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Robotic-assisted laparoscopic anterior pelvic exenteration in patients with advanced ovarian cancer: Farghaly’s technique

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Summary

The safety and efficacy of the robotic-assisted laparoscopic approach to anterior pelvic exenteration is evaluated in patients with advanced ovarian cancer undergoing anterior pelvic exenteration for involvement of the urinary bladder during primary cytoreduction surgery. All patients undergo preoperative lab work, imaging studies and bowel preparation prior to surgery. The Davinci surgical system is used to perform urinary cystectomy, total hysterectomy, bilateral salpingo-oophorectomy, bilateral pelvic adenectomy (including obturator, hypogastic, external iliac, and common iliac lymph nodes). In addition, debulking to less than 1 cm is performed. The anterior pelvic exenteration procedure involves wide perivesical dissection. Then the robot is locked, and ileal conduit is performed via a 6 cm lower midline incision. Operative time can be maintained in 4.6 hours with a mean blood loss of 215 ml and hospital stay of five days. Farghaly’s technique of robotic-assisted laparoscopic anterior pelvic exenteration in patients with advanced ovarian cancer is safe, feasible, and cost-effective with acceptable operative, pathological and short- and long-term clinical outcomes. It retains the advantage of minimally invasive surgery.

Introduction

Patients and surgical characteristics

Patients with advanced ovarian cancer undergo preoperative lab work, imaging studies (e.g., chest X-ray and abdominal and pelvic cross-sectional and positron emission tomography (PET) imaging). PET scan is valuable in primary staging of ovarian cancer, and diagnosis of advanced ovarian cancer. Patients with advanced ovarian cancer involving the wall of the urinary bladder are selected for this surgical technique.

Instruments

The SILS (TM) electrosurgical instrument is utilized (Covidien, Mansfield, MA). This is a multi-instrument access port that allows up to three laparoscopic instruments (three 5-mm cannulas or two 5-mm, and one 12-mm cannula) to be used simultaneously through separate flexible channels. The cannula positions are adjustable within the flexible port, and separate channel allows for CO₂ gas insufflation. After the insertion of the port and insufflation of the abdomen with CO₂ gas a blunt 5-mm trocar is placed into the most cephalad channel and a round table 30° laparoscope is introduced (Endoeye, Olympus America Inc., Center Valley, PA). A blunt 10-mm trocar is inserted through the most cephalad channel of the robotic scope (Intuitive Surgical, Sunnyvale, CA), and additional 5-mm and 8-mm trocars are inserted caudally and laterally to this for the robotic trocars/instruments. A single-channel Gelport (Applied Medical, Rancho Santa Margarita, CA) is used. The Gelport consists of two parts: the outer rigid Gelcap (10 cm in diameter) visualized, and the Alexis Wound Retractor over which the Gelcap fits. The wound retractor has an inner flexible retraction ring which can be inserted into small incisions and can accommodate a variety of port diameters from 1-10 cm to a rigid extra-peritoneal retraction ring onto which the Gelcap fits to provide a large surface area for trocar placement and superior exposure. The combination of the rigid ring of the Alexis wound retractor with a Gelseal® cap help to maintain the pneumoperitoneum during multiple instrument exchanges. A blunt 5-mm trocar is placed at the most caudal aspect of the Gelport. The abdomen is insufflated with CO₂ gas to 15 mmHg, and a 5-mm 30° Olympus laparoscope is inserted. A 10-mm robotic scope is introduced through a 10-mm blunt trocar and two standard robotic-trocars (5-mm and 8-mm, respectively) are placed through the Gelport in a triangulated distribution. In addition a V-care uterine manipulator is used.

Technique

Once the patient is anesthetized, she is placed in the low lithotomy position in yellowfin stirrups with her arms tucked at her side. After prepping and draping the patient, a standard Vcare Uterine Manipulator (Conmed Endosurgery, Utica, NY) is placed and a foley catheter is inserted into the urinary bladder. A 3-cm incision is made at the umbilicus, a Gelport is inserted into the incision and trocars are introduced through the port with robotic instruments. The patient is then placed in the steep Trendelenburg position and the da Vinci surgical system (Intuitive Surgical, Sunnyvale, CA)
is docked between her legs. A 10-mm robotic 30° scope is used through the 10-mm port and a robotic monopolar hook and bipolar Maryland instruments are used through the triangulated robotic ports to perform the procedure. The assistant intermittently places an endoscopic suction device directly through the port. Ovarian cancer tumors and local metastases are debulked to less than 1 cm in diameter. The round ligaments are ligated bilaterally, and retroperitoneal spaces are developed. The infundibulopelvic ligaments are skeletonized and transected. A bladder flap is developed, and the uterine arteries and their tributaries are skeletonized and ligated. Pelvic and paraaortic lymph nodes are dissected. Anatomical margins for the lymph node dissection include: medially the ureter, laterally the body of the psoas muscle and genitofemoral nerve, posteriorly, the obturator nerve, inferiorly, the deep circumflex iliac vein, and cephalic of the midportion of the common iliac artery. The superior limit of the paraaortic dissection is the inferior mesenteric artery. Farghaly’s technique for anterior pelvic exenteration is employed to create free margins as the ovarian cancerous tumor spreads to the bladder wall. The bladder is dissected with its covering. The peritoneum in the cave of Retzius and ureters are clipped and cut. The vagina is cut with harmonic shears and this cut is extended anteriorly into the urethra and the entire specimen is disconnected. The paracolpos is cut with a ligasure till the levator ani muscle with endopelvic fascia is seen. The entire specimen; uterus, ovarian tumor tissues, fallopian tubes and all lymph nodes, are removed through the vagina by placing it in an endocatch bag, and the vagina is packed to prevent carbon dioxide gas leakage. The urinary reservoir is formed by dissecting the terminal ileum about 12 cm from the ileocecal valve and the large colon is dissected 15-20 cm distal to the hepatic flexure. The transsection site of the large colon is performed before the middle colonic artery. The distal portion of the ileum is used for the continent mechanism of the reservoir. The isolated bowel tract is washed using normal saline solution, Ringer’s lactate and antiseptic povidone-iodine solution. The isolated bowel tract is then filled with 200 ml of normal saline, and six teniamyotomies are performed. The tenia is sectioned across the whole width to the submucosal layer with, 6 cm between each teniamyotomy. The teniamyotomies are left open to increase the reservoir capacity of the pouch. The spatulated ureters are sutured together at the medial site of spatulation to create a trapezoidal plane which is anastomosed to the reservoir as the distal ileum is used as an effenter segment of the pouch. The distal ileum is cannulated with a 14 Fr catheter. The ileocecal valve is reinforced with 2/0 prolene sutures. The tapered ileum is then brought to the anterior abdominal wall. A pelvic drain is introduced through the 10 mm port and ports were removed under vision. The vagina is closed by intracorporeal suturing with 2-0 vicryl and by taking continuous interlocking sutures. The fascia is closed using 0 vicryl sutures and the skin is closed with a running 4-0 monocryl subcuticular stitch. Estimated operative time is 4.6 hours, and average blood loss is 210 ml. The pelvic drain is kept in place for 24-48 hours depending on the drainage. Hospital stay is about five days.

Discussion

The advantage of using the robotic system is that it enables the surgeon to dissect deeply in the narrow pelvic floor. Also, it offers better visualization with the binocular optics generating 3-D stereoscopic vision. Utilization of a harmonic scalpel allows for control of the pelvic sidewall vessels and transaction of the ligament attachments around the pelvic structures. The articulating wristed robotic instrument allows for fine sewing. Robotic surgery for advanced ovarian cancer can be achieved by rotating the operating table and relocking the robot at the patient’s head. This position will allow dissection and removal of the paraaortic lymph nodes, resection of the upper abdominal metastases, and debulking of the diaphragm and liver involvement [1]. It has been shown that robotic radical prostatectomy provides a significant advantage in terms of its learning curve, especially for surgeons with little or no advanced laparoscopic experience [2]. It requires only 12 cases to achieve proficiency in performing robotic-assisted radical prostatectomy.

Total cystectomy with urinary diversion remains the treatment of choice for organ-confined muscle invasive cancer of the urinary bladder. Gil et al. [3] reported laparoscopic radical cystectomy, bilateral lymphadenectomy, and ileal conduit diversion with the entire procedure carried out by an intracorporeal laparoscopic technique.

There have been few case reports of laparoscopic anterior pelvic exenteration [4, 5]. It has been shown that the procedure is feasible even if combined with intracorporal urinary diversion. Overall morbidity and hospitalization have considerably decreased. It is worth noting that the goal of extensive surgery, anterior pelvic exenteration, should always be resection of the tumor with tumor-free margins. Prototypes of the robotic operative systems that come down from the ceiling and have the ability to rotate 360° have been proposed to allow more flexibility and better visualization of the operating field.

Conclusion

Farghaly’s anterior pelvic exenteration technique offers benefits such as improved surgeon dexterity, enhanced ergonomics and 3-D optics. The utilization of an ileal conduit formation for urinary diversion is technically feasible with good results. Moreover, it is safe, cost effective, with acceptable operative, pathological and short- and long-term clinical outcomes. It retains the advantage of minimally invasive surgery. Randomized controlled studies are necessary to define surgical and oncologic outcomes obtained from this surgical technique, and to assess the relative benefits of this technique compared with more conventional minimally invasive approaches.
References

Effects of third-line chemotherapy for women with recurrent ovarian cancer who received platinum/taxane regimens as first-line chemotherapy


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Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy, accounting for 8,000 new diagnoses and 4,000 deaths annually in Japan [1]. Early diagnosis of EOC is extremely difficult because most patients with early-stage disease are asymptomatic, so that as many as 80% of patients present with advanced disease. The patients are usually treated with cytoreductive surgery, followed by platinum and paclitaxel chemotherapy. Initial response rate for these treatments exceeds 70% [2]. Despite initial responses, the majority experience relapse, with a median disease-free interval of 18 to 24 months. The recurrent cases are classified into three categories: platinum-sensitive (relapse ≥ 6 months from initial platinum therapy), platinum-resistant (relapse within 6 months from initial platinum therapy), and platinum-refractory (stable disease or progressive disease during initial platinum therapy). Based on the National Comprehensive Cancer Network (NCCN) guideline, platinum-based combination therapy should be considered for platinum-sensitive recurrences; however, for platinum-resistant/refractory cases, non-platinum monotherapy is recommended [3].

