneous increase in all three tumour markers was seen in almost all cases of malignancy. It should be noted that increase in all of those three tumour markers was found in a small number of patients. It was also found that increased CA 15-3 was associated mostly with malignancy. The tumour markers’ results were less accurate than ultrasound, physical examination, patients’ age, and menopausal status [23].

In a large British study with a sample size of 200,000 postmenopausal women comparing the diagnostic sensitivity and specificity of CA-125 and transvaginal ultrasonography (TVUS), it was observed that sensitivity, specificity, and positive predictive value of CA 125 in all primary tumours of the ovary and fallopian tube cancers obtained were 89.4%, 99.8%, and 43.3%, respectively; while these values were 84.9%, 98.3%, and 2.8% for TVUS alone. However, from an economic point of view, there is no consensus in using a combination of these two methods in screening of ovarian tumours [24].

Milojkovic et al. in 2003 reviewed 212 patients to assess the value of CA 125 in differentiation of benign from malignant tumours before surgery, and concluded that measuring this tumour marker before surgery is an effective method in diagnosis of benign and malignant pelvic masses [25].

Behtash et al. studied 75 patients with adnexal masses using ultrasound and CT scan, and found that transabdominal ultrasound is a sensitive method for detection and staging of suspected ovarian tumours, but CT scans and tumour markers do not add further information about the nature of the masses [26]. Biomarkers, as screening tools, are usually most useful in people with high risk and family history of BRCA 1, BRCA2, and family history of colorectal cancer [8].

In an Iranian study performed by Yousefi et al. in 2007, it was observed that 42.1% of patients with ovarian tumours had high serum level of CA 125, while the level of CEA showed no increase in any of those patients [27].
Evaluation of serum CA 125 level and its relation to surgical, histopathologic and ultrasonographic findings in patients with pelvic mass

Conclusion

Patients with pelvic mass who present with either of bilateral involvement, ascites, higher stage, inability for complete resection, or postoperative complications had significantly higher serum concentrations of CA 125. There was no relationship between serum level of CA 125 and such variables as tumor size and abdominal ultrasound findings. Using CA 125 as a diagnostic and prognostic tool in patients with newly-discovered pelvic mass can be helpful in some aspects, but cannot estimate size of the tumor and solid/cystic status of it. It also cannot predict post-surgical complications of malignant pelvic masses.

References


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Case Reports

Squamous cell carcinoma arising in mature cystic teratoma of the ovary: report of two cases with molecular analysis

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³Institute of Biomolecular Chemistry, Cancer Genetics Unit, C.N.R., Sassari (Italy)

Summary
Mature cystic teratoma (MCT) is the most frequent ovarian tumor and it is generally composed of well-differentiated elements which, nevertheless, have the potential for malignant transformation. The authors report two cases of squamous cell carcinoma (SCC) arising on ovarian MCT. In the present study, no mutation of the CDKN2A gene, whose impairment may deeply affect either the p16CDKN2A–CyclinD1–pRb cascade or the p14CDKN2A–mdm2–p53 cascade, was observed in tumor tissues from our cases’ collection. This suggests that changes in the protein levels for the above-described candidate effectors may be somehow due to epigenetic alterations into the mechanisms controlling their expression. Analogously, no genetic modification among the two main genes (EGFR and KRAS) upstream the MAPK signalling pathway, which has been widely reported to play a major role in both development and progression of vast majority of malignant tumours, was detected in this series. Additional genes and pathways should be therefore investigated in order to identify genomic impairments underlying the MCT malignant transformation.

Key words: Ovary; Mature Cystic teratoma (MCT); Squamous Cell carcinoma (SCC); Double-colour FISH analysis.

Introduction
Mature cystic teratoma (MCT) is the most frequent ovarian tumor, accounting approximately for 10% to 20% of all ovarian tumors [1, 2]. It is generally composed of well-differentiated elements which, nevertheless, have the potential for malignant transformation. Such event is globally rare, occurring in 0.3% to 6.67% of MCTs [3, 4]. The most common malignant tumor arising on MCT is squamous cell carcinoma (SCC), which derives from malignant ectodermal transformation, and accounts for 70% to 85% of all the MCT malignancies [5, 6]. Numerous other less common malignant forms have been reported, including adenocarcinoma, carcinoid, carcinosarcoma, melanoma, small cell carcinoma, chondrosarcoma, and others [5, 6]. Generally, tumors arising by malignant MCT transformation have greater mean dimensions than benign MCTs, and involve younger individuals [7].

The clinical and oncological management of SCCs arising on MCT is generally challenging. This is because no evidence-based guidelines exist, given the rarity of these tumors and the small number of cases reported in literature. Furthermore, preoperative diagnosis is extremely difficult, as neither clinical nor radiological pathognomonic elements have been described. Moreover, pathological diagnosis can be demanding in some cases, despite that morphological and immunohistochemical patterns of SCCs have been extensively described [8]. In addition, the prognosis of the disease is really poor, and invasive and radical surgical operations in early stages represent the only hope for cure [9]. These characteristics make the management of patients with SCC of ovarian MCT challenging and delicate.

A consistent number of cases of SCC arising on MCT have been recently published containing a increasing amount of useful clinical and oncological data. Nevertheless, very little knowledge exists concerning the molecular and pathophysiological mechanisms of MCTs’ malignant transformation. In this regard, the deregulation of the cyclin-dependent kinase inhibitor 2 (CDKN2A) gene seems to play a role in such a type of tumorigenesis. The CDKN2A gene encodes two proteins, p16CDKN2A and p14CDKN2A, that act as tumor suppressors [10]. In particular, p16CDKN2A is part of the G1–S cell cycle checkpoint mechanism, whose final effectors are CyclinD1 and pRb proteins, whereas p14CDKN2A exerts its tumour suppressor effect through activation of the antiapoptotic p53 protein. Alteration of the p16CDKN2A and p53 expressions have been reported in MCT malignant transformation [5]. An additional pathway usually associated with the pathogenesis of carcinomas is the mitogen-activated protein kinase (MAPK) signal transduction cascade, whose main effectors are represented by EGFR, KRAS, and BRAF gene products [11].
The authors report two cases of SCC arising on ovarian MCT, with the aim to describe the clinical and oncological features of these tumors and to discuss the findings of the molecular analysis performed.

Cases Report

Case one
The first patient was a 47-year-old Caucasian woman, P0000, married, without significant previous pathology or surgical history, menarche at the age of 12 years, and menstrual regularity. She underwent clinical evaluation for pain in the low abdomen quadrants in the last three months. Gynecological examination showed normal conditions of the vulva and vagina, but the uterus, despite regular in size, was posteriorly displaced by a swelling mass of hard consistency extending to the umbilical line cross.

Ultrasound examination evidenced a 117 x 123 x 90 mm left ovary-linked cystic mass with an inhomogeneous, strongly distorted echo-structure. Areas of solid appearance mixed to granular and heterogeneous hypoechoic areas were also evidenced. The bladder was displaced towards the anterior pelvic wall. Power and color-Doppler ultrasound showed a poorly represented vascular tree with high strength velocimetric flow waves. The uterus, located caudally and in slight anti-flexion, presented dimensions a little higher than normal values and a globular shape with strongly inhomogeneous echo-structure due to fibromatosis. The endometrium appeared proliferative and 7.2 mm thick. No alterations of the right ovary were detected. Magnetic resonance imaging (MRI) confirmed these findings. Serum Ca19-9 was 341.37 > U / ml [normal range: 0.00-37.00], while no alterations of other tumor markers were found.

The patient underwent laparotomy and total hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy. The postoperative course was uneventful and she was discharged at the 7th postoperative day. No complications were observed during hospital stay.

Gross examination of the specimens evidenced a partially cystic mature teratoma of the ovary containing hair, sebaceous yellowish material, and a solid component. The wall of the cyst showed keratinized stratified squamous epithelium, hair follicles, sebaceous glands, and a vast foreign-body giant cell reaction. Furthermore, a vegetant SCC with wide necrotic areas and marked cytological atypias, infiltrating the wall of the dermoid cyst (but not the capsule), was also evidenced (Figure 1). Neoplastic endovascular emboli were found; no lymph node or omental metastasis were detected.

The patient underwent subsequently six cycles of platinum-based adjuvant chemotherapy and taxol and she is alive and disease-free 48 months after diagnosis. Currently the woman is free from disease.

Case two
The patient of the second case was a 57-year-old Caucasian woman, P4004 (four spontaneous deliveries), menarche at the age of 13 years, and menstrual regularity. She underwent operative hysteroscopy and excision of a submucosal myoma three years prior. She was referred for gynaecological evaluation due to the presence of pain in the right iliac fossa for more than a year.

She underwent clinical evaluation for pain in the right iliac fossa for more than a year. A computed tomography (CT) scan of the abdomen and pelvis was performed showing a voluminous cystic lesion of about 10 cm in greatest diameter in relation to the right adnexa. The lesion had regular margins and a partially cystic appearance with areas of fluid or fatty tissue. There were not substantial densitometric modifications after contrast administration, unless a modest parietal enhancement. There were not relevant pathologic findings in pelvic and abdominal viscera or lymph nodes. The bladder appeared imprinted by the mass on the right antero-lateral wall. An MRI of the pelvis was also performed, showing the mass of the right adnexa, which appeared hyper-intense on long TR images and hypo-intense on T1, but containing a hypo-intense on T2 and heterogeneously hyper-intense on T1 area (fat tissue). These features appeared suspicious for a MCT with a mixed fluid and fatty tissue component.

The patient underwent adhesiolysis, total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy. She was discharged at the 8th postoperative day, as no complications were observed during hospital stay.

Gross and histological examination of the specimens evidenced findings similar to those of the first case. In the context of the wall of the cystic part of the lesion, proliferation of keratinized epithelial elements with numerous atypical mitoses and areas of necrosis was evidenced (Figure 2). Some neoplastic emboli were also found, but nor infiltration of the capsule neither lymph node metastasis were detected.

The patient underwent the same chemotherapy as the previous case, but she died 30 months after surgery because of liver metastasis.