After second-line chemotherapy, most patients have a progressive course. The length of response in the second-line approach using a platinum-based regimen is reported to be shorter than that in primary chemotherapy [4]. After second relapse, third-line chemotherapy is usually performed; however, a standard regimen has not been determined yet. It remains unclear whether third-line chemotherapy can prolong the survival or have clinical benefit. Many reports showed the effect of salvage chemotherapy; however, most of the reports focused on the efficacy of particular drug regimens and only a few reports demonstrated the efficacy of third-line chemotherapy [5-7].

Thus, in this study we aimed to evaluate the effects of third-line chemotherapy and its prognostic factors.

Materials and Methods

Patients

We retrospectively reviewed the medical records of women with recurrent ovarian cancer who received third-line chemotherapy. Forty patients who started to receive third-line chemotherapy between February 1998 and October 2008 were included in this study. All patients underwent initial surgery and primary chemotherapy consisting of a platinum/taxane regimen, and received second-line chemotherapy after the first relapse. Patients who underwent surgery at relapse were not included. All patients were followed up at the Department of Obstetrics and Gynecology, Keio University Hospital, Tokyo. Treatment decisions for third-line chemotherapy were usually made by the attending clinician. Data were collected on age, International Federation of Obstetricians and Gynaecologists (FIGO) staging, histologic type, the extent and outcome of surgery, prior chemotherapeutic treatments and disease responses, recurrent sites, intervals between primary, secondary and tertiary treatments and overall survival after receiving the third-line drug.

Summary

Purpose: At present, it remains unclear whether the third-line chemotherapy has clinical benefit. In this study, we retrospectively evaluated the effect of third-line chemotherapy. Methods: We reviewed the medical records of 40 women with recurrent epithelial ovarian cancer (EOC) who received third-line chemotherapy after platinum/taxane regimens as first line chemotherapy. Results: In the first recurrence, 23 cases were platinum-sensitive and 17 cases were platinum-resistant. The cases for which the treatment-free interval from second-line chemotherapy (TFI) was ≥ 3 months had a higher non-PD rate than those with TFI < 3 months (86% vs 33%, p = 0.002). In addition, the median overall survival (OS) was longer for TFI ≥ 3 months than for TFI < 3 months (1195 days vs 235 days, p = 0.004). Finally, TFI was an independent significant prognostic factor by univariate (HR 3.28, p = 0.006) and multivariate (HR 3.21, p = 0.018) proportional hazard tests. Conclusion: TFI from second-line chemotherapy may predict a survival benefit of third-line chemotherapy.

Key words: Epithelial ovarian cancer; Recurrence; Third-line chemotherapy.
Definition of chemotherapy sensitivity of primary chemotherapy

“Refractory,” “resistant,” and “sensitive” in the first recurrence were defined as follows: Refractory: progression, partial remission, or stable disease on primary chemotherapy; Resistant: complete remission and relapse < 6 months after stopping primary chemotherapy; Sensitive: complete remission and relapse ≥ 6 months after stopping primary chemotherapy.

The treatment-free interval (TFI) before third-line chemotherapy was defined as the interval between the end of second-line chemotherapy to the start of third-line chemotherapy.

Evaluation of response of third-line chemotherapy

Response was based on two-dimensional measurements of the lesions based on computed tomography (CT) or magnetic resonance imaging (MRI). A complete response (CR) was defined as no evidence of disease on imaging studies, with normalization of the serum CA125 level. Partial response (PR) was defined as a > 50% decrease in tumor size. Progressive disease (PD) was defined as a > 25% increase in tumor size or the appearance of a new lesion. Stable disease (SD) was defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. The CA125 response criteria were not used; however, the patients were not considered as having partial response or no change if there was an increase of CA125. Overall survival (OS) was defined as the interval from the first day of administration of third-line drug to the day of death.

Statistical analysis

The relationship between response rate or non-PD rate and age, histology, regimen, and TFI were analyzed by Fisher’s exact test. Patients were categorized by age (young vs old), histology (serous vs non-serous), regimen (monotherapy vs combination therapy), and TFI (< 3 months vs ≥ 3 months). Factors influencing overall survival were analyzed by Cox’s proportional hazard test and the Kaplan-Meier test. After investigation of the multicollinearity of these factors, multivariate Cox’s proportional hazard test was applied. A value of p < 0.05 was considered statistically significant. Statistical calculations were performed using SPSS Statistics software version 17.0 for Windows (SPSS, Chicago, IL).

Results

Patients

Median age at the time of third-line chemotherapy was 57 years (range: 34-78). Clinical stage and histology were as follows: clinical stage (I, 3; II, 5; III, 23; IV, 9); histology (serous, 24; clear cell, 7; endometrioid, 5; mucinous, 1; undifferentiated, 3). In the first recurrence, 23 patients were platinum-sensitive and 17 patients were platinum-resistant. Twenty-one patients received platinum/taxane regimen, nine patients received cisplatin+irinotecan, four patients received cisplatin+doxorubicin+cyclophosphamide, two patients received irinotecan, and one patient each received liposomal doxorubicin, paclitaxel, docetaxel, or carboplatin as second-line chemotherapy.

Clinical backgrounds of second relapse patients are shown in Table 1. Second recurrent sites were as follows: Abdominal cavity, 9; lymph node, 8; pelvic cavity, 7; liver, 3; pleura, 2; lung, 1 and ten patients had multiple sites. TFI from the last day of second-line chemotherapy to the day of the start of third-line chemotherapy was 0-3 months in 18 cases, 3-6 months in two cases, and ≥ 6 months in 20 cases. Performance status (PS) was 0-1 in 36 cases and 2 in four cases at third-line chemotherapy. Nineteen patients received single agent chemotherapy (taxane, 9; irinotecan, 4; liposomal doxorubicin, 3; other, 3) and 21 patients received combination chemotherapy (platinum/taxane, 15; cisplatin+irinotecan, 3; cisplatin+doxorubicin+cyclophosphamide, 2; other, 1) as third-line chemotherapy. The median number of treatments with third-line chemotherapy was four cycles (range, 1-15).

Table 1. — Patient characteristics of third-line chemotherapy.

<table>
<thead>
<tr>
<th>Clinical factor</th>
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<tr>
<td>Sensitivity of primary chemotherapy*</td>
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<td>Sensitive</td>
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<td>Refractory/Resistant</td>
<td>17</td>
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<td>TFI</td>
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<td>0-3m</td>
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<td>3-6m</td>
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<td>6m-</td>
<td>20</td>
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<tr>
<td>Recurrent site</td>
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<tr>
<td>Solitary</td>
<td>30</td>
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<tr>
<td>Abdominal cavity</td>
<td>9</td>
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<td>Lymph node</td>
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<tr>
<td>Pelvic cavity</td>
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<td>Liver</td>
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<td>Pleura</td>
<td>2</td>
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<tr>
<td>Lung</td>
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<td>Multiple</td>
<td>10</td>
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<td>Therapeutic regimen</td>
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<td>Mono</td>
<td>19</td>
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<td>Taxane</td>
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<td>Irinotecan</td>
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<td>Liposomal doxorubicin</td>
<td>3</td>
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<tr>
<td>Other</td>
<td>3</td>
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<tr>
<td>Combination</td>
<td>21</td>
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<tr>
<td>Platinum/taxane</td>
<td>15</td>
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<td>Cisplatin+irinotecan</td>
<td>3</td>
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<td>Cisplatin+doxorubicin+cyclophosphamide</td>
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<td>Other</td>
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*Chemosensitivity: Sensitivity to primary chemotherapy.

Relationships between clinical factors and the response rate or non-PD rate of third-line chemotherapy

Relationships between clinical factors and the response rate or non-PD rate of third-line chemotherapy are shown in Table 2. In total, response rate and non-PD rate in all cases were 23% and 63%, respectively. In all cases, age, regimen, histology, and chemosensitivity of first-line chemotherapy were not related with response rate or non-PD rate in third-line chemotherapy. The cases for which TFI > 3 months had a higher non-PD rate than the cases with TFI < 3 months (86% vs 33%, p = 0.002). In platinum-sensitive cases, the response rate and non-PD rate were 30% and 70%, respectively. TFI was significantly
related with non-PD rate (88% vs 17%, \(p = 0.007\)); however, age, regimen, and histology were not related with non-PD rate. In resistant cases, the response rate and non-PD rate were 12% and 53%, respectively. TFI and regimen tended to be related with non-PD rate (80% vs 45%; 70% vs 29%); however, these differences were not statistically significant. Age and histology were not related.

**Relationship between clinical factors and overall survival**

The median overall survival for all cases was 498 days (29-3335 days). Twenty-four patients died, 13 were alive with disease, and three were alive with no evidence of disease at the last day of February 2009. The median OS was significantly longer in non-PD cases than in PD cases (1110 days vs 164 days, \(p < 0.001\)) (Figure 1). The OS was significantly related to TFI, but not to histology, chemosensitivity of first-line chemotherapy, chemotherapy regimen, or age. The OS was longer in cases for which TFI \(\geq 3\) months than in TFI < 3 months (1195 days vs 235 days, \(p = 0.004\)) (Figure 2). TFI was an independent significant prognostic factor by univariate (HR 3.28, \(p = 0.006\)) and multivariate (HR 3.21, \(p = 0.018\)) proportional hazard tests (Table 3).

**Discussion**

Despite a high clinical complete remission rate, ovarian cancer patients still have high recurrence rate and require second-line chemotherapy. The Gynecologic Oncology Group has divided the disease into categories of sensitive and resistant disease at six months of TFI [8]. The definition of sensitivity to treatment is initially described for
platinum drugs, but this criterion is generally applied to other chemotherapeutic regimens, such as paclitaxel, as well [9]. Based on the NCCN guideline, platinum-based combination therapy should be considered for platinum-sensitive recurrences; however, for platinum-resistant/refractory cases, non-platinum monotherapy is recommended [3]. For sensitive cases, the aim of second-line chemotherapy is to prolong survival, while for resistant cases the aim is mainly palliation. However, the length of response in the second-line approach using the platinum-based regimen is generally reported to be shorter than that in primary chemotherapy [4], while the role of third-line chemotherapy remains unclear.