Molecular analysis
Paraffin-embedded tumour tissues from the two cases were used for both isolating genomic DNA for mutation analysis and preparing tissue sections for fluorescence in situ hybridization (FISH)
analysis. For mutation analysis, the full coding sequences and splice junctions of CDKN2A (exons 1, 2, and 3), EGFR (codons 19-21), and KRAS (exons 2-3) were screened for mutations through direct sequencing using an automated fluorescence-cycle sequencer. Double-colour FISH analysis was performed using probes specific for CyclinD1 and EGFR genes, as previously described [12]. Mutation analysis and FISH were performed for either the malignant and the benign squamous component of MCT in both cases, in order to evidence different molecular alterations. No mutation or genomic rearrangement in candidate genes was detected in this series, with the exception of a polymorphism in exon 3 of CDKN2A (C500G) for the second case only.

Discussion

MCT is the most common ovarian tumor comprising ten to 20% of all tumors of the ovary, and it is bilateral in nine to 16% of cases [1, 2, 13]. Despite it is composed by well-differentiated cells, malignant transformation occurs in 0.3% to 6.67% of cases and can arise in any of the three germ layers [3, 4]. The ectoderm is most commonly involved in malignant transformation; therefore SCC is the most frequent malignancy, accounting for 70% to 85% of cases [5, 6]. Adenocarcinoma accounts for approximately seven to 15% of cases, while numerous other less common malignancies have been described [5, 6, 13]. Sporadic cases of multiple synchronous tumors arising in dermoid cysts have also been described [10].

MCT-related SCC arises frequently in post-menopausal women, as opposed to MCT which generally affects younger women. In the study of Futagami et al. the mean age of patients with MCT-related SCC was 42.5 years, and that of patients with benign MCT 34.2 years, while in the study of Kikkawa et al. the respective figures were 55.2 and 37.5 years [7, 14]. Hachethal et al. in a review of 277 published cases found that the mean age of patients with SCC malignant transformation was 55 years [9]. It was speculated that malignant transformation occurs in long-standing benign MCTs and this explains the tendency to affect older women. This tendency was confirmed also in the present cases.

The clinical presentation is generally similar to that of benign MCTs, characterized by abdominal pain and a mass in the pelvis, as observed in the present cases. Furthermore, signs and symptoms of compression or invasion of other anatomical structures, like bowel, ureters and bladder may be found [8, 9]. Compressive symptoms may be more frequent in cases of malignant transformation as these lesions are generally more voluminous than classic MCTs. Weight loss or fever can also be observed. These clinical manifestations may pose the suspect of a MCT, which is relatively frequent, but do not give any information on the existence of malignant transformation.

The role of several tumoral markers in the preoperative assessment of the disease has been investigated. In both the present cases, serum CA 19-9 was found increased, and CA 125 and CEA were found within normal ranges. Other authors have reported CA 19-9 increment in SCC malignant transformation with extremely variable percentages [6, 9]. More interesting appears the role of SCC antigen, despite it cannot be used for early diagnosis as it depends on the volume of the tumor [4]. It has been speculated that the combination of increased serum SCC antigen levels (> 2.5 ng/ml) and age > 40 years suitably predict malignant transformation in MCT [15]. In the study of Hackethal et al., serum SCC antigen was increased in 86.5% of the cases examined; the corresponding figures for CA 125, CA 19-9 and CEA were 71%, 77% and 67%, respectively [9]. The authors did not evidence any correlation between the serum levels of these markers and the FIGO stage of the disease.

Concerning imaging, CT and MRI represent the radiological tools most frequently used. These techniques permit generally to diagnose MCTs preoperatively. Nevertheless, differential diagnosis between a benign and malignant MCT is extremely challenging. CT and MRI can provide useful information on the extension of the disease, but their precise impact on the management of patients with MCT-related SCC must be better assessed [17].

On gross examination, MCTs generally present a mixed composition, with variable solid and cystic components and often filled with pultaceous material, hair, cartilage, bone, and other differently differentiated tissues. The surface can be smooth and regular or distorted by tumoral invasion and adhesions. Events like malignant transformation, haemorrhage, and necrosis involve mainly the solid component. The former must be carefully ruled out, because of its influence on prognosis. The diagnosis of squamous malignant transformation is based on the identification of architectural and

Figure 2. — Malignant squamous cells with marked anaplasia and atypical mitoses (H&E x400).
cytological morphologic patterns which resemble those of the normal squamous mucosae. Well-differentiated squamous elements may present keratinization and intracellular bridging, as well as a polygonal shape and central nuclei with one or two central nucleoli. These features may be partially identifiable in less differentiated forms; immunohistochemistry becomes mandatory to reach diagnosis in these cases, as well as in cases with confusing elements like papillations, cysts, pseudoglands, and polypoid or insular patterns [8]. Typical immunohistochemical findings in SCCs are high-molecular-weight cytocheratin positivity (e.g. cytocheratins 5/6 and 34BE12) and p63 positivity [8]. In the study of Iwasa et al. 21 cases of MCT-related SCC were analysed and immunoreactivity for p53, MDM2, p21, and CyclinD1 was evidenced in 67%, 43%, 14%, and 57%, respectively. The same authors report decreased expression of p16 and Rb protein in 86% and 48% of cases, respectively [5]. In the present study, no mutation of the CDKN2A gene, whose impairment may deeply affect either the p14CDKN2A-mdm2-p53 cascade, was observed in tumour tissues from the cases’ collection. This suggests that changes in the protein levels for the above-described candidate effectors may be somehow due to epigenetic alterations into the mechanisms controlling their expression. Analogously, no genetic modification among the two main genes (EGFR and KRA4) upstream the MAPK signalling pathway, which has been widely reported to play a major role in both development and progression of vast majority of malignant tumours, was detected in the present series. Additional genes and pathways should be therefore investigated in order to identify genomic impairments underlying the MCT malignant transformation.

Surgery represents the most important treatment method for SCC arising on MCT. Unfortunately, the diagnosis of the disease is commonly made by postoperative pathological examination of the surgical specimen, because the clinical and radiological elements mentioned above do not allow pre-operative diagnosis. This has a negative impact on the surgical planning. Nevertheless, encouraging results have been described, especially in early stages. Abdominal hysterectomy with bilateral salpingo-oophorectomy, omentectomy, and lymphadenectomy has been proposed as the surgical procedure of choice, but in younger patients who desire to conserve the possibility to procreate and with Stage Ia disease, a monolateral approach seems to offer good oncological results [18]. The rationale of the surgical treatment of MCT-related SCC depends on the necessity for an appropriate staging of the disease and for an optimal debulking. Several authors reported that cytoreduction was one of the factors that impacts positively on survival, especially when followed by adjuvant therapy [18, 19]. This was also the rationale of the surgical strategy in both the present cases, but it was demonstrated effective only in one case.

Concerning adjuvant treatments, several chemotherapeutic and/or radiotherapeutic approaches have been proposed, but the low incidence of the disease renders evaluation and standardization of these treatments difficult. The most effective and diffusely used agent seems to be cisplatin [18]. Both the present patients underwent cisplatin-based adjuvant chemotherapy, which resulted effective, in association with previous surgery, in one case. Our oncologists and radiotherapists did not prescribe radiation therapy, as no convincing evidence exists on its usefulness [18, 19]. Some novel combined adjuvant approaches with encouraging results have been recently proposed; the most important seem to be cisplatinum-taxane chemotherapy and chemoradiation with nedaplatin [18, 20]. Several factors have been described to influence prognosis of MCT-related SCC; the most relevant seem to be age, tumor markers (SCC antigen and CA 125), tumor size, clinical stage, grade of differentiation, capsular, and/or vascular invasion and cytoreduction [9, 21]. In patients with favourable conditions and a localized, well-differentiated and well capsulate lesion, who underwent successful surgical resection prognosis is globally good, as observed in the first patient. In cases of extra-capsular invasion, prognosis is generally poor, despite surgical and chemotherapeutic efforts. This picture reflects the absolute need to improve and to invent diagnostic means for early detection of these tumors, which seems to be the only way to reduce mortality.

On the other hand, it is very important to better understanding the pathophysiology and molecular mechanisms of squamous cell malignant transformation, in order to enlarge the actual therapeutic possibilities. Some authors believe that the transformation process occurs through dysplastic changes in the squamous epithelium or in the columnar epithelium that had undergone squamous metaplasia [21]. Complex chromosome aberrations, alterations in the p53 and p16 genes, and in expression of cyclooxygenase-2 have been reported to play substantial roles in this setting, but the causes and the exact molecular mechanisms are not known [5, 22].

References

Squamous cell carcinoma arising in mature cystic teratoma of the ovary: report of two cases with molecular analysis


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Angiomyofibroblastoma of the vulva

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Summary

Background: Angiomyofibroblastoma (AMF) is a rare benign mesenchymal neoplasm that arises in the pelviperial region. Case: A patient presented with a painless mass in the right vulva. Under the preoperative diagnosis of Bartholin cyst, she underwent a simple tumor excision. Pathological examination revealed an AMF. Immunohistochemical examination showed that tumor cells were positive for estrogen receptor, progesterone receptor, vimentin, and CD34. She has been with no evidence of local recurrence for ten months after surgery. Conclusion: AMF of the vulva is a distinctive mesenchymal tumor that is curable with a simple excision.

Key words: Angiomyofibroblastoma; Immunohistochemistry; Vulva.

Introduction

Angiomyofibroblastoma (AMF) is a benign, well-circumscribed myofibroblastic neoplasm that usually arises in the pelvic and peritoneal regions, especially in the vulva, of the middle-aged women. This tumor is often misdiagnosed as a Bartholin gland cyst, lipoma, and aggressive angiomyxoma. Despite the difficulty in the precise preoperative diagnosis, it is clinically important to distinguish AMF and aggressive angiomyxoma with a high propensity for local infiltration.

Here, the authors present a case of AMF arising in the vulva with the findings of pathological and immunohistochemical analyses.

Case Report

A 42-year-old woman presented with a painless mass in the right vulva which she had noticed ten months ago. Physical examination revealed a ruby mass arising from the right labia majora with no tenderness, measuring six cm in the diameter. She was diagnosed as having a Bartholin cyst and underwent a simple excision. She has been with no evidence of recurrence for ten months since surgery.