Tangjitgamol et al. evaluated the efficacy of third-line chemotherapy and reported that response rate and non-PD rate were 16% and 47%, and median OS after third-line treatment was 13.8 months [6]. Bodnar et al. evaluated the effect of topotecan as third-line chemotherapy and reported that response rate and non-PD rate were 15% and 45% [10]. In addition, Spannuth et al. performed a prospective phase II study of topotecan for secondary platinum-resistant recurrent ovarian cancer as third-line chemotherapy and showed that response rate and non-PD rates were 14% and 55% [11]. As we reported in this study that response rate and non-PD rate were 23% and 63%, and median OS duration after third-line chemotherapy was 498 days, the data described above were concordant with our study.

Villa et al. reported a high response rate (48%) of third-line chemotherapy; however, that report included only patients who received second-line chemotherapy for platinum-sensitive [5]. Nishio et al. also showed a high response rate (41%) of third-line chemotherapy [7]. Our response rate of 23% was much lower than that reported by Nishio et al. This might be due to the differences in patient population or chemotherapy regimen. For example, in Nishio’s report 69% were platinum-sensitive cases, while in our report 58% were sensitive cases. In addition, in the present study, the median OS was significantly longer in non-PD cases than in PD cases. However, the two reports above demonstrated that there was no significant difference in OS between third-line responders and non-responders [6, 7]. Two reasons are proposed, as follows: 1) The clinical factors, including patient population and therapeutic regimen, differ among these retrospective studies; 2) Previous reports evaluated the relationship between chemotherapy response (CR+PR) and OS, while our study evaluated the relationship between non-PD and OS. We believe that stable disease is a clinical benefit in the third-line setting. In our study, the response group (CR+PR) consisted of only nine cases and there was no significant relationship between response rate and OS (p = 0.197). Further study will be required to clarify these observations.

The NCCN guideline recommends that platinum-based combination therapy should be considered for platinum-sensitive recurrences and, non-platinum monotherapy for platinum-resistant/refractory cases [3]. However, there is no definitive guideline for the third-line setting. In the present study, TFI from second-line chemotherapy can be used to predict the effect of third-line chemotherapy. We classified TFI into the two categories of less and more than three months, because the second relapse usually occurs in a shorter interval than the first recurrence, as mentioned, and only two patients were in the category of TFI 3-6 months. The OS was longer in patients with TFI ≥3 months than in TFI <3 months, and the TFI was an independent significant prognostic factor by univariate and multivariate proportional hazard tests. Nishio et al. reported that primary disease-free interval (DFI) was a useful predictor of the survival benefit of third-line chemotherapy; however, secondary DFI was not related with OS [7]. This discrepancy remains unclear. Further study will be needed.

**Conclusion**

The non-PD rate of third-line chemotherapy was related to OS. TFI from second-line chemotherapy may predict the survival benefit of third-line chemotherapy. In the third-line setting, we must consider TFI and clinical benefit before administration.

**References**


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Predictive factors for the detection of CIN II-III in the follow-up of women with CIN I

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Hospital Sant Joan de Déu, University of Barcelona, Esplugues (Barcelona)

Summary

Purpose of investigation: To determine which factors may increase the risk that women diagnosed with CIN I may later develop CIN II-III. Methods: A prospective study of 174 women with a grade 1 intraepithelial lesion (CIN I) confirmed by biopsy, with a follow-up time of at least one year. The following factors were studied: age, HPV infection, HPV infection by a high-risk genotype, the HPV genotypes involved, coinfection by several HPV genotypes and duration of follow-up. These factors were correlated with later detection of CIN II-III by biopsy during follow-up. Statistical analysis was performed using SPSS. Results: CIN II-III was detected at the follow-up in 24 of 174 women included in the study (13.7%), in four cases by colposcopically directed biopsy and in 20 by LLETZ. Correlation of the factors studied with the incidence of CIN II-III in this group showed that the only statistically significant factors were overall HPV infection and HPV infection by genotypes 31 and 70 (Chi-square and Fisher’s test, p < 0.05, respectively), while the duration of follow-up came close to statistical significance (Student’s test, p = 0.052). Conclusion: HPV infection and duration of follow-up are predictive factors for the detection of CIN II-III in follow-up care for women with CIN I.

Key words: Papillomavirus; CIN I; CIN II-III; Risk factors; Cervix.

Introduction

Follow-up and treatment of women with a low-grade squamous intraepithelial lesion (SIL) is often complex. On the one hand, these lesions have a low potential – approximately 1% [1] – for becoming malignant, and a risk of progression to CIN II-III of 5-11% [1, 2]. On the other hand, although low-grade SIL is initially detected in cytology and confirmed by colposcopy and directed biopsy, high-grade SIL is not infrequently found in subsequent follow-up testing [3]. According to one study [4], occult or de novo high-grade SIL was found in 23-55% of cases. For this reason, the management of low-grade SIL in young women tends to be very conservative, since it is believed that low-grade SIL is simply an indicator of HPV infection, and since this infection is often transitory [5, 6] the lesion will subside. After age 40, however, treatment tends to be more aggressive and the transformation zone is excised, since it is assumed that in such cases the infection is persistent and the lesion is less likely to resolve spontaneously [7]. In such cases, there is increased risk that it may develop into high-grade SIL, or that there may be occult high-grade SIL.

The objective of this study was to determine which factors may increase the risk that women with low-grade SIL will present later with high-grade SIL, in order to develop objective parameters that will facilitate identification of those patients who should be treated.

Material and Methods

This was a prospective study of a group of women who were referred by their primary care physicians for abnormal cytology results. All the patients underwent repeat cytology, human papillomavirus (HPV) testing, and colposcopy. In cases of atypical colposcopy results, they also underwent a biopsy. For the study group, 174 women were chosen who had a grade 1 intraepithelial lesions (CIN I) confirmed by colposcopically guided biopsy. All women had been followed for at least one year.

The following factors were studied in the study group: age, HPV infection, HPV infection by a high-risk genotype, the HPV genotypes involved, co-infection by several HPV genotypes and duration of follow-up. These factors were correlated with detection of CIN II-III in a colposcopically directed biopsy, limited excision of the transformation zone (LETZ), or cone biopsy performed during the follow-up period. LETZ was indicated in cases of persistent CIN I for a period longer than one year.

Colposcopy: Colposcopy was performed following application of 2% acetic acid solution. The classification proposed by the International Federation of Cervical Pathology and Colposcopy (FCPC) in Rome in 1990 [8] was used.

Human papillomavirus detection and genotyping: Samples were obtained using a swab applied to the surface of the cervix. The sample was then dissolved in 0.5 ml saline solution (pH 7.2) and HPV genotyping was performed using a new microarray-based molecular technique (Genomicin®) that permits detection of the 35 most prevalent genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85, 89).

Statistical analysis

Statistical analysis was carried out using the SPSS program (version 12.0), Fisher’s exact test, Chi-square and the Student’s t-test. The level of statistical significance was established at p < 0.05.
Results

The patients included in the study were 174 women. Their average age was 33.89 years, with an age range between 16 and 62 years. In 134 cases, the reason for referral was low-grade SIL. Reasons for referral in the remaining cases are shown in Table 1. Mosaic pattern was the abnormal colposcopic image most frequently detected, accounting for 61 cases (35%), followed by leukoplakia in 32 cases (18.3%) and punctation in 17 cases (9.7%). Colposcopically guided biopsies were performed in all women of the group and diagnosis of CIN I was confirmed in all the patients. HPV infection was detected in 119 (68.3%) women using a microarray-based molecular technique (Genomica®). High-risk or probable high-risk genotypes were present in 84% of cases. The most frequently isolated genotype was 16, followed by 53 and 51 (Table 2). Infection by more than one HPV genotype was found in 63 (36.2%) women.

During follow-up, 47 women with abnormal colposcopic images underwent a second colposcopically guided biopsy. In 37 cases the results of the second biopsy showed CIN I, in four cases CIN II-III, and in six cases the results were negative. In 101 cases, treatment was indicated. LETZ was performed in 87 cases and conization in eight, and six women underwent LETZ followed by cone biopsy, four of them because the surgical margin was involved.

CIN II-III was detected in 24 women (13.7%), in four cases by colposcopically directed biopsy and 20 by LETZ. Of the 24 women, 18 were infected by high-risk HPV genotypes, but genotype 16 was detected in only six women and genotype 18 in one case (Table 2). The mean duration of follow-up was 712 (SD 392.25) days.

Statistical analysis was performed to correlate detection of CIN II-III with the variables studied (Table 3). Only HPV infection (p = 0.03, Chi-square test), infection by HPV genotype 31 (p = 0.02, Fisher’s exact test) and infection by type 70 (p = 0.035, Fisher’s exact test) were statistically significant. Association with HPV genotype 53 came close to statistical significance (p = 0.065; Fisher’s exact test). Another parameter of near-statistical significance was the duration of follow-up. The longer the follow-up period, the greater the risk of developing CIN II-III (p = 0.052; Student’s t-test).

The other parameters studied – age, the presence of high-risk HPV infection, and co-infection by various HPV genotypes – did not significantly increase the risk of detecting CIN II-III.

Table 1. — Initial cytology results of the 174 women included in the study and percentage of HPV infections in each cytologic diagnosis.

<table>
<thead>
<tr>
<th>Cytology results</th>
<th>Number</th>
<th>Percentage</th>
<th>HPV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-grade SIL</td>
<td>149</td>
<td>85.6%</td>
<td>67.1%</td>
</tr>
<tr>
<td>AGUS</td>
<td>1</td>
<td>0.5%</td>
<td>0%</td>
</tr>
<tr>
<td>ASCUS</td>
<td>24</td>
<td>13.7%</td>
<td>66.6%</td>
</tr>
</tbody>
</table>

Table 2. — Genotypes isolated in the 174 women with an initial diagnosis of CIN I and in the 24 women in whom CIN II-III was detected.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total group Number</th>
<th>Percentage*</th>
<th>24 women with CIN II-III Number</th>
<th>Percentage*</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>32</td>
<td>18.3%</td>
<td>6</td>
<td>25%</td>
</tr>
<tr>
<td>53</td>
<td>27</td>
<td>15.5%</td>
<td>7</td>
<td>29.1%</td>
</tr>
<tr>
<td>51</td>
<td>26</td>
<td>14.9%</td>
<td>4</td>
<td>16.6%</td>
</tr>
<tr>
<td>66</td>
<td>20</td>
<td>11.5%</td>
<td>2</td>
<td>8.3%</td>
</tr>
<tr>
<td>31</td>
<td>13</td>
<td>7.4%</td>
<td>5</td>
<td>20.8%</td>
</tr>
<tr>
<td>33</td>
<td>10</td>
<td>5.7%</td>
<td>3</td>
<td>12.5%</td>
</tr>
<tr>
<td>58</td>
<td>9</td>
<td>5.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>8</td>
<td>4.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>8</td>
<td>4.6%</td>
<td>1</td>
<td>4.1%</td>
</tr>
<tr>
<td>84</td>
<td>8</td>
<td>4.6%</td>
<td>1</td>
<td>4.1%</td>
</tr>
<tr>
<td>18</td>
<td>7</td>
<td>4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>3.4%</td>
<td>2</td>
<td>8.3%</td>
</tr>
<tr>
<td>70</td>
<td>6</td>
<td>3.4%</td>
<td>3</td>
<td>12.5%</td>
</tr>
<tr>
<td>81</td>
<td>6</td>
<td>3.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>2.2%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The percentages do not add up to 100 because 36.2% of the women were infected by more than 1 HPV genotype.

Table 3. — Statistical analysis of the possible factors correlated with CIN II-III detection.

<table>
<thead>
<tr>
<th>Factors</th>
<th>CIN III positive*</th>
<th>CIN III negative*</th>
<th>Test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV Infection inf by a high-risk genotype*</td>
<td>18/21</td>
<td>84/98</td>
<td>Fisher’s</td>
<td>0.3</td>
</tr>
<tr>
<td>Genotype 6 inf</td>
<td>2/24</td>
<td>4/150</td>
<td>Fisher’s</td>
<td>0.19</td>
</tr>
<tr>
<td>Genotype 11 inf</td>
<td>0/24</td>
<td>5/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 16 inf</td>
<td>6/24</td>
<td>26/150</td>
<td>Fisher’s</td>
<td>0.39</td>
</tr>
<tr>
<td>Genotype 18 inf</td>
<td>1/24</td>
<td>6/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 31 inf</td>
<td>5/24</td>
<td>8/150</td>
<td>Fisher’s</td>
<td>0.02</td>
</tr>
<tr>
<td>Genotype 33 inf</td>
<td>2/24</td>
<td>8/150</td>
<td>Fisher’s</td>
<td>0.63</td>
</tr>
<tr>
<td>Genotype 35 inf</td>
<td>0/24</td>
<td>2/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 39 inf</td>
<td>0/24</td>
<td>1/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 42 inf</td>
<td>0/24</td>
<td>5/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 44 inf</td>
<td>1/24</td>
<td>3/150</td>
<td>Fisher’s</td>
<td>0.45</td>
</tr>
<tr>
<td>Genotype 45 inf</td>
<td>0/24</td>
<td>1/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 51 inf</td>
<td>4/24</td>
<td>22/150</td>
<td>Fisher’s</td>
<td>0.76</td>
</tr>
<tr>
<td>Genotype 52 inf</td>
<td>0/24</td>
<td>8/150</td>
<td>Fisher’s</td>
<td>0.6</td>
</tr>
<tr>
<td>Genotype 53 inf</td>
<td>7/24</td>
<td>20/150</td>
<td>Fisher’s</td>
<td>0.065</td>
</tr>
<tr>
<td>Genotype 54 inf</td>
<td>1/24</td>
<td>3/150</td>
<td>Fisher’s</td>
<td>0.45</td>
</tr>
<tr>
<td>Genotype 56 inf</td>
<td>0/24</td>
<td>10/150</td>
<td>Fisher’s</td>
<td>0.36</td>
</tr>
<tr>
<td>Genotype 58 inf</td>
<td>0/24</td>
<td>9/150</td>
<td>Fisher’s</td>
<td>0.61</td>
</tr>
<tr>
<td>Genotype 59 inf</td>
<td>0/24</td>
<td>1/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 61 inf</td>
<td>1/24</td>
<td>7/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 62 inf</td>
<td>0/24</td>
<td>1/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 64 inf</td>
<td>0/24</td>
<td>1/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 66 inf</td>
<td>2/24</td>
<td>18/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 68 inf</td>
<td>0/24</td>
<td>1/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 70 inf</td>
<td>3/24</td>
<td>3/150</td>
<td>Fisher’s</td>
<td>0.035</td>
</tr>
<tr>
<td>Genotype 73 inf</td>
<td>0/24</td>
<td>2/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 81 inf</td>
<td>0/24</td>
<td>6/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Genotype 82 inf</td>
<td>1/24</td>
<td>1/150</td>
<td>Fisher’s</td>
<td>0.25</td>
</tr>
<tr>
<td>Genotype 84 inf</td>
<td>1/24</td>
<td>7/150</td>
<td>Fisher’s</td>
<td>1</td>
</tr>
<tr>
<td>Coinfection**</td>
<td>12/21</td>
<td>51/98</td>
<td>Chi-square</td>
<td>0.64</td>
</tr>
<tr>
<td>Mean age</td>
<td>32.25 years</td>
<td>34.15 years</td>
<td>Student’s t</td>
<td>0.39</td>
</tr>
<tr>
<td>Mean follow-up</td>
<td>861 days</td>
<td>690.5 days</td>
<td>Student’s t</td>
<td>0.052</td>
</tr>
</tbody>
</table>

* inf = infection.
| ** only cases with high-risk genotype infection. |
| ** only cases with coinfection. |
| * group with progression to CIN II-III (number of cases/total). |
| ** group with no progression to CIN II-III (number of cases/total). |
Discussion

According to the literature, the prevalence of HPV infection in women with cytology results showing low-grade SIL ranges between 52.5% and 76.1% [9, 10]. In our study, the prevalence of this infection was 67.1%. Another study reports that CIN I detected by biopsy is more frequently associated with HPV infection in as many as 93% of cases [3]; this percentage was considerably lower in our study, 68.3%.

It is noteworthy that 85.7% of the genotypes infecting women with CIN I were high-risk types, and by contrast with other studies of women with CIN II-III in which by far the dominant HPV genotype was 16 [9, 11], in our study there was no great difference in the frequency of genotypes 16, 51 and 53. Genotypes 6 and 11, which are more closely associated with transitory HPV infection, represented a very small percentage of all genotypes detected in our study.

The percentage of women treated for CIN I who were later diagnosed with CIN II-III was also similar to the tendency observed in the literature, between 23% and 55% [4]. In our study, this was the case for 24 of the 101 women (23.7%).

Age was not a statistically significant risk factor for CIN II-III in our cases. This places in question the recommendation that women over the age of 40 are at higher risk and should be treated for CIN I [7]. The average age of the women with CIN I and those with CIN II-III was quite similar, 34 and 32 years, respectively. Al-Nourhji et al. [12] also observed that women whose cytology results showed low-grade SIL and were later diagnosed with CIN II-III confirmed by biopsy were younger than those not diagnosed with CIN II-III (an average age of 25 as opposed to 30 years). Age, therefore, does not eliminate the risk of developing CIN II-III for women with a cytology diagnosis of low-grade SIL. For these reasons, we would recommend follow-up colposcopy, and in the event of an abnormal result, a biopsy to establish the diagnosis. In our study, during follow-up colposcopy and colposcopically directed biopsy detected four (16.6%) of the 24 cases of CIN II-III.

The only risk factors for detection of CIN II-III were overall HPV infection, and HPV infection by genotypes that are not, oddly, among the most prevalent: one that is probable high-risk, 70, and 31, a high-risk genotype. Infection of high-risk genotypes 16 and 18 were not correlated in the study with risk of detecting CIN II-III in women diagnosed with CIN I.

The duration of follow-up is also important. Though not statistically significant, it came close (p = 0.052). The longer the follow-up period, the greater the risk, probably because CIN I is persistent.

In conclusion, age is a relatively unimportant factor in deciding which patients with CIN I should be treated.

HPV testing and genotyping may be useful in deciding whether or not to treat, not as an isolated factor but in conjunction with other clinical parameters (cytology, colposcopy and biopsy results) and the persistence of the lesion. Infection of high-risk genotypes other than 16 and 18 are correlated in the study with risk of detecting CIN II-III.

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References


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DNA cytometry as a first-line method for diagnosis of cervical precancer with respect to clinical behaviour

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Summary

Background: The objective of this study was to estimate DNA image cytometry (DNA-ICM) as a first-line diagnostic method for diagnosis of cervical precancer with respect to its clinical behaviour. Methods: 30 consecutive patients with Papanicolaou smears that yielded diagnoses of LSIL or HSIL and showed single cell or stem line aneuploidy were included in a prospective cohort study. Slides were classified according to the Bethesda system. DNA-ICM was performed according to the consensus reports of the European Society of Analytical Cellular Pathology. Results: 24 (80%) patients with DNA aneuploid cervical epithelial cell abnormalities had cervical intraepithelial neoplasia (CIN) (CIN I: n = 5; CIN II: n = 6; CIN III n = 13). Six (20%) patients showed no evidence of CIN in subsequent biopsies. During follow-up of three years none of the six patients with negative histology developed cervical precancer or cancer. All 24 (100%) lesions confirmed as CIN by histology showed DNA aneuploidy in cytology. Conclusions: DNA-ICM should be used as an objective first-line diagnostic tool for predicting cervical precancer. Yet, due to immune response, DNA aneuploid cervical cell abnormalities do not seem to be enough to predict the definitive clinical outcome in each patient.