Macroscopically, the excised tumor was well-circumscribed, elastic soft, and 63 x 43 x 19 mm in size. The cut surface was solid, yellowish-white in color, and homogeneous with no areas of hemorrhage or necrosis. Microscopic examination revealed AMF of the vulva. Tumor was well demarcated by a thin fibrous pseudocapsule and showed hypocellular areas with an abundant, loose edematous stroma in the periphery (Figure 1A), and collagenous, hypercellular areas in the central (Figure 1B). In the hypocellular areas, thin-wall blood vessels were distributed in the reticular pattern, and thin wavy collagen fibers were scattered. Tumor cells were composed of primitive spindle-to-stellate cells with scanty cytoplasm and spindle-to-rounded cells with eosinophilic cytoplasm including multinucleated cells. Typically, tumor cells were concentrated around vessels (Figure 2A) and clustered with an epithelioid appearance (Figure 2B). Occasionally, mitoses were found (Figure 2C). Furthermore, mature adipocytes were focally seen (Figure 2D). No mucin in the edematous stroma was stained with Alcian blue at pH 2.5.

A panel of immunohistochemical analysis with estrogen receptor (ER), progesterone receptor (PR), vimentin, CD34, α-smooth muscle actin (SMA), desmin, H-Caldesmon, S-100, and AE1/AE3 was performed using a streptavidin-biotin method. The majority of tumor cells were positive for ER (Figure 3A), PR (Figure 3B), and vimentin (Figure 3C), and spindle-to-stellate cells in the edematous stroma were weakly positive for CD34.

Discussion

AMF is an uncommon benign mesenchymal tumor that usually occurs in the female genital tract. By reviewing 71 cases of AMF, Sims et al. summarized that the mean age of AMF at presentation was 45 years and that the lesions were equally distributed between the left (52%) and right (48%), with the mean diameter being 5.9 cm [1]. The most common diagnosis is a Bartholin gland cyst (46%) or lipoma (15%) [1]. The differential diagnosis includes aggressive angiomyxoma, cellular angiofibroma, fibroepithelial stromal polyp, and epithelioid leiomyoma [2]. It is crucial to distinguish AMF from aggressive angiomyxoma because aggressive angiomyxoma has a marked tendency to local recurrence [3]. The treatment of choice of AMF is a simple total excision [1], while the wide surgical excision with tumor-free margin is the traditional treatment of choice in aggressive angiomyxoma [4].

In the present case, microscopic findings were in agreement with the reported histological features of AMF [5]. Histological findings of AMF are characterized by the presence of variable hypocellular and hypercellular areas, edematous stroma containing wavy collagen fibers, tumor cells varying in shape from spindle, stellate, plasmatoitid to epithelioid appearance, isolated or grouped tumor cells in cords or nests typically around blood vessels, the presence of small- to medium-sized and dilated vessels, the mixture of lipomatous elements in a few cases, and minimal mitotic activity [1, 5-12].
AMF mimics aggressive angiomyxoma due to the presence of spindle-shaped stromal cells, myxoid stroma, and abundant blood vessels [2]. However, Fletcher et al. described that AMF can be distinguished from aggressive angiomyxoma by its circumscribed borders, much higher cellularity, more numerous blood vessels, frequent presence of plump stromal cells, minimal stromal mucin, and rarity of erythrocyte extravasation [5].

Figure 1. — Microscopic findings of AMF. (A) Hypocellular area in the edematous stroma. (B) Hypercellular area in a dense collagenous stroma (H&E stain, original magnification x10).

Figure 2. — Microscopic findings of AMF. (A) The concentration of the tumor cells was seen around a degenerated vein. (B) The tumor cells were clustered. (C) Mitosis was seen (arrow). (D) Mature adipocytes were focally contained in the tumor (H&E stain, original magnification (A)x20, (B)x40, (C)x40, (D)x20).
In the present case, immunohistochemical analysis showed that tumor cells were positive for ER, PR, vimentin, and CD34. Previous reports have demonstrated that AMF is strongly positive for vimentin and desmin, and usually expresses ER and PR, but is negative for cytokeratin, α-SMA, and S-100 protein [5-8, 11-14]. In contrast, aggressive angiomyxoma was shown to be positive for vimentin [15,16], α-SMA [15, 16], ER [16], and PR [16], but negative for S-100 and desmin [15, 16]. Nagai et al. speculated that AMF and aggressive angiomyxoma are derived from the stromal stem cell with a capacity for hormone-inducing myofibroblastic differentiation [7]. Unlike microscopic evaluation, the differential diagnosis between AMF and aggressive angiomyxoma with immunohistochemistry seems to be difficult because of the overlapping of immunostaining pattern.

Collectively, AMF is a rare mesenchymal tumor that occurs mainly in the vulva. Histological evaluation is important to distinguish AMF from aggressive angiomyxoma with a propensity for local recurrence.

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Mitotically active cellular fibroma of the ovary: a case report and a review of the literature

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Summary
Mitotically active cellular fibroma (MACF) is characterized by increased cellularity, mitotic activity, and less frequently, nuclear atypia, which comprises 10% of ovarian fibromatous tumors. The authors report the case of a 76-year-old woman who presented at the present hospital with a two-month pelvic mass. B ultrasound disclosed a 75 × 52 × 41 mm mass in the right accessories. A hysterectomy and bilateral salpingo-oophorectomy was performed. Histologically, the tumor was composed of a densely cellular proliferation of fibroblastic-like cells with bland nuclear features and arranged in a fascicular pattern. There were more than four mitotic figures per ten high-power fields (HPFs). The histological diagnosis for the mass of the right ovary was MACF. MACF should be distinguished from ovarian fibrosarcoma. MACF is a recent histopathologic entity. Despite the high count of mitotic figures, the clinical course of the tumor is typically uneventful. Long-term clinical follow-up is recommended.

Key words: Ovary; fibroma; Mitotically active cellular fibroma; Outcome.

Introduction
Fibromatous tumors of the ovary are comprised predominantly of fibroma, cellular fibroma, mitotically active cellular fibroma (MACF) and fibrosarcoma [1]. The majority of these neoplasms are benign fibromas and the diagnosis is usually straightforward. The MACF is defined by increased cellularity, mitotic activity, and less frequently nuclear atypia, which should be distinguished from fibrosarcoma as their prognosis and therapy are different [2]. Since the first report described by Irving et al., only three cases have been reported to date [3-5]. Herein, the authors report a patient with a rare ovarian fibrous tumor with a large number of mitotic figures but without severe nuclear atypia.

Case Report
A 76-year-old woman presented with a two-month pelvic mass and requested gynecological consultation. B ultrasound in the locality revealed a 75 × 52 × 41 mm liquid dark area in the right adnexa and the capsule was intact. The patient was then admitted to the present hospital and B ultrasound was repeated. Ultrasound examination showed an irregular low-echo mass measuring 77 × 44 × 62 mm on the right adnexa, which containing fairly abundant, cord-like color blood stream, and irregular echo (Figure 1). The mass had an indistinct capsule. The right adnexa tumor was considered. Serum levels of tumor markers including CA125, CA153, CA199, AFP, CEA, and SCC were within the normal range.

At laparotomy, a 6 × 5 × 5 cm solid, white, and smooth mass was detected in the right ovary. The right oviduct, the left ovary and oviduct, and the uterus appeared normal. No ascitic fluid and peritoneal dissemination were observed. Right adnexectomy was performed. Intraoperative frozen section of the right adnexa confirmed an ovarian malignant tumor consistent with Sertoli-Leydig stromal cell tumor. Subsequently, a hysterectomy and bilateral salpingo-oophorectomy were performed.

Macroscopically, the tumor was 9 × 6 × 5 cm and firm, fibrous, well-demarcated. The external surface was smooth. On cut surface, the tumor was grey-white in colour without gross necrosis and hemorrhage. Microscopically, the tumor was comprised predominantly of densely spindle fibroblastic-like cells with focal edematous areas (Figures 2a, 2b). The spindle cells arranged in intersecting fascicles with mild nuclear atypia (Figure 2c). The cells had scant cytoplasm and indistinct borders and nucleoli. Interestingly, the mitotic index varied from five to nine per ten high-power fields (HPF) (Figure 2d). Immunohistochemical analysis showed that tumor cells were positive for vimentin, alpha-inhibin (Figure 3), ER, PR, and focally for CD56 and CD99, while cytokeratin, EMA, CD10, HMB45, S-100, calretinin, CD34, CD117, and Dog-1 were negative. The Ki-67 labeling index reached up to 10%.

Discussion
Ovarian stromal tumors are comprised of a pure proliferation of fibroblastic cells usually consisting of fibromas, cellular fibromas (CFs), and hardly ever of fibrosarcomas [1]. The majority of these neoplasms are benign fibromas and rarely pose any diagnostic difficulty and are readily considered benign in most cases. However, about 10% of fibromatous tumors exhibit increased cellularity, mitotic activity, and less frequently, nuclear atypia [4]. The presence of one or a combination of these features may result in difficulty to classify a case within the group of fibromatous tumors. In 1981, Prat and Scully studied 17 cellular fibrothecomatous lesions of the ovary and identified the mitotic count as the most important feature for distinguishing between benign and malignant lesions [6].

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Figure 1. — B ultrasound showing an irregular low-echo mass measuring 77 × 44 × 62 mm on the right adnexa, which containing fairly abundant, cord-like color blood stream and irregular echo.

Figure 2. — Histopathologic features of MACF. a) tumor is composed predominantly of densely spindle fibroblastic-like cells which is arranged in intersecting fascicles. b) Focal edematous and sparse cell areas are seen within the tumor. c) On high-power field, the tumor cells are spindle with oval or spindle nuclear and scant cytoplasm, but no moderate and severe atypia. d) Mitotic figure can been readily seen within the tumor.

Figure 3. — Tumor cells immunoreactive with alpha-inhibin.
suggested that a tumor containing fewer than three mitotic figures per ten HPFs should be diagnosed as a cellular fibroma whereas a tumor containing more than four mitotic figures per 10 HPFs should be diagnosed as a fibrosarcoma. Tsuji et al. [7] suggested that the proliferative activity labeled by MIB-1 in tumor cells was an additional useful parameter for distinguishing between fibromas and fibrosarcomas of the ovary.