Key words: Cervix; Cancer; Natural history; DNA cytometry.

Introduction

None of the established diagnostic methods (cytology, HPV testing, molecular markers, colposcopy, and even guided biopsy) can be relied on for an accurate diagnosis of cervical intraepithelial neoplasia (CIN). The positive predictive value (PPV) of these methods is low [1,2]. Colposcopy can evaluate only lesions limited to the ectocervix. The diagnostic accuracy of colposcopically guided biopsies depends entirely on the sites from which they are taken. Biopsy cannot always detect glandular involvement or invasion. Endocervical curettage shows only extension of CIN into the cervical canal.

DNA aneuploidy, as identified by DNA image cytometry (DNA-ICM), represents the quantitative cytometric equivalent of chromosomal aneuploidy and has been widely accepted as an objective marker of malignant cell transformation [3-7]. However, CIN represents a heterogeneous group of lesions, particularly with respect to their clinical behaviour. CIN in individual women can undergo any of four possible options: 1: regression; 2: persistence, 3: progression, and 4: recurrence.

The objective of this prospective cohort study was to estimate DNA-ICM as a first-line diagnostic method for diagnosis of cervical precancer with respect to its clinical behaviour.

Material and Methods

Patients

Between February and June 2006, Pap smears from 59 women yielded diagnoses of low-grade squamous intraepithelial lesions (LSIL) or high-grade squamous intraepithelial lesions (HSIL) at the Cytological Laboratory of the Department of Obstetrics and Gynecology, Medical University of Graz, Austria. Cytological samples were obtained consecutively from routine input at this institution. DNA-ICM was performed in all Pap smears. The median patient age was 34 years (range, 16-78 years).

Sample processing and assessment

Samples from the uterine cervix were obtained using a spatula and/or cervix brush. Colposcopy generally was not performed. The specimens were fixed in alcohol, subjected to Pap staining, and screened by medical technical assistants. The 2001 Bethesda system was used for cytological classification [6].

After morphologic investigation, the smears underwent destaining and restaining according to the method described by Feulgen [8]. Measurements of nuclear DNA content were performed as previously described using a computer-based image analysis system consisting of a Zeiss Axioplan 2 microscope (Zeiss, Jena, Germany) with a 40x objective (numeric aperture, 0.75; Köhler illumination) and a charge-coupled device black-and-white video camera with 572 lines of resolution (VariCam CCIR; PCO Computer Optics, Kehlheim, Germany).

The software package used in the current study was the AutoCyte QUIC-DNA-Workstation (AutoCyte Inc., Burlington, NC), which provides shading and glare correction. The latter was performed at a rate of 2.2%. In each case, at least 30 intermediate squamous cells with normal appearance were measured as internal reference cells. Using squamous cells as an internal reference, latent human papillomavirus (HPV) infection must be considered as a potential cause of a slightly changed peridiploid DNA content [9, 10]. Since a clonal change would be unlikely in cells with normal appearance, latent viral infection should increase the coefficient of variation of reference cells rather than shifting the respective DNA histogram peak. The former potential confounder was limited in the current study, because the coefficient of variation for reference cells was always ≤ 5%. At least 200 epithelial cells with abnormal (i.e., hyperchromatic), enlarged or polymorphic nuclei were

Revised manuscript accepted for publication
measured, starting with encircled areas. To increase the detection rate of 9c-exceeding events (9cEEs), all Feulgen-stained smears were considered during measurement. All technical instruments and all software used in the study met the standard requirements of the consensus reports of the European Society for Analytical Cellular Pathology (ESACP) [9, 11-13].

Two parameters were assessed for diagnostic interpretation [3-6]. DNA stem line is the G0/G1 cell phase fraction for a proliferating cell population (with a first peak and a second doubling peak or with nuclei in the doubling region). DNA stem line aneuploidy was defined as the modal value of DNA stem line in c units (c = DNA content). DNA stem line aneuploidy was assumed if the modal value of a stem line was < 2.20c or > 4.40c. Rare DNA events included the 9cEEs, which were defined as the number of cells with a DNA content > 9c. Single cell aneuploidy was diagnosed when at least one cell per slide had DNA content > 9c (9cEE > 1) [14].

Follow-up

The reference standard was histologic examination. The following procedure was agreed upon with the gynaecologists sending samples to the cytological laboratory: Depending on the location of the transformation zone, each patient should have one or more biopsies and/or endocervical curettages within six months after cytological diagnosis. Histological diagnoses were classified according to the CIN system [15]. For patients whose histology showed no evidence of CIN, we considered cytological follow-up at time intervals of six months. Final data retrieval of follow-up cytology was carried out in August 2009.

Results

In 40 of the 59 patients with LSIL or HSIL, histology was performed within six months after cytological examination. Nineteen patients were lost to follow up due to different reasons. In 24 (60%) of 40 patients histology showed CIN. None of the patients had invasive cancer.

In the collective of 40 patients with subsequent histology, DNA-ICM showed DNA aneuploidy in 30 (75%) of the patients (stem line n = 12, single cell n = 12) whereas ten patients (25%) had normal DNA histograms.

Twenty-four (80%) of the 30 patients with DNA aneuploidy had CIN (CIN I: n = 5; CIN II: n = 6; CIN III: n = 13) and six (20%) patients were negative for CIN (Table 1). During follow-up of three years, none of the six patients with negative histology developed cervical precancer or cancer.

Table 1. — DNA-ICM characteristics of six women with DNA aneuploid cervical epithelial cell abnormalities showing regressive behaviour during follow-up of three years.

<table>
<thead>
<tr>
<th>Patients</th>
<th>9c exceeding events</th>
<th>DNA stem line ploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>no</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>no</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>2.52 (12 cells)</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>2.25 (37 cells)</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>4.68 (35 cells)</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>no</td>
</tr>
</tbody>
</table>

Discussion

The International Consensus Conference on the Fight Against Cervical Cancer (International Academy of Cytology Task Force 8) recommended DNA-ICM as a useful adjunctive method for identifying cervical intraepithelial lesions which require further clinical management [16]. Studies that applied a retrospective or prospective design reported high PPVs (84-100%) for the development of in situ or invasive carcinoma from mild to moderate cervical dysplasias with proven DNA aneuploidy [3-5, 7, 14, 17]. Compared to DNA-ICM, HPV testing and surrogate molecular markers of HPV infection (p16INK4a) may also help identify cases that are associated with underlying CIN; however, the PPVs of these diagnostic methods are reported to be much lower [1].

In the current study, all 24 (100%) lesions confirmed as CIN by subsequent histology had preceding DNA aneuploid smears. This confirms the high value of DNA-ICM for identification of CIN in cervical cytology. In contrast, six (20%) patients with DNA aneuploid cell abnormalities showed negative in subsequent histology and during follow-up of three years. This is interpreted as morphological regression, showing that CIN has a substantial variability in prognosis. Four of six patients with regressive morphological follow-up revealed DNA stem line aneuploidy and two had single cell DNA aneuploidy.

DNA stem line aneuploidy reflects the clonal expansion of cells with distinct chromosomal aneuploidy. Abnormal stem lines have also been reported in most invasive cervical carcinomas and have exhibited some degree of correlation with tumour grade and histologic subtype [18-20]. In addition to stem line abnormality, rare events may indicate DNA aneuploidy. These events are likely to be attributable to nonproliferating abnormal cells with different chromosomal aneuploidies and abnormally high numbers of chromosomes [10]. Therefore, rare events may also serve as markers of malignant cell transformation, even if they are not relevant to tumour growth [7].

From the clinical viewpoint, immune surveillance plays a critical role in spontaneous regression, persistence or progression for CIN. Lesions are cleared as a result of a successful cell-mediated immune response directed against early human papilloma virus (HPV) proteins [21]. The HPV type and other partly unknown factors play a role in this. The most comprehensive review of the literature on progression, regression, and persistence rates for CIN comes from a compilation of all studies on the natural history of CIN dated from 1952-1992 [22]. In this review, regression rates for CIN 1 were 60%; for CIN 2 they were 40% and for CIN 3 they were 33%.

Our DNA cytometric finding of a 20% regression rate in patients with DNA aneuploid cell abnormalities is well comparable with the findings reported by Ostor [22] and may reflect a special feature of squamous intraepithelial lesions with HPV protein expression. In squamous intraepithelial lesions without HPV protein expression (i.e., oral cavity), a closer relationship between DNA aneuploidy and clinical outcome was reported [23].
In summary, DNA-ICM should be used as an objective first-line diagnostic tool for predicting cervical precancer. Yet, due to immune response, DNA aneuploid cervical cell abnormalities do not seem to be sufficient to predict the definitive clinical outcome in each case. Longer follow-up is necessary to confirm these findings.

Acknowledgement
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References

Human telomerase RNA gene (TERC) gain and polysomy of chromosome 3 in cervicovaginal liquid-based pap preparations: a fluorescence in situ hybridization study

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Summary

Purpose. This study investigated human telomerase RNA gene (TERC) gain and polysomy of chromosome 3 in cervicovaginal liquid-based pap preparations in Guilin, China, and assessed the relationship between FISH findings and clinical diagnoses.

Methods. Slides prepared from 63 liquid-based preparations with cytologic diagnoses of negative for squamous intraepithelial lesion or malignancy (NILM n = 9), atypical squamous cells of undetermined significance (ASCUS, n = 18), low-grade squamous intraepithelial lesion (LSIL, n = 14), high-grade squamous intraepithelial lesion (HSIL, n = 9), and cervical squamous cell carcinoma (SCCA, n = 13) were analyzed for TERC gain and polysomy of chromosome 3 using a commercially available two-color FISH probe. The results of the cytologic analysis and those of concurrent or subsequent biopsies, when available, were compared with the FISH findings. The Mann-Whitney test was used to assess associations between FISH findings and diagnoses.

Results. TERC gain and polysomy of chromosome 3 were significantly associated with the cytologic diagnosis (p < 0.001). Patients with HSIL or SCCA cytology diagnoses had a significantly higher percentage of cells with TERC gain and polysomy of chromosome 3 than did patients with NILM, ASCUS or LSIL cytologic diagnoses. Those abnormal cases with CIN1 histological diagnosis had a significantly lower percentage of cells with TERC gain and polysomy of chromosome 3 than did patients with a CIN2, CIN3 and SCCA histological diagnosis.

Conclusions. TERC gain and polysomy of chromosome 3 may be important associated genetic events in cervical intraepithelial neoplasia and carcinoma. FISH is a potential tool for the diagnoses of uterine cervix disease.

Key words: FISH; TERC; Chromosome 3; Pap preparations; Uterine cervix disease.

Introduction

Despite substantial progress in understanding cervical carcinogenesis and the development of advanced preventive measures, cervical carcinoma continues to be a leading cause of cancer death among women worldwide [1]. There are about 510,000 cases of cervical carcinoma reported annually: 68,000 in Africa, 77,000 in Latin America, and 245,000 in Asia [2]; The People’s Republic of China has historically been considered at relatively low risk for cervical carcinoma, but nationwide mortality surveys show a variable pattern of risk across the country, which is on the increase among younger women, particularly in urban settings [3].

For the last 50 years, cervical cytology has provided the cornerstone of cervical carcinoma prevention programs, but the limited sensitivity of cervical cytology makes these programs difficult and expensive to maintain [4]. Fortunately, astonishing progress has been made in our understanding of the pathogenesis of cervical carcinoma. For example, infection with human papilloma virus (HPV) is considered to be the initiating factor in the carcinogenesis of uterine cervix, and HPV prevalence in any given population correlates well with cervical carcinoma risk [5, 6]. In addition, numerical chromosomal aberrations, such as aneuploidy and tetraploidy, are the most prevalent genetic changes observed in human solid tumors [7], have been reported in women diagnosed with precancerous and cancerous cervical lesions [8]. Recently, some researches have documented that chromosome arm 3q gain is a common feature of squamous cell carcinoma, with an overlapping area of gain at 3q26 having been reported in squamous cell carcinoma at different anatomic sites, including the lung, head and neck, esophagus, and cervix of the uterus [9]. Interestingly, chromosome arm 3q26 region contains the human telomerase RNA gene (TERC), encoding one main component of telomerase, which is of interest to research because of the correlation between telomerase activity and tumorigenesis in later years [10]. However, a study dealing with these genetic events of cervical squamous cell lesions in different regions worldwide, including both preneoplastic/preinvasive proliferations and early-stage invasive carcinomas of the cervix is still lacking. In this study, we investigated the prevalence of TERC gain and polysomy of chromosome 3, as measured by fluorescence in situ hybridization (FISH), in routine liquid-based cytologic preparations in Guilin, China, and assessed the associations between the FISH finding and clinical diagnoses.
Table 1. — Summary of TERC gain according to cytology.

<table>
<thead>
<tr>
<th>Cytology diagnosis</th>
<th>No. of cases</th>
<th>No. of cases with TERC gain</th>
<th>Mean of cells with TERC gain (%)</th>
<th>Mean of cells with polyosity 3 (%)</th>
<th>Range of cells with TERC gain (%)</th>
<th>Range of cells with polyosity 3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NILM</td>
<td>9</td>
<td>2</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ASC-US</td>
<td>18</td>
<td>10</td>
<td>1.2</td>
<td>0.1</td>
<td>0-6</td>
<td>0-1</td>
</tr>
<tr>
<td>LSIL</td>
<td>14</td>
<td>8</td>
<td>1.3</td>
<td>0.1</td>
<td>0-6</td>
<td>0-1</td>
</tr>
<tr>
<td>HSIL</td>
<td>9</td>
<td>9</td>
<td>15.6</td>
<td>10.4</td>
<td>2-40</td>
<td>1-27</td>
</tr>
<tr>
<td>SCCA</td>
<td>13</td>
<td>13</td>
<td>42.8</td>
<td>24.9</td>
<td>6-96</td>
<td>6-74</td>
</tr>
</tbody>
</table>

Methods and Materials

Cervicovaginal specimens

The 63 cases of specimens made from liquid-based preparations and collected from the cervix were confirmed cytology diagnoses and the cases with abnormal cytology results were further confirmed by histological diagnosis. The residual material of liquid-based preparations was prepared for FISH analysis. All these 63 patients were informed of the study’s goals and all signed a consent form.

In the performance of cytologic diagnosis, the SUREPATH (TriPath Imaging, Inc., Burlington, NC) liquid-based preparations were stained using the papanicolaou screened by a cytotecnologist, and interpreted according to the Bethesda System [11] as follows: negative for squamous intraepithelial lesion or malignancy (NILM, 9 cases); atypical squamous cells of undetermined significance (ASC-US, 18 cases); low-grade squamous intraepithelial lesion (LSIL, 14 cases); high-grade squamous intraepithelial lesion (HSIL, 9 cases); and cervical squamous cell carcinoma (SCCA, 13 cases). The histologic diagnoses were obtained from the pathology database of Guilin 181st Hospital, China, and categorized as follows: negative for squamous intraepithelial lesion or malignancy (NILM), intraepithelial neoplasia (CIN1, CIN 2, and CIN 3), and SCCA.

Cervical cell collection and preparation

The cervicovaginal specimens which were residual material of liquid-based preparations were centrifugated and rinsed in 10 ml physiological saline two times, then digested in 5 ml collagenase at the temperature of 37°C (0.5) for 10 min, and centrifugated again, abandoning the supernate fluid, and hypotension with the solution of KCl (0.075 mol/l) at the temperature of 37°C (0.5) for 20 min, and then centrifugated again, abandoning the supernate fluid, and fixed in 5 ml of amethanol: acetic acid mixture (3:1) two times. After centrifugation, and abandoning the supernate fluid until the volume was 0.2~0.6 ml, the cells were misce bene slightly. At last, the cervical cell suspension was dropped onto clean glass microscope slides, open-air dried, and baked at 56°C [1] for 30 min.

FISH analyses were performed in cooperation with the manufacturer of China Medical Technologies, Inc. (Beijing, China). The commercially available two-color FISH probe consists of two probes: 3q26 (Rhodamine, red) covering the whole gene of TERC and the centromeric chromosome 3 (FITC, green). The FISH fixed glass microscope slides were placed in 2 × sodium saline citrate (2 × SSC, pH 7.2) at room temperature for 3 min, digested in a protease solution (20 mg/ml, pH 2.0) for 5-12 min at 37°C, rinsed in 2 × SSC at room temperature for 3 min, and fixed in 1% formaldehyde for 10 min at room temperature. After dehydration in a graded series of concentrations of ethanol, the slides were dried in the open-air. To denature DNA, the slides were placed in 78.5°C preheated 70% formamide/2 × SSC for 8 min and then were dehydrated in a graded series of concentrations of ethanol which were precooled in -20°C. After being dried in the open-air, 10 μl of probe destructured at 75.5°C for 7 min was applied onto each slide, which was then coverslipped and sealed with rubber cement, then hybridized overnight at 42.8°C. After 16-18 h of hybridization, the slides were washed in 46°C preheated post-hybridization buffer (2 × SSC/0.1% sodium dodecyl sulfate) for 5 min and rinsed in 70% ethanol. After air-drying (out of direct light), the slides were counterstained with 15 μl DAPI/anti-fade solution and coverslipped.

FISH analyses were performed by cytotecnologists who were blinded to the clinical diagnoses at the time of evaluation. The slides were scanned using an OLYMPUS BX51 fluorescent microscope (OLYMPUS BX51, Japan) equipped with a 100-watt mercury lamp and filter set to detect DAPI, FITC (chromosome 3), and Rhodamine (TERC) at 1000×; 100 epithelial cells were noted for the signal numbers of both TERC and centromeric chromosome 3. To avoid counting split signals as two signals, the distance between any two signals had to be at least the diameter of one signal for them to be counted as separate signals. A cell scored as amplification of TERC was defined as a ratio > 1.0 between the TERC and the chromosome 3 copy number; polysomy 3 by the finding of more than two specific signals for chromosome 3.

The Mann-Whitney test was used to assess associations between FISH findings and diagnoses. All computations were carried out using SPSS 13.0 for windows.

Results

Cervicovaginal specimens were obtained from 63 women, ranging in age from 23 to 63 years (mean age, 42 years). Twenty-seven of the 63 cases had HPV (16 and 18) tested. Of the nine NILM cases, none were HPV positive; of the 18 ASC-US cases, all showed CIN1 on biopsy; of the 14 LSIL cases, 13 showed CIN1 and one case showed CIN2 on biopsy and two cases were HPV positive. Of the nine HSIL cases, three cases showed CIN2, six cases showed CIN3, and one case showed SCCA on biopsy, and two cases were HPV positive. All SCCA cytologic diagnoses were confirmed by biopsy and eight cases were HPV positive.

Representative features of TERC gain and polysomy 3 are depicted in Figure 1. Table 1 summarizes TERC gain and polysomy of chromosome 3 results by FISH. Only two of NILM cases showed one to two epithelial cells with TERC gain, and none of them showed epithelial cells with polysomy of chromosome 3. Ten of ASC-
US cases showed one to six epithelial cells with TERC gain, and three cases showed one epithelial cell with polysomy of chromosome 3. Eight of LSIL cases showed one to six epithelial cells with TERC gain, and only one case showed one epithelial cell polysomy of chromosome 3. All HSIL cases showed two to 40 epithelial cells with TERC gain and one to 27 epithelial cells with polysomy of chromosome 3. All of SCCA cases showed three to 96 epithelial cells with TERC gain, and six to 96 epithelial cells with polysomy of chromosome 3.

The FISH finding (TERC gain and polysomy of chromosome 3) were significantly associated with the cyto logic diagnosis and histological diagnosis ($p < 0.001$; Table 1). Cases with HSIL or SCCA cytologic diagnoses had a significantly higher percentage of cells with TERC gain and polysomy of chromosome 3 than did patients with a NILM, ASC-US or LSIL cytologic diagnosis. Those abnormal cases with CIN1 histological diagnoses had a significantly lower percentage of cells with TERC gain and polysomy of chromosome 3 than did patients with a CIN2, CIN3 and SCCA histological diagnosis.