Since the Prat and Scully study, subsequent ovarian fibrosarcoma with a benign clinical course have been reported in the literature. In 2001, Huang et al [8] reported a case of primary ovarian fibrosarcoma that was free from disease for six years. In the following year, Cinel et al. [9] reported a 45-year-old postmenopausal woman with ovarian fibrosarcoma which showed densely cellular and average six mitotic figures per ten HPFs. The patient had shown no evidence of recurrent disease for five years. Choi et al. [10] in 2006 reported two cases of primary ovarian fibrosarcoma. After surgery, both patients received several courses of combination chemotherapy. Neither patient demonstrated any evidence of disease recurrence during follow-up for ten years and five years, respectively. These collecting cases showed the diagnosis was based primarily on a mitotic count of four or more than mitotic figures per 10 HPFs, not more than nuclear atypia showing mild to moderate atypia in these reported cases.

In a 2006 study, Irving et al. [2] reviewed 75 cases of cellular fibroma of the ovary and they found 40 cases of cellular fibroma characterized by four or more than mitoses per ten HPFs but with no or mild atypia and were classified as MACF. The entity had an uneventful clinical outcome except for local recurrence in three cases which they presented with ovarian surface adhesions or extraovarian involvement.

Since the first study published by Irving et al. in 2006, only three cases of MACF have been reported to the best of the authors’ knowledge. Kaku et al. [3] in 2007 reported a unique fibrous tumor of the ovary which showed two-circumscribed component. Of the component, there were 17 mitotic figures per 10 fields HPFs but no obvious atypia which consistedent with MACF. The patient had shown no evidence of the disease for one year after surgery. In 2009, Bucella et al. reported a case of MACF with recurrence occurring six years after primary surgery [4]. Monterio et al. [5] in 2012 reported a 13-year-old girl with significant ascites who was not given any additional treatment and was remained well with no signs of recurrence after 3 years of follow-up. These results further suggested that MACF was associated with a favorable outcome.

Considering the benign nature of MACFs, a more aggressive treatment was not recommended. However, in the study by Irving et al. [2], local recurrence was observed in a small proportion of cases with ovarian surface adhesions/rupture or extraovarian involvement. In addition, long-term local recurrence can also be seen in the MACF case reported by Bucella et al. [4], which showed no tumoral rupture or surgical difficulty. Thus, the long-term clinical follow-up is recommended.

Since current experience with primary ovarian MACF is so highly limited, treatment modalities are still not well established. Regular follow-ups with transabdominal pelvic ultrasound every six to 12 months seems reasonable. Besides, prognostic factors for MACF have also not yet been characterized, thereafter, further studies with a large number of cases are required for the identification of prognostic factors for ovarian MACF.

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Laparoscopic radical trachelectomy (LRT) with round ligament and ascending branches of uterine artery preservation: case report

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Summary
Fertility sparing surgery might be a benefit for young patients with early-stage cervical cancer. The authors herein describe a case of adenocarcinoma of cervix Stage IA2 treated with laparoscopic radical trachelectomy (LRT) with round ligament and uterine artery preservation.

Key words: Cervical cancer; Radical trachelectomy; Laparoscopy; Fertility.

Introduction
Although cervical cancer screening programs have resulted in a decline of cervical cancer incidence, there has been an increase in young patients with early-stage disease. It is estimated that 15% of all cervical and 45% of Stage IB cervical cancer were diagnosed in patients younger than 40 years of age [1].

To preserve fertility, radical trachelectomy is to remove the cervix, upper two cm of the vagina, and the parametrium in a similar manner to type III radical hysterectomy, except by sparing the uterine body.

The original technique is radical vaginal trachelectomy, initiated by professor Daniel Dargent in 1987 [2]. The tumor size suitable for radical vaginal trachelectomy is two cm or smaller. Radical abdominal trachelectomy, another technique, may result in wider parametrial specimen than in radical vaginal trachelectomy [3]. According to this advantage, the patient with tumor size two to four cm could be in the eligible criteria for radical abdominal trachelectomy [4].

The more recent technique, laparoscopic radical trachelectomy (LRT), was first described by Pomel et al. in 2002 [5]. Theoretically the radicality of parametrial resection is comparable between the radical abdominal trachelectomy and LRT. However the advantages of laparoscopy are better cosmetic wound, less pain, faster recovery, less blood loss, and less intra-peritoneal adhesion compared to the open technique. In addition the laparoscopic technique, by magnification, can offer the chance to preserve ascending branches of uterine arteries.

The aim of the present case report is to describe the authors’ experience in LRT for conservative treatment of early-stage cervical cancer.

Materials and Methods
A 33-year-old nulliparous woman came to the hospital with Pap test showing atypical glandular cells, favoring neoplasia (endocervical cell type). She underwent colposcopy, cervical biopsy, and endocervical curettage. Histopathology showed only chronic cervicitis. Loop electrosurgical excision procedure (LEEP) was performed and the histopathology showed adenocarcinoma, endocervical cell type, measuring 0.35 cm. in depth and 0.7 cm. in length. The endocervical and stromal resected margins were not free from adenocarcinoma. The magnetic resonance imaging at six weeks after LEEP showed a 4.9 x 3.2 mm. ill-defined lesion at mid anterior lip of endocervix about five mm above endocervical os. After counseling for all options of treatment, she decided to undergo LRT with bilateral pelvic node dissection.

The operative time was 340 minutes, including time for frozen section. Estimated blood loss was 700 ml with no other serious intraoperative complication. The specimen consisted of cervix measuring three cm. in length and two by two cm. in diameter, anterior and posterior vaginal cuff measuring one and 1.5 cm. with the right and left parametrium measuring three x 2.5 cm. Sections showed reactive changes without residual tumor. Fifteen right pelvic lymph nodes and six left pelvic lymph nodes were negative for metastatic tumor.

Postoperatively, the patient had urinary retention which lasted five weeks. Otherwise, the recovery was uneventfully. She had her period on the ninth day after surgery and had regular menstruation every month during follow up time. The new cervix healed satisfactorily. She had sexual intercourse at fifth month after surgery. However since she did not wish to become pregnant during this period, hence condom was used for contraception. During the 22-month follow-up period, history taking, physical examination, pelvic examination, and cervical Pap test revealed normal findings.
After general anesthetic method, the patient was in lithotomy position. The uterine elevator was applied to the cervix to help traction of the uterus. The ten-mm trocar was inserted at umbilicus and air insufflation was performed. After steep Trendelenburg position the two five-mm trocars were inserted to both sides of lower abdomen in the line between anterior superior iliac spine and umbilicus and two-finger breadths above the anterior superior iliac spines under direct visualization. The ten-mm trocar was inserted at the suprapubic area. The five-mm trocar was inserted at the left paramedian line about two-finger breadths below the umbilicus.

Right round ligament was elevated by grasper forceps and anterior leaf of broad ligament was opened and widened. Systematic bilateral pelvic lymphadenectomy, including deep inguinal, external iliac, internal iliac, and obturator nodes was performed. All pelvic nodes were sent for frozen section. LRT was initiated after the frozen section result of the pelvic nodes was negative for tumor. The right and left paravesical, pararectal, and vesico-uterine space were formed. The right ureter was identified and mobilized from the peritoneum down to where it crossed under the right uterine artery. By dissecting the right ureteric tunnel and the loose tissue between the right ureter and the right uterine artery, the right ureter was separated from the right uterine artery. The right uterine artery was preserved. The urinary bladder was extended caudally until the adequate margin of the upper vagina and laterally to identify the bladder pillar. The right ureteric tunnel was further unroofed up to the level where the ureter entered the urinary bladder. The left ureter was manipulated as the same manner. Then the peritoneum just above the rectum was incised by scissors to create the rectovaginal space. Then the peritoneum was incised laterally along both sacro-uterine ligaments and the rectum was separated from posterior vagina and bilateral sacro-uterine ligament. There was some adhesion at recto-vaginal septum due to endometriosis. After separating both ureters laterally, both sacro-uterine ligaments were divided close to the sacrum. Subsequently, bilateral parametriaums and paracolpiums were divided at two cm below to the tip of cervix. The descending branches of both uterine arteries were coagulated by bipolar forceps at the level of isthmus and the ascending branches were preserved. The uterine elevator was taken off and then replaced by vaginal tube to prevent $CO_2$ leakage. Subsequently, the vagina was incised by monopolar was taken off and then replaced by vaginal tube to prevent and the ascending branches were preserved. The uterine eleva-

![Figure 1. — Cervical specimen.](image)

**Figure 1.** — Cervical specimen.

**Discussion**

LRT is basically identical to laparoscopic radical hysterectomy except it preserves the body of uterus, bilateral round ligament, and bilateral ascending branches of uterine arteries. For this reason, LRT should theoretically yield adequate parametrium and vaginal cuff-like radical abdomi

nal tracheectomy. Although one of the advantages of laparoscopic surgery is the magnification of the laparoscope providing the chance to preserve the uterine artery, there were three reports of LRT that of all of these patients’ uterine arteries were severed [6-8]. However, there were other reports demonstrating LRT with uterine artery preservation [5, 9-10]. The authors use the advantage of magnification of laparoscope to save bilateral ascending branches of uterine arteries and normal menstrual pattern returned immediately. Although after bilateral uterine artery ligation, there are collateral circulations from ovarian vessels, most of the patients in the literature indicated normal menstrual pattern after radical tracheectomy. However, endometrial microcirculation and fertility capacity might be impaired after this procedure [11]. Ungar et al. reported that menstruation pattern did not return normal in six percent of the patients who underwent radical abdominal tracheectomy with coagulation of uterine vessels [12]. This would indirectly indicate that preservation of the uterine arteries may be beneficial for pregnancy outcome.

In the presented case the authors preserved round ligaments which are the one of the uterine supports. While some authors sacrificed the round ligament [10, 13, 14], Kim et al. preserved it [5].

The other advantages of laparoscopy are less pain, better cosmetic results, less blood loss, faster recovery [5], and less intraperitoneal adhesion [15] compared to open surgery. Less intraperitoneal adhesion should translate to the better fertility outcome that is the aim to preserve body of uterus.