**Discussion**

The main findings of our investigation can be summarized as follows: (a) TERC gain and polysomy of chromosome 3 may be two early genetic events associated with cervical affection; (b) TERC gain and polysomy of chromosome 3 may be involved in the progression of cervical carcinogenesis, and they are allowed distinction of cervical affection to some extent. (c) FISH may be an adjunct to cytology screening to detect patients with high-grade lesions.

A great deal of evidence has shown that infection with distinct types of HPV is the primary risk factor for the development of cervical carcinoma [12-15]. The most critical molecules in HPV replication are E6 and E7, which functionally inactivate the products of two important tumor suppressor genes, p53 and pRb, respectively. Both oncoproteins induce proliferation, immortalization, and malignant transformation of the infected cells [16]. However, evidence supports the theory that cervical squamous cell carcinoma is not simply caused by a single transforming event, and some researchers have documented that chromosomal instability as manifested by increases...
in aneuploidy and structural chromosome aberrations could be found in most cervical carcinomas, especially chromosome 3q which contains the human telomerase RNA gene (TERC) [17-19].

Heselmeyer-Haddad et al. used FISH to investigate previously stained papanicolaou tests for the amplification of TERC. They took images of abnormal cells that were situated somewhere in previously stained papanicolaou tests. After FISH detecting (using the same previously stained papanicolaou tests), the same cells were again imaged and analyzed if they were a positive gain of TERC. Their findings constituted a comprehensive retrospective evaluation of papanicolaou tests in an attempt to validate amplification of TERC [17]. Caraway et al. performed FISH on cervicovaginal liquid-based preparations to analyze the most atypical 25 cells for abnormal signal numbers with a 3q26 gain, and they found that gain of 3q26 was associated with high-grade squamous intraepithelial lesions and cervical SCCA [18]. Alaharski et al. performed FISH to analyze cervical cell samples collected from 143 cases of patients with diagnoses ranging from NILM to HSIL, and found that the frequencies of cells exhibiting either tetrasomy or aneusomy for chromosomes 3 & 17 increased significantly with disease progression [8]. There are also other similar researches [19-21]. In our research, we analyzed abnormal cells on liquid-based cervicovaginal preparations for TERC gain similar to that used by Caraway et al. However, our research is not simply repeating their works because we used another probe (centromeric chromosome 3) defining a cell scored as TERC gain by a ratio > 1.0 between the TERC and the chromosome 3 copy number, which could discern gene gain from polysomy. The cells selected for the assessment of TERC gain in this research were different from Caraway et al. as well; we chose more cells and analyzed 100 epithelial cells for the assessment of signal numbers of both TERC and centromeric chromosome 3 for each case. In addition, the genetic makeup, lifestyle, and individual sanitation of Chinese people are somewhat different from other races, thus all of these are needed to be further researched. From our data, it can be seen that two cases with NILM had one or two abnormal cells with another copy of TERC. This indicates that evaluation for the threshold of positive TERC gain is needed because those abnormal signals may be caused by the experiment itself or there may be little clinical value with only a small proportion of abnormal cells. In LSIL cases, there were eight cases with abnormal cells of TERC gain, and only one case with polysomy of chromosome 3. It may be that polysomy of chromosome 3 is a later genetic event compared to TERC gain. However this research can not provide enough evidence to support it.

In short, TERC gain and polysomy of chromosome 3 are significantly associated with clinical diagnosis; FISH may be an adjunct to cytology screening to detect high-grade lesions and assess the risk of cervical carcinogenesis. Although this study can not prove the association between multiple HPV infection and findings, our work may help with early detection of cervical carcinogenesis.

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References


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Prognostic significance of topoisomerase II alpha and collagen IV immunoexpression in cervical cancer

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Introduction

Cervical cancer continues to remain a major health problem worldwide. According to the latest WHO statistics, cervical cancer is second only to breast cancer among the most frequent female malignancies worldwide [1].

The prognosis of cervical cancer is determined by several predictors, and according to the recent task force on prognostic factors in cervical cancer, there is an urgent need for more specific markers capable of predicting the disease outcome in individual patients.

As the major prognostic factor, the depth of invasion is one of the most widely used parameters of cervical cancer that shows an evident statistically significant relationship with the presence of lymph node metastasis, recurrence and death from cancer in many studies. All other prognostic factors, such as horizontal spread, involvement of lymphovascular space, type of invasion, cell type and additional factors are considered as non-independent prognostic factors. Certain biological factors have been suggested lately that might predict the course of cervical cancer, topoisomerase II alpha and collagen IV being two of them [2, 3].

In the present study, the immunohistochemical expression of topoisomerase II alpha and collagen IV was studied in cervical cancer. The results of IHC expression for both markers were compared to the clinical and histological factors predicting the course of disease. The major aim of this study was to find which of the analyzed factors has the highest predictive power for predicting cervical cancer outcome in individual patients.

Material and Methods

Patients

Clinical and morphologic data were obtained from 114 patients with cervical cancer, treated at the Department of Gynecology and Obstetrics, University Medical Centre Ljubljana, between 1995 and 1999. The mean patient age was 42.3 ± 11.5 (SD) years (range 25-88 years). After the diagnosis was confirmed, the patients were treated by radical hysterectomy, conization or radiotherapy. After therapy, the patients were closely followed-up; mean follow-up time was 69.64 ± 27.71 (SD) months, range 7-80 months.

Histology and immunohistochemistry

Both colposcopy biopsies and surgical samples were fixed in 10% buffered formalin, embedded in paraffin, and processed for 5-μm-thick paraffin sections stained with hematoxylin-eosin for routine diagnosis. The morphological and morphometric char-
The formalin-fixed, paraffin-embedded tissue sections were stained with monoclonal antibodies to topoisomerase II alpha (Ki-S1) and collagen IV with a standard streptavidin immunohistochemical technique, with antigen retrieval to assess the presence of enzymes. The results were based on nuclear staining and percentage of positivity. Figures 1-2 show tumor cells in the cervix after IHC staining.

Statistical analyses

The chi-square analysis was used to test the relationship among the variables. Multivariate analysis by Cox’s proportional hazards model was used to rank the importance of each prognostic factor in prescribing treatment. Kaplan-Meier survival analysis was used to calculate the survival times. Statistical significance was set at \( p < 0.05 \).

Results

Clinical characteristics of patients and results of pathomorphological analysis

The frequency of histologic types according to the WHO classification and frequency of cases grouped into four histologic subtypes, grade, clinical stage according to FIGO, state of lymph nodes, different intensity of defense reaction, and lymphovascular invasion in 114 patients are shown in Table 1.

Results of immunohistochemical staining

The frequency of immunostaining intensity and the percentages of tumor cells stained for topoisomerase II alpha and to collagen IV are shown in Table 2.
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Somatase II alpha was significantly decreased in adenocarcinoma compared to intense IHC in squamous cell carcinoma and was significantly associated with nuclear grade \( (p = 0.038) \) and defense reaction \( (p = 0.001) \).

Using the chi-square test we found statistically significant correlations between various degrees of defense reaction in the presence of carcinoma as well as between the intensity of IHC expression of collagen IV and the percentage of cells stained for collagen IV. In less intense IHS to collagen IV, which reflects the loss of components of the basal membrane and the thinning of collagen fibres in invasive carcinomas, a well expressed defense reaction was found present.

Table 3 shows histologic types compared with percentage of positive immunostaining for topoisomerase II alpha and nuclear grade and defense reaction compared with intensity of immunostaining for topoisomerase II alpha.

Survival

The mean patient age was 42.3 years (± SD 11.5 years, range 25-88 years). According to data from the Cancer Registry of Slovenia, 83 patients (72.8%) were alive, 23 (20.2%) had died, and the information for eight (7%) patients was lost by 30 April 2004, the end of the observation period. The mean survival time by then was 69.64 months (± 27.71 months).

The intensity and share of immunostaining for topoisomerase II alpha had no significant influence on mean patient survival time. However the mean survival time was significantly shorter in cases with negative and weak immunostaining for collagen IV \( (p = 0.011) \).

Kaplan-Meier analysis showed statistically significantly better survival in women with initial stages of cervical cancer \( (p = 0.001) \), higher degree of tumor differentiation \( (p = 0.049) \), more shallow depth of tumor invasion \( (p = 0.004) \), smaller horizontal tumor spread \( (p = 0.001) \), in cases without lymph node metastases \( (p = 0.001) \) and lymphovascular space invasion \( (p = 0.001) \), in younger age groups \( (p = 0.001) \) and in women with regular menstrual cycle \( (p = 0.001) \).

Results of multivariate analysis

To determine the importance of each individual prognostic factor in deciding on the treatment of cervical cancer, multivariate analysis with the Cox proportional hazards model was performed.

In the logistic regression model we put the following variables: FIGO stage of disease, patient age, tumor differentiation degree, defense reaction, lymphovascular space invasion, and intensity of IHC staining for topoisomerase II alpha and collagen IV. The patient survival time was the dependent variable. Using the step-by-step method we found that the most important variable in the survival time prediction for women with cervical cancer was lymphovascular space invasion followed by intensity of IHC staining to collagen IV (Table 4).

Table 3. — Histologic types compared with percentage of positive immunostaining for topoisomerase II alpha and nuclear grade and defense reaction compared with intensity of immunostaining for topoisomerase II alpha.