To the authors’ knowledge, this is the first case report of laparoscopic radical tracheectomy with round ligament and ascending branches of uterine artery preservation in South East Asia. This case adds data to accumulating series demonstrating that preservation of round ligaments and ascending branches of uterine arteries is feasible in such treatment.
Conclusion
Combining with the results in the literatures the authors believe LRT is feasible and safe for selected cervical cancer patients.

References

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Placental site trophoblastic tumor on endometrial polyp: a case report

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Summary
Placental site trophoblastic tumor (PSTT) is the least common form of gestational trophoblastic disease (GTD), and is biologically different from other forms of GTD. There is a wide clinical spectrum of presentation and behavior ranging from benign conditions to an aggressive disease with a fatal outcome. The authors document a case of PSTT on an endometrial polyp. A 51-year-old woman had abnormal vaginal bleeding for the duration of two months. Her past history included a vaginal delivery in 1998. Her physical examination was normal. Tumor markers were at normal levels. Serum β-human chorionic gonadotropin (hCG) level was 19 mIU/ml and human placental lactogen (hPL) level was in the normal range. The patient underwent an operative hysteroscopy. On examination the uterine cavity appeared to be occupied by a pedunculated polyoid neoformation measuring about 2.5 cm in diameter which was removed and later determined to be a PSTT. There were occasional mitotic figures (0-1/10 high power field). The patient underwent hysterectomy and bilateral salpingo-oophorectomy. The patient has no evidence of disease six months after surgery. The authors conclude that a high mitotic count and atypical undifferentiated pathological features are significant poor prognostic factors for survival in PSTT. Hysterectomy represents the gold standard of treatment in all cases of disease confined to the uterus.

Key words: Placental site trophoblastic tumor; Gestational trophoblastic disease; Mitosis; Hysterectomy.

Introduction
Placental site trophoblastic tumor (PSTT) is a rare form of gestational trophoblastic disease that accounts for one to two percent of gestational trophoblastic disease (GTD) and is biologically different from other forms of GTD with approximately 200 cases reported in the literature [1-6]. There is a wide clinical spectrum of presentation and behaviour ranging from benign conditions to an aggressive disease with fatal outcome. While most cases confined to the uterus have a benign clinical course, some cases with metastasis are clearly associated with poor prognosis.

Case Report
In November 2011, a 51-year-old woman (gravida 1, para 1) was referred for recurrent uterine bleeding of two months’ duration. Her past history included a vaginal delivery in 1998 and after a full-term pregnancy of 40 weeks, she delivered a male infant.

Ultrasound examination showed an overhanging 2.5 cm heterogeneous mass in the uterine cavity with moderate to minimal blood flow with colour Doppler flow imaging (CDFI). The uterine adnexa were normal and the Douglas pouch was empty of fluids.

A diagnostic hysteroscopy was performed showed the presence of an endometrial pedunculated neoformation that was blackish in colour and taut in consistency. The biopsy sample histological examination of the neoformation proved to be an endometrial polyp.

The patient was readmitted one month later for metrorrhagia. On ultrasound examination, the uterus measured 82 x 53 x 69 mm. The endometrium was 18.8 mm at its thickest point and colour Doppler examination showed a poorly vascularised non-homogeneous ecosctructure. The left ovary was normal and there was a simple cyst in the right ovary. There were no abdominal fluids.

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the left annex and the right tube appeared within morpho-volumetric norms. The right ovary was the site of a three-cm cystic formation with regular walls. A frozen section was negative for neoplasia.

Histology was reviewed by an expert pathologist in the present centre and PSTT was not performed. The histological examination results showed: endometrium with proliferative aspects; chronic cervicitis; bilateral ovarian tissue with no neoplastic localisation; tubular tissue with no neoplastic localisation. A pre-operative β-hCG test showed 33 mU/ml (NR< 5 mU/ml).

The patient is on regular follow-up. Since the procedure, blood samples have been taken for β-hCG tests every 15 days. Only one of these samples resulted positive: β-hCG: 2.54 mIU/ml (NR< 2.4 mIU/ml). Clinical examination, pelvic abdominal sonography and control CT have shown no signs of local or systemic disease in the six months since surgery.
Placental site trophoblastic tumor on endometrial polyp: a case report

Discussion

PSTT is a rare form of GTD that originates from the implantation site of an intermediate trophoblast. It accounts for about one percent of all GTDs with an estimated incidence of one per 100,000 pregnancies [7]. PSTT was originally termed “atypical chorioepithelioma” by Marchand in 1895 [8]. In 1976, under the title “trophoblastic pseudotumour of the uterus”, Kurman et al. [9] recognized the entity as a form of trophoblastic disease, distinct from choriocarcinoma. Five years later, Scully and Young [10] introduced the term “placental site trophoblastic tumour” to indicate possible malignant behaviour.

Although the age of most patients at presentation was reproductive age [11,12], a few cases have been reported in post-menopausal women [13].

The mean age at diagnosis is 31 to 33 years, and the disease can appear following any type of pregnancy [14, 15]. The antecedent pregnancy is full term normal in 53% of the cases [12, 15] or molar pregnancy seen in 21% of cases [12, 16]. The mean interval from the last pregnancy and diagnosis of PSTT can vary from several weeks to up to 15 years [11].

Unlike choriocarcinoma, the level of serum $\beta$-hCG in PSTT correlates with neither tumor burden nor the malignant behavior. $\beta$-hCG thus appears to have no predictive value and the disease may still progress even if levels are not raised [3, 11].

Irregular vaginal bleeding is the most common presenting feature, although a wide range of other symptoms has also been reported, including galactorrhea, virilization, nephritic syndrome, and polycythemia [17].

The outcome of PSTT as reported in literature is highly variable [18]. All cases of metastasis to vital organs, such as the brain, result in mortality despite all forms of treatment.

In the majority of cases, PSTT behaves in a benign fashion, with only about 10-15% being clinically malignant.

Extraterine spread of the disease appears to be the most useful prognostic factor for progression [19,20]. The interval from the last known antecedent pregnancy appears to be a second major prognostic variable in PSTT. In a multivariate analysis, the risk for unfavorable behavior of the disease increased considerably with the length of this interval [18,21]. Diagnosis less than two years from the antecedent pregnancy, and the disease localized to the uterus, are associated with better outcomes [12,18]. How et al. found that the likelihood for fatal outcome was 14 times higher if the mitotic count was well above 5 [20]. Baergen et al. [2] reporting on 55 cases, showed that a high mitotic rate was more likely to be associated with metastasis or death. They noted that mitotic count was one of the strong predictors of survival with 88% to 100% of surviving patients having mitotic counts of less than 2.5/10 HPFs versus 48% to 52% survival in those patients with mitotic counts greater than 6/10 HPFs.

<table>
<thead>
<tr>
<th>TNM Classification</th>
<th>FIGO Staging system</th>
<th>Description</th>
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<tr>
<td>TX</td>
<td>Primary tumor cannot be assessed</td>
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<tr>
<td>T0</td>
<td>No evidence of tumor primary</td>
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<tr>
<td>T1</td>
<td>Disease limited to uterus</td>
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<tr>
<td>T2</td>
<td>Disease outside of uterus but limited to genital structures (ovary, tube, vagina, and broad ligaments)</td>
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<td>M1a</td>
<td>III</td>
<td>Lung metastases</td>
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<td>M1b</td>
<td>IV</td>
<td>All other distant metastasis</td>
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On first being hospitalized, the present patient presented recurrent uterine bleeding and other significant adverse prognostic factors, such as interval from antecedent pregnancy > two years and age > 40 years.

On a subsequent second hospitalization, histological examination revealed the presence of PSTT on an endometrial polyp.

Generally studies conducted to establish a general staging of PSTT (Table 1) revealed that 65.5% of patients are affected by the disease confined to just the uterus, 11.8% extension to the pelvis (Stage II), 15.1% with lung metastases (Stage III), and 7.6% with metastases in other sites (Stage IV).

In contrast to choriocarcinoma, PSTT is relatively resistant to chemotherapy. Consequently surgery is the mainstay of treatment. In other cases the treatment of metastases can be successful through chemotherapy, although some PSTT cases exhibit fatal course even with treatment.

In conclusion, PSTT is difficult to diagnose by its symptoms and diagnosis is usually delayed.

High mitotic count and atypical undifferentiated pathological features are significant poor prognostic factors for survival in PSTT. The only approach at present remains hysterectomy which represents the gold standard of treatment in all cases of disease confined to the uterus.

References


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Diagnostic laparoscopy identifies a peritoneal adenomatoid-like mesothelioma masquerading as ovarian cancer: a case report

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Summary

The authors report a rare case of peritoneal adenomatoid mesothelioma in a woman with no history of asbestos exposure. A 61-year-old woman was originally suspected of having a bilateral ovarian tumor based on chest radiography and magnetic resonance imaging (MRI). Upon referral to our hospital, the presence of two solid masses was confirmed by enhanced MRI and ¹⁸F-fluorodeoxyglucose positron-emission tomography/computed tomography (¹⁸F-FDG-PET/CT). Physical examination was normal, as were serum concentrations of the tumor markers CA 19-9, CA 125, and CEA. Laparoscopic surgery showed a right ovarian tumor and laparoscopic right salpingo-oophorectomy and adhesiometry were performed. Two months later, the patient underwent laparoscopic segmental resection of the sigmoid colon, with histological analysis identifying an adenomatoid-like tumor. The final diagnosis was peritoneal adenomatoid-like mesothelioma with invasion of the right ovary. This case report demonstrates that imaging techniques must be coupled with laparoscopic surgery for an accurate diagnosis of peritoneal mesothelioma.

Key words: Laparoscopic surgery; Mesothelioma; Adenomatoid-like tumor.

Introduction

Mesotheliomas are aggressive tumors arising from serous surfaces, including the pleura (65%–70%), peritoneum (30%), tunica vaginalis testis, and pericardium (1%–2%) [1]. The main cause of malignant mesothelioma is exposure to asbestos. Its current incidence in the United States is 2,500 patients per year, with an expected worldwide peak in 2020, reflecting the increased use of asbestos in the second half of the 20th century [2].

Mesothelioma is generally diagnosed by imaging methods, including computed tomography (CT), magnetic resonance imaging (MRI), and ¹⁸F-fluorodeoxyglucose positron-emission tomography/computed tomography (¹⁸F-FDG-PET/CT). A definitive diagnosis requires laparoscopy or open surgery with biopsy, to obtain tissue for histological and immunocytochemical analyses. Consequently, the diagnosis can be easily overlooked, especially in patients with no previous exposure to asbestos.

The authors describe a woman with no previous exposure to asbestos, who was preoperatively diagnosed with bilateral ovarian tumor, but, after laparoscopic surgery, was found to have a rare peritoneal adenomatoid mesothelioma.

Case Report

A 61-year-old woman visited a hospital for treatment of left coxarthrosis. MRI suggested a bilateral ovarian tumor based on chest radiography and magnetic resonance imaging (MRI). Upon referral to our hospital, the presence of two solid masses was confirmed by enhanced MRI and ¹⁸F-fluorodeoxyglucose positron-emission tomography/computed tomography (¹⁸F-FDG-PET/CT). The size and position of the masses were confirmed by both techniques, but were significantly more visible on MRI than on ¹⁸F-FDG-PET/CT (Figures 1A-B). On the basis of this information, there was no reason to doubt the diagnosis of bilateral ovarian cancer. Nonetheless, they conducted additional tests to determine the spread of the cancer to other tissues. Because malignant bowel obstruction is a common complication of ovarian cancer, the patient was examined by gastroscopy and fibroscopy, but these tests showed no evidence of any gastrointestinal tumor. Furthermore, serum concentrations of the tumor antigens CA 19-9, CA 125, and CEA were within normal limits, which did not support a diagnosis of cervical carcinoma. Based on these results and her clinical course, this patient could not be diagnosed with malignant ovarian tumors.

Because non-invasive approaches could not clarify the diagnosis of this patient, the authors performed laparoscopic surgery to remove the tumors and identify the type of cancer. First, a laparoscopic right salpingo-oophorectomy (RSO) was performed, with histopathological analysis identifying an adenomatoid tumor (Figures 2A, B). The left ovary was found to adhere to the sigmoid colon. An adhesiometry was performed, and the left ovary was found to be atrophic. Moreover, a sigmoid colon...
tumor, preoperatively diagnosed as a left ovarian tumor, was found to be present (Figures 2C, D). The patient was discharged from the hospital four days after the operation. Two months later, she underwent a laparoscopic sigmoid colon segmental resection. After discharge, she received no additional therapy.

The tissue specimens collected during the two surgical procedures were analyzed to identify the type(s) of cancer. Microscopic examination revealed that the right ovarian tumor and the colon tumor were both solid yellowing masses (Figures 3A, B). Specimens from each tumor were fixed in formalin, stained with hematoxylin and eosin, and examined by light microscopy. Histologically, both tumors had characteristics of adenomatoid tumors, with a typical microcystic pattern accompanied by a papillary structure and microcystic pattern (Figures 3C, D). The tumors were therefore diagnosed as adenomatoid tumors. Immunohistochemical assays showed that the tumors were weakly positive for epithelial membrane antigen (EMA), a marker for mesothelioma. The tumors were also positive for the mesothelioma marker calretinin (Figure 3E), which is also a good marker of the mesothelioma and weakly positive for Ki-67, a marker of cell proliferation (Figure 3F) [3, 4].

Based on these findings, the authors diagnosed this patient as having a peritoneal adenomatoid-like mesothelioma, which was surprising, since this patient had no history of asbestos exposure. The right ovarian tumor was diagnosed as a metastatic lesion.

**Discussion**

Mesothelioma can be macroscopically divided into localized and diffuse types. The localized type is usually benign, whereas the diffuse type is malignant. However,
Figure 3 – Microscopic findings. The resected tissues were fixed in 10% formalin. Both the right ovarian (A) and left colon (B) tumors were solid and yellowish in color. Histologically, the right ovarian tumor (C) and colon tumor (D) were similar in that both had microcystic patterns accompanied by a papillary structure. Immunohistochemically, the tumors were weakly positive for EMA and calretinin (E) and positive for Ki-67 (F).
transformation of localized to diffuse mesothelioma has been reported [5-7]. Moreover, localized mesotheliomas have the potential of malignant transformation [8].

Histologically, mesotheliomas are classified as epithelioid, sarcomatoid, and biphasic types. Some variant forms are recognized, including multicystic mesothelioma, adenomatoid tumor, desmoplastic-type mesothelioma, and well-differentiated papillary mesothelioma [9]. Immunohistochemical analysis is required for differential diagnosis.

Adenomatoid mesothelioma is a usual variant of epithelioid malignant mesothelioma that may mimic other tumors histologically, including benign adenomatoid tumors [10]. Adenomatoid tumors are usually located in the genitourinary tract and are often detected incidentally during pelvic surgery [11]. Since adenomatoid mesotheliomas look very similar to other adenomatoid lesions, adenomatoid mesotheliomas have been so designated because of their resemblance to adenomatoid tumors of the genital tract [12]. Diffuse and multicystic adenomatoid lesions are thought to be malignant mesothelioma, whereas local and simple tumors are thought to be adenomatoid tumors.

Accurate diagnosis of diffuse malignant mesothelioma requires a comprehensive diagnostic workup, including assessments of its gross appearance, histology, histochemistry, immunocytochemistry, and electron microscopy [13]. Pathologically, positive immunostaining for calretinin and EMA are considered markers of malignancy when differentiating epithelioid malignant mesothelioma and mesothelioma-in-situ from reactive mesothelial hyperplasia [14]. Ki-67 is strictly associated with cell proliferation [4]. The right ovarian tumor in the present patient was clearly positive for EMA and weakly positive for calretinin and Ki-67. This patient was diagnosed immunocytochemistry as a tumor of low malignant potential and therefore did not receive adjuvant therapy. The right ovarian tumor may have been a metastatic lesion of the left colon tumor, although both may have appeared simultaneously.

Mesothelioma is difficult to diagnose, although it can be diagnosed by histopathological examination of the sample taken during laparoscopy [15-17]. Laparoscopy is therefore an important tool in the diagnosis of primary mesothelioma. In contrast, other diagnostic modalities, such as ultrasonography, CT, and cytology of the ascites fluid, are less accurate. Since laparoscopy can complicate management by facilitating tumor dissemination, non-invasive imaging should be performed prior to laparoscopic surgery [18].

Conclusion

The authors report a patient with a malignant adenomatoid methothelioma who was diagnosed after laparoscopic surgery by immunocytochemistry. Laparoscopy is an important tool in the diagnosis of mesothelioma.

References

Bilateral Krukenberg tumor in a 16-week pregnant woman

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Summary
The authors present the case of a G2P1001 who presented in 16-week gestation with bilateral Krukenberg tumor, abdominal pain, and iterative vomiting episodes. Although a few cases of Krukenberg tumor in pregnant women have been reported, no case reports asymptomatic and free of disease at 18 months were found in the English literature. Early detection followed by surgery and chemotherapy during pregnancy could possibly result in a favorable outcome in such patients.

Key words: Chemotherapy; Krukenberg tumor; Pregnancy.

Introduction
Only a small minority of adnexal masses detected at pregnancy is malignant. Krukenberg tumor refers to gastrointestinal cancer metastatic to the ovaries and accounts for one to two percent of all ovarian tumors [1]. Thus they are a diagnostic and treatment challenge to the physician. The authors present a case report of a woman 16 weeks pregnant with bilateral ovarian tumors, abdominal pain, and iterative vomiting episodes.

Case Report
A 31-year-old woman, gravida 2 para 1, was admitted to the present hospital with intensive abdominal pain and iterative vomiting episodes. The patient complained of mild bloating and early satiety for one year before pregnancy, and these complaints were attributed to gastritis and gastroesophageal reflux. Thus, one year ago she underwent gastric endoscopic biopsies and the pathology result was erosive gastritis. The patient received antacid medication.

The physical examination, radiological imaging, and ultrasound examination showed mobile tumors in both ovaries. Transabdominal color Doppler examination showed bilateral asymptomatically encapsulated solid ovarian masses, with the right ovary measuring 15.5 x 12.5 x 7 cm and the left ovary measuring 9.6 x 5.7 x 6.3 cm. Color Doppler ultrasonography showed few vascular structures inside the ovarian masses. Pelvic magnetic resonance imaging revealed a gravid uterus and bilateral adnexal mass lesions which were hypointense in T1-weighted images and hyperintense in T2-weighted images (Figure 1).

Because of suspected ovarian semitorsion, the authors performed urgent surgery. At laparotomy, bilateral ovarian tumors and ascites were identified. No visceral metastases were noted. The right ovary and fallopian tube were removed and a wedge biopsy was taken from the left ovary. Histological examination of the specimen yielded the diagnosis of Krukenberg tumor. Upper gastrointestinal endoscopy and sigmoidoscopy were performed to locate the primary tumor. This revealed a small malignant ulcer with a diameter of 12 mm at the corner of the greater curvature of the stomach. A biopsy was performed and microscopic examination of the stomach specimens revealed an epithelial malignant tumor. At the postoperative period, patient’s family did not consent to terminate the pregnancy. Thus, the authors began postoperative chemotherapy in the 26th week with two cycles of docetaxel (75 mg/m²), carboplatin (five AUC) and 5-fluorouracil (500 mg/m²) every three weeks. At 33 weeks, the patient underwent an elective cesarean delivery and left oophorectomy (There was a ten-cm tumor in left ovary). A virilized female infant weighing 1,850 g was delivered by cesarean section with Apgar scores of 6 and 8 at one and five min, respectively (Figure 2). Beginning at two weeks after surgery, the patient was given five cycles of the same chemotherapy drugs schedule every four weeks. No metastases were detected after chemotherapy. However, the primary gastric neoplasm did not recover. Therefore, the patient underwent total gastrectomy.