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>100%</th>
<th>75%</th>
<th>50%</th>
<th>25%</th>
<th>&lt; 25%</th>
<th>Negative</th>
<th>Not estimated</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>25 (36.2)</td>
<td>18 (26.1)</td>
<td>14 (20.3)</td>
<td>3 (4.3)</td>
<td>2 (2.9)</td>
<td>3 (4.3)</td>
<td>4 (5.8)</td>
<td>69 (100.0)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>11 (29.7)</td>
<td>4 (10.8)</td>
<td>8 (21.6)</td>
<td>0 (0.0)</td>
<td>1 (2.7)</td>
<td>8 (21.6)</td>
<td>5 (13.5)</td>
<td>37 (100.0)</td>
</tr>
<tr>
<td>Others</td>
<td>2 (100.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>Adeno&amp;squamous cell carcinoma</td>
<td>1 (16.7)</td>
<td>2 (33.3)</td>
<td>1 (16.7)</td>
<td>2 (33.3)</td>
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<td>Total</td>
<td>3 (34.2)</td>
<td>24 (21.1)</td>
<td>23 (202)</td>
<td>5 (4.4)</td>
<td>3 (2.6)</td>
<td>11 (9.6)</td>
<td>9 (7.9)</td>
<td>114 (100.0)</td>
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<th>Nuclear grade</th>
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<th>75%</th>
<th>50%</th>
<th>25%</th>
<th>&lt; 25%</th>
<th>Negative</th>
<th>Not estimated</th>
<th>Total</th>
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<tbody>
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<td>Grade 1</td>
<td>9 (23.7)</td>
<td>5 (13.2)</td>
<td>12 (31.6)</td>
<td>9 (23.7)</td>
<td>3 (7.9)</td>
<td>38 (100.0)</td>
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<td>Grade 2</td>
<td>2 (3.0)</td>
<td>5 (7.6)</td>
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<tr>
<td>Total</td>
<td>11 (9.6)</td>
<td>11 (9.6)</td>
<td>38 (33.3)</td>
<td>45 (39.5)</td>
<td>9 (7.9)</td>
<td>114 (100.0)</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Defense reaction</th>
<th>100%</th>
<th>75%</th>
<th>50%</th>
<th>25%</th>
<th>&lt; 25%</th>
<th>Negative</th>
<th>Not estimated</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>6 (25.0)</td>
<td>5 (20.8)</td>
<td>6 (25.0)</td>
<td>7 (29.2)</td>
<td>0 (0.0)</td>
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<tr>
<td>Poor</td>
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<td>4 (10.5)</td>
<td>17 (44.7)</td>
<td>15 (39.5)</td>
<td>0 (0.0)</td>
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<td>Moderate</td>
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<td>2 (7.1)</td>
<td>11 (39.3)</td>
<td>13 (46.4)</td>
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<tr>
<td>Strong</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (33.3)</td>
<td>4 (66.7)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not estimated</td>
<td>1 (5.6)</td>
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<td>2 (11.1)</td>
<td>6 (33.3)</td>
<td>9 (50.0)</td>
<td>18 (100.0)</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>11 (9.6)</td>
<td>11 (9.6)</td>
<td>38 (33.3)</td>
<td>45 (39.5)</td>
<td>9 (7.9)</td>
<td>114 (100.0)</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 4. — Results of logistic regression step by step method - regression model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient</th>
<th>Standard deviation</th>
<th>p value</th>
<th>Odds ratio with 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphovascular space invasion</td>
<td>3.302</td>
<td>1.141</td>
<td>0.004</td>
<td>27.1 (2.9-254.3)</td>
</tr>
<tr>
<td>Negative and weak expression of IHC</td>
<td>2.145</td>
<td>0.954</td>
<td>0.025</td>
<td>8.5 (1.3-55.4)</td>
</tr>
<tr>
<td>staining to collagen IV</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
In our study a specific prognostic factor for lymphovascular space invasion, which is a poor prognostic factor as it permits lymphogenic carcinoma metastasis, proved to be the intensity of the IHC staining to collagen IV.

In the multivariate analysis the intensity of IHS to collagen IV was significantly associated with lymphovascular invasion (OR = 5.906; 95% CI 2.18-15.96).

Discussion

Cervical cancer is the only cancer that can be efficiently prevented by detection of precancerous lesions [4]. Efficient early detection and treatment of cervical cancer involves knowledge of molecular mechanisms [5, 6]. Currently, the main focus is given to the interaction between the tumor and the matrix, interaction between growth factors and components of intercellular, processes of angiogenesis and biochemical markers [5, 6]. Determination of these biochemical markers may be helpful in the diagnosis and prediction of the disease course, and in the decision on the treatment modality, and in early detection of recurrent and metastatic disease.

The stage of cervical cancer is the most important prognostic factor for the course of disease, even if it is detected early (stage IB, IIA) and treated surgically. A greater tumor size represents a higher risk of lymph node metastases and predicts a poorer course of disease [7]. Thus, in our study the women with early stages of disease at diagnosis (IA and IB) also had a significantly longer survival time than those with the disease diagnosed at Stage II or higher (p = 0.001).

Depth of invasion ≥ 5 mm combined with horizontal spread ≥ 7 mm meant a significantly shorter mean survival time. Depth of invasion was manifest as increased intensity of IHC-stained topoisomerase II alpha expression, the quicker the carcinoma growth. The same conclusion was obtained by Gibbons et al. after they investigated topoisomerase II alpha and MIB-1 expression in changed squamous epithelium of the uterine cervix and assessed their power as new proliferation markers. A number of recent studies have shown that increasing depth of invasion in cervical cancer increases the risk of lymph node metastases, recurrence of disease and death by cervical cancer [8]. The depth of invasion is one of the major prognostic factors for lymph node metastases [9].

The mean survival time of the women enrolled in our study was significantly longer in women with endometrioid type of adenocarcinoma or with squamous cell carcinoma. The survival time was the shortest in women with small cell carcinoma. This is in line with other authors claiming that small cell non-differentiated carcinomas have a high malignant potential and an extremely quick spread of metastatic lymph nodes via lymphovascular spaces [10, 11].

Metastases of cervical cancer in neighbouring or remote lymph nodes are among the major prognostic factors for the outcome of cervical squamous cell and adenocarcinomas [12]. Numerous studies over the past years aimed at finding the complicated mechanisms of morphologic reactions in lymph nodes, occurring as the host’s immune response to carcinoma [12]. According to many authors vascular invasion is a poor prognostic factor for the course of cervical cancer [11]. In our series the women without lymph node metastases had a significantly longer mean survival time than the women with detected metastases.

Stromal reaction, the so-called defense reaction around carcinoma, as an interaction between the tumor and the host is still insufficiently investigated. Roughly we know two types of stromal reactions: reaction of the connective tissue and infiltration with immunocompetent cells. Sano et al. have found that an increased number of plasma cells, reticular fibres and a high collagen/collagenase ratio act as a protective mechanism against the invasion of carcinoma [13].

Proliferation tumor activity was in our study manifested as increased intensity of IHC-stained topoisomerase II alpha and good defense reaction around the tumor. When the defense reaction is good, collagen is thinned due to increased number of plasma cells, reticular fibres and a high collagen/collagenase ratio act as a protective mechanism against the invasion of carcinoma [13].

In 114 women the presence of topoisomerase II alpha and collagen IV in tumor tissue was determined using IHC. When the percentage of fibres stained for collagen IV using IHC was 100%, the intensity of topoisomerase II alpha expression was negative, and vice versa when the fibres stained for collagen IV remained uncolored, the intensity of topoisomerase II alpha expression was the highest. Topoisomerase is a marker of cell proliferation, thus the higher the intensity of topoisomerase II alpha expression, the quicker the carcinoma growth. The same conclusion was obtained by Gibbons et al. after they investigated topoisomerase II alpha and MIB-1 expression in changed squamous epithelium of the uterine cervix and assessed their power as new proliferation markers. The number of positive nuclei in the epithelium increased according to progressive changes in the epithelium from the basal layer to its entire depth [15].
The intensity of topoisomerase II alpha expression and the percentage of stained tumor cells were significantly higher in women after conization than in those after hysterectomy or radiotherapy. This indicates a high degree of proliferation activity of early-stage cervical carcinomas with a small volume, which should not be underestimated for this very activity. The fact that we are dealing with malignant neoplasmas is confirmed by the development of carcinoma in lymphovascular spaces in cases of initial invasion of carcinoma Stage IA when the depth of invasion does not exceed or is even less than 3 mm. It can be presumed that increased carcinoma volume leads to relatively poorer blood supply, hence the decreased carcinoma growth, and consequently the poorer expression of proliferation markers.

The percentage of cell nuclei stained for topoisomerase II alpha was significantly lower in adenocarcinomas than in squamous cell carcinomas. Also, the course of disease was more favorable to the women with the former compared to those with the latter carcinoma. The results of studies on the predictive value of histologic carcinoma subtype for the course of cervical cancer are contradicting [20]. Villoglandular adenocarcinoma, adenoid and basal cell carcinomas all predict a good course of disease. Poorer is the prognosis for the course of serous papillary carcinoma. Waldenstrom et al. have recently published survival results for women with adenocarcinoma in Sweden [20]. The 5-year survival rate of women with adenocarcinoma was 64%, and of those with squamous cell carcinoma 66%. The authors concluded that the survival rates are similar under the condition that their treatments are similar.

In our study we have found statistically significant correlations between the intensity of topoisomerase II alpha expression and degrees of tumor differentiation. With grade 1, the intensity of expression was negative in 23.7%, whereas with grade 3, it was highly positive in 50% of cases. Today, the degree of tumor differentiation is generally not considered to have an important predictive value in squamous cell carcinoma [21], whereas in adenocarcinoma it is considered a reliable prognostic factor for the course of cervical cancer [14].

Collagen IV plays an important role in tumor invasion and metastasis; immunohistochemically it is displayed in the basal membrane on the borderline between the epithelium and the stroma. Collagen IV shows whether the basal membrane in the cancerous tissue is discontinued or completely erased. Therefore, a negative and weak IHC expression to collagen IV reflects a complete absence of collagen fibres or decreased collagen in the basal membrane and in the connective stroma around the cancerous tissue of invasive carcinoma [19, 22-26].

Favret et al. analyzed the distribution of laminin, collagen IV and fibronectin in dysplasias and neoplastic changes on the uterine cervix using the IHC method [22]. The analysis was made on normal cervical tissue (16 cases), in cervical dysplasia (14 cases) and in invasive carcinomas (45 cases). It was found that in normal and dysplastic epithelium the distribution of laminin and collagen IV in the basal membrane was linear