Discussion
Krukenberg tumors in pregnancy are very rare and their management can present a dilemma for the obstetrician and gynecologist. Krukenberg tumor refers to a gastrointestinal cancer metastatic to the ovaries and accounts for one to two percent of all ovarian tumors [1]. The persistent gastrointestinal symptoms mimicking the early nausea and vomiting of pregnancy mask the presentation of a tumor in the stomach. Growth of the fetus leading to abdominal distension masks the presence of the metastatic ovarian tumor in the pelvic cavity. For this reason, early diagnosis of the tumor may be delayed [2, 3]. Persistent unusual gastrointestinal symptoms require careful evaluation by ultrasound and panendoscopic examination. Fetal virilization may occur during pregnancy as the result of advanced malignant disease and ovarian Krukenberg tumor. Mechanism of the androgen overproduction in this exceptional condition is still poorly understood [4].

Tamussino et al. reported that the cornerstone of the management of these tumors is the localization of the gastrointestinal primary tumor, the prognosis being worsened when the primary tumor is identified after the ovarian metastasis is discovered [5]. However, 18

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months passed since the initial diagnosis of metastatic gastric carcinoma in the presented patient and she remains asymptomatic and free of disease. This unexpectedly favorable clinical outcome suggests that a patient may benefit from resection of gastric carcinoma metastatic to ovary when no other sites of metastatic disease are present and the patient receives chemotherapy with invasive surgery.

Early detection followed by surgery and chemotherapy could possibly result in a favorable outcome in such patients.

References


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A 29-year-old woman with complex atypical hyperplasia and polycystic ovary syndrome: a challenging issue

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Summary

Background: The standard treatment for complex atypical hyperplasia is hysterectomy and bilateral salpingo-oophorectomy. Although radical surgery offers high survival prospects, it also eliminates any chance of further fertility, thus in young nulliparous women who wish to preserve their childbearing potential, a conservative progestin therapy is preferable. Case report: The authors report a case of complex atypical hyperplasia in a 29-year-old nulliparous woman with polycystic ovary syndrome treated with norethisterone acetate in order to preserve her childbearing potential. The specimens sampled during the follow-up demonstrated inactive endometrium with pseudodecidual changes and no ultrasonographic images exhibited abnormal endometrial thickness. Conclusion: According to literature and to the authors' experience, they can affirm that progestin treatment is the most reasonable option for young nulliparous women affected by complex atypical hyperplasia who desire to maintain their fertility potential, showing its efficacy also in patients with an associated polycystic ovary syndrome.

Key words: Complex atypical hyperplasia; Uterus-sparing management; Norethisterone acetate; Young woman; Polycystic ovary syndrome.

Introduction

Endometrial carcinoma (EC) is the most common gynecological malignancy in western countries. Typically the disease is referred in postmenopausal women, but 25% of cases occur in premenopausal women with 2.5-14.4% under the age of 40 [1]. Endometrial hyperplasia (EH) is an abnormal proliferation of the uterine endometrial glands due to unopposed estrogen exposure and is classified as simple or complex, with or without cytological atypia [2]. Complex atypical hyperplasia (CAH) is estimated to progress in 15%-75% of patients if left untreated [3]. Moreover, invasive endometrial cancer can already be found in approximately 40% of patients initially diagnosed with CAH [1]. The occurrence of the disease at young age can be related to several conditions associated to prolonged unopposed estrogen exposure, such as obesity, nulliparity, and polycystic ovary syndrome (PCOS) [4] and chronic anovulation [1, 5]. It can be also related to exogenous estrogen exposure as hormone therapy [6]. Furthermore, the association between the excessive estrogen of obesity, PCOS, ovarian stimulation, endocrine disruptors, and CAH/EC with the growing spread of obesity, assisted reproductive technology (ART) practice and pollution suggests that the incidence of the endometrial neoplasm will likely increase in the future. Although the standard surgical procedure to treat CAH and EC is hysterectomy and bilateral salpingo-oophorectomy, for young nulliparous women a conservative hormonal therapy should be considered [7]. Different progestins can be used such as norethisterone acetate, megestrol acetate, and medroxyprogesterone acetate. Megestrol acetate or medroxyprogesterone acetate are the most commonly used [1, 8]. Intrauterine device-delivered progestin (IUD) is another option that seems promising in offering control of the disease, and has been successfully used in the prevention and treatment of endometrial hyperplasia [3, 8-10], but there is not sufficient evidence to recommend it as the treatment of choice [9]. Many studies in literature have investigated the efficacy of progestins as a primary therapy for EH and EC [1-3, 5, 7, 8, 10-16]. The authors report a case of CAH in a young woman with PCOS treated with norethisterone acetate in order to preserve her childbearing potential.

Case Report

In April 2009, a 29-year-old nulliparous woman with polycystic ovary syndrome came to the authors’ observation for a significant and worsening menometrorrhagia of 20 days duration, although an oral administration of tranexamic acid was prescribed for a week. She had menarche at the age of 12 and her menstrual cycles were irregular since then. Her weight was 58 kg (BMI 19.84 kg/m2) and she denied a history of previous surgeries and smoking, but her paternal grandmother died of uterine cancer. At the gynecologic examination, only the presence of blood within the vaginal cavity was found and there was no evidence of pelvic pain. A transvaginal ultrasound revealed a regular-sized uterus, but a thickened endometrial lining of 6.2 mm, abnormal for the phase of the cycle, with irregular ultrasonic echo signal and ovaries with polycystic appearance. Thus, an office hysteroscopy and an endometrial biopsy were carried out, which showed polypoid-like features of the uterine cavity mixed with blood clots and the histological examination of her specimen was reported as CAH. The possible progression from CAH to EC should suggest the need of a radical hysterectomy, but considering patient’s age and her strong desire to preserve fertility, a conservative management was proposed. Therefore, a transvaginal ultrasound was performed to rule out myometrial invasion and concurrent ovarian lesions and a medical treatment with
norethisterone acetate (10 mg/die from the 14th to the 24th day of the menstrual cycle) for six months was carried out. Two vaginal ultrasounds, three and six months after the beginning of the therapy, were performed revealing a thin endometrium and she observed menstrual blood loss dramatically decreased. Moreover, at the sixth month, at the end of the therapy, an endometrial cytology was conducted showing numerous samples of proliferative endometrium with no signs of atypia. Then the patient stopped the hormonal therapy for the following six months, but as she stopped the treatment she experienced episodes of irregular uterine bleeding. One year later from the diagnosis and six months after the end of the therapy (2010), during the follow-up, a transvaginal ultrasound performed at the 14th day of the menstrual cycle showed an endometrial lining of 6 mm with non-homogeneous pattern. Then a second diagnostic hysteroscopy and an endometrial biopsy were performed, with the result of a proliferative endometrium according to the phase of the cycle and tiny fragments of a hyperplastic polyp. Thus, the authors decided to administer norethisterone acetate for other six months. After this second cycle of therapy, she stopped the progestins again and six months since the end of the therapy, and two years since the diagnosis (2011), the thickness of the endometrial lining, detected with a vaginal ultrasound, was 6.3 mm with a homogeneous pattern, but she complained heavy menstrual period and the authors decided to administer norethisterone acetate for other six months. In 2012, she underwent a third hysteroscopy with an endometrial biopsy, which showed a uterine cavity with hypertrophic and richly vascularized endometrium with a polypoid aspect. The histological examination of the specimen detected a fragment of endometrial polyp characterized by the presence of pseudodecidualized stroma and glands with proliferative aspects. The successive follow-up controls and transvaginal ultrasounds conducted every six months during the first year after the end of the hormonal therapy were negative, she had not complained of menometrorrhagic periods in the following months and she had not undergone any other hormone therapy.

Discussion

Young women with CAH are frequently nulligravid with a history of infertility and a strong desire to preserve their fertility potential. Hysterectomy with bilateral salpingo-oophorectomy is not acceptable in these young patients and should be performed in women after completion of childbearing, in patients with co-morbidities precluding surgery or in those without an appropriate response to the hormonal therapy. Hence, in young women with CAH or grade 1 EC who wish to preserve fertility, non-surgical management is preferable [7, 8, 11, 12]. Several studies indicate that 60%-80% of premenopausal women with CAH and grade 1 EC respond to progestin treatment [3, 10, 16]. In a study conducted in 2012, 15 fertile women with a diagnosis of early-stage EC or CAH were treated with megestrol acetate (80-160 mg/daily) or medroxyprogesterone acetate (500-1000 mg/daily) for at least 12 weeks. Among them, 11 women had a complete pathological remission of the disease and 4 pregnancies were attained in 4 women. Three of them showed progression and underwent definitive surgery within 18 months, one underwent hysterectomy after 24 months and showed no response after three cycles of progestins [7]. A systematic review and metaanalysis recently conducted by Gallos I.D. et al., considering the regression, relapse, and live birth rates of early-stage EC and CAH with fertility-sparing treatment, noticed that the conservative approach was a safe and valid option [12]. Several studies evaluated women with CAH, comparing those with prescribed progestin to those with-not prescribed progestin, showing that the regression of the disease was greater in the first case than in the second one [2, 13]. However, uterine-sparing treatment with progestins bears the risk of progression and relapse [2, 3, 5, 12, 17, 18] and some cases of conservatively treated CAH of the endometrium have progressed to high stage EC [5, 17, 18]. Fertility-preserving therapy followed by ART can be a good option in selected patients with fertility desires [14]. ART gives the chance of a pregnancy and minimize the time before a hysterectomy, which could prevent them from relapse [7, 12]. Pregnancy itself is a highly effective treatment, with the endocrine placenta as a natural continuous progestin source [7, 12]. The management of these patients is a dilemma for clinicians: there is uncertainty about the treatment regimen (progestational agent, dose, schedule, and clinical outcomes) [2, 11, 12, 18], the follow-up [11, 12] and there are no good pathologic indicators that are predictive of response [2, 18]. In general, progestin treatment should be continued for no less than six months [3]. In the authors’ experience, they performed an interrupted therapy (every six months) and in literature there is some evidence that interrupted, rather than continuous, progestin therapy may added benefit by inducing the highest apoptotic death rates [19]. It should be noted that the development of EH in the presented 29-year-old patient was supposedly related to the excessive estrogen of PCOS and to the shortness of her luteal phase with a scarce exposure of the endometrium to the progestin action. Indeed, PCOS may be associated with chronic anovulation and EH and there is an increased risk of EC through chronic unopposed estrogen production [8]. In patients with PCOS and CAH, it has been demonstrated that progestin therapy is particularly feasible and effective not only for reducing the menstrual blood loss, but also for improving the pathological findings [8], thus having positive effects, both on EH and PCOS. In summary, this case report shows that premenopausal women with CAH who are strongly motivated to preserve their childbearing potential may be treated successfully with progestins alone as primary therapy.

Conclusion

Although there are no guidelines regarding the use of progestins and most of the studies do not report a long follow-up after the hormonal therapy, the authors believe that the most reasonable option for young nulliparous women affected by CAH should be the progestin treatment, which can be also useful to treat patients with an associated PCOS, producing positive effects on both diseases. Moreover, conservative therapy followed by ART can be a good option in selected patients with fertility desires, while hysterectomy...
with bilateral salpingo-oophorectomy is recommended in women who have completed their childbearing or if fertility-sparing treatment has failed. Type and duration of the progestin treatment should be decided depending on the characteristics of the patients. Although the risk of progression to carcinoma during the therapy is low, before starting the treatment, it is crucial to exclude extraterine disease. Women should be informed about the risks, such as the progression of the disease during hormonal therapy and a close follow-up, including clinical examination, endometrial biopsies, transvaginal ultrasound and/or magnetic resonance imaging is mandatory to monitor any failure and to change into alternative, usually surgical, therapies.

References


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Primary carcinoid tumor of the ovary arising in a mature cystic teratoma: a case report

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Summary

Primary ovarian carcinoid tumors are rare entities, they may appear with other teratomatous components, and can be often being mistaken as part of mature cystic teratomas. Consistent with their rarity and low incidence, imaging clues that could have led to suspicion of this tumor are not well-documented. Herein, the authors present a rare case of primary ovarian carcinoid tumor in a mature cystic teratoma, who initially presented with complaints of abdominal distension for months. Contrast-enhanced computerized tomography (CT) demonstrated a multilobular mass with different density components including fat, soft tissue, and calcification materials, as well as rich vascular supply from the right ovarian vein. Serum tumor markers were within normal limits. Bilateral salpingo-oophorectomy was performed and the pathological diagnosis was mature cystic teratoma with coexisting primary ovarian carcinoid tumor, insular type. The patient has remained well with no residual disease for over one year of follow-up.

Key words: Carcinoid tumor; Computerized tomography; Teratoma.

Introduction

Ovarian carcinoid tumors are rare, accounting for only 0.3 to one percent of all carcinoid tumors, and 0.1% of all ovarian neoplasms [1]. They may appear with other teratomatous components and can be often be neglected as part of mature teratomas [2]. Few women may present with teratomatous ovarian masses that are not properly diagnosed as carcinoid tumor until the time of histopathological analysis. Prior knowledge of correlated images of ovarian carcinoid tumor may be helpful to arouse alert of coexistent malignant component before operation. Nonetheless, literature that described detailed image findings of primary ovarian carcinoid tumors is sparse [3-8]. Herein, the authors present a rare case of primary ovarian carcinoid tumor in a mature cystic teratoma, and describe its detailed findings on computed tomography (CT).

Case Report

A 72-year-old woman experienced abdominal distension with bearing down sensation for several months. Ultrasonography showed a huge heteroechoic cystic pelvic tumor with acoustic shadows. Contrast-enhanced CT showed a right ovarian multilobulated mass with rich vascular supply from an engorged and tortuous right ovarian vein (Figure 1). Solid parts with miniscule streaks of vascular enhancement, calcification, and fat density were also found within the tumor (Figure 2). Serum tumor markers were within normal limits (carcinoembryonic antigen, 2.7 ng/ml; cancer antigen 125, 19.66 U/ml).

Exploratory laparotomy revealed a right well-circumscribed ovarian tumor (11 cm × 9 cm × 9 cm) with solid and cystic parts. The cystic part contained greasy, sebaceous-like material, and hair- and teeth-like structures, whereas the solid part showed tan-brown speckles with focal hemorrhage but no visible necrotic change (Figure 3). Owing to its mature teratomatous appearance, bilateral salpingo-oophorectomy was performed. The uterus and the left ovary were atrophic. Histopathological examination revealed right ovarian mature cystic teratoma with coexisting primary ovarian carcinoid tumor of the insular type (Figure 4). The carcinoid tumor cells stained positive for cytokeratin, synaptophysin, and chromogranin A. The patient has remained in good health without tumor recurrence at one-year follow-up.

Discussion

The appearance of ovarian carcinoid tumors on various imaging modalities is not characteristic enough for easy distinction from other solid tumors. Takeuchi et al. described ovarian carcinoid tumor as a solid component of homogenous soft tissue enhancement on CT [3]. On contrast-enhanced CT scans, the hypervascularity of carcinoid tumors can be observed as intense enhancement [3]. The isoattenuating density of the ovarian solid part compared to that of the psoas muscle, right tortuous and engorged ovarian vein (Figure 1), and miniscule streaks within the solid part (Figure 2) found in the presented case may represent the clinical characteristics of hypervascularity, indicating malignant ovarian tumor.

In the authors’ knowledge, only a few case reports described their vague imaging findings of coexistent ovarian carcinoid tumors in mature cystic teratomas (Table 1) [5-8]. Most authors described their ovarian neoplasms with calcification and fat contents. Only one case report mentioned about adnexal mass with the solid component, which was later confirmed as the carcinoid tumor [5].
Figure 1. — Computerized tomography revealed a multilobulated mass with different density components. The tortuous and engorged ovarian vein (white arrows) courses along the right ureter and eventually ends in the tumor mass.

Figure 2. — Miniscule streaks of vascular enhancement (white arrows) are seen within the solid component.

Figure 3. — Right ovarian tumor with a whitish-yellow solid component and a cystic, greasy component.

Figure 4. — Histopathological examination of the right ovarian carcinoid tumor revealed uniform, monotonous tumor cells arranged in an organoid manner, insular to focally trabecular patterns (H&E, x100).

Table 1. — Clinical features of patients with an ovarian carcinoid tumor in a mature cystic teratoma.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Authors</th>
<th>Age (years)</th>
<th>Clinical presentations</th>
<th>Image findings</th>
<th>Management</th>
<th>Follow-up period</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Guney et al. [5]</td>
<td>54</td>
<td>Abdominal fullness, abnormal uterine bleeding</td>
<td>US: right ovarian cystic mass with solid components CT: right adnexal mass of solid and cystic components with calcifications</td>
<td>TAH, BSO</td>
<td>3 months</td>
<td>NED</td>
</tr>
<tr>
<td>2</td>
<td>Torre et al. [6]</td>
<td>76</td>
<td>Abdominal fullness</td>
<td>US: left ovarian tumor CT: left ovarian tumor with invasion to the uterine serosa</td>
<td>TAH, BSO, Pelvic LN dissection</td>
<td>70 months</td>
<td>NED</td>
</tr>
<tr>
<td>3</td>
<td>Kester et al. [7]</td>
<td>25</td>
<td>Urinary frequency, urgency, nocturia</td>
<td>US: left ovarian tumor CT: a left ovarian mass with fat and calcification</td>
<td>Left oophorectomy</td>
<td>NA</td>
<td>NED</td>
</tr>
<tr>
<td>4</td>
<td>Ansell et al. [8]</td>
<td>54</td>
<td>Bilateral ankle edema, elevated jugular vein pressure, systolic murmurs</td>
<td>US: right ovarian mass CT: dermoid cyst-like ovarian mass</td>
<td>TAH, BSO, mesenteric LN biopsy, appendectomy</td>
<td>8 months</td>
<td>NED</td>
</tr>
</tbody>
</table>

*BSO: bilateral salpingo-oophorectomy; CT: computerized tomography; LN: lymph node; NA: not available; NED: no evidence of disease; TAH: total abdominal hysterectomy; US: ultrasound.
Only one-third of the insular type ovarian carcinoid tumors are symptomatic with flushing, diarrhea, bronchospasm, or dyspnea [9]. Therefore, diagnosis of carcinoid tumors based on symptom presentations is not reliable.

Although an ovarian tumor with hair, bone, and sebaceous contents is usually believed to be a mature teratoma; however, any concomitant ovarian cystic tumor with solid component and/or hypervascularity on CT scan should prompt the consideration of intraoperative frozen section examination, which was reported to be useful in determining the nature of any suspicious ovarian lesions with a good sensitivity and specificity of 91.2% and 98.6%, respectively [10]. Despite the good prognosis of ovarian carcinoid tumor, intraoperative confirmation of carcinoid tumor can prompt immediate gynecologic staging surgery without delay [11]. Aggressive debulking surgery is advised, followed by standard post-operative work-up including gut hormone analysis and radionuclide scintigraphy with Octreoscan [12].

Conclusion

In conclusion, carcinoid tumor and ovarian mature cystic teratoma may exist concomitantly, and prompt intra-operative investigation (such as frozen section examination) should be performed in cases of ovarian tumor with solid component and hypervascularity on CT scan despite their mature teratomatous appearance.

References


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Foreword

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

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

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

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

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

M. MARCHETTI

Contents

Chapter 1: Epidemiology of Ovarian Cancer: An Update
Jennifer Permuth-Wey, Andrea Besharat, Thomas A. Sellers

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

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

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

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

The 14 chapters of this textbook thoroughly examine all the “aspects” related to prevention and early detection. From the general information on this neoplasia, through primary prevention, HIV infection, risk factors, methods of screening, study of biomarkers, organization of training for personnel involved in screening programs, to the general instruction for prevention, this manual offers a complete contribution to improve women’s health.

Contents


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

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


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

Chapter 7: Cervical cytology and alternative methods of screening. A. Longatto-Filho.

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Chapter 10: Basic concepts of quality and accreditation in Health Care Services. M. Branca.


Chapter 13: Instruction and training of personnel in a cervical cancer screening program. M. Branca and A. Longatto-Filho.

Chapter 14: Universal hygienic measures and precautions for infection prevention in gynecological ambulatory centers and hospitals. M. Branca.

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

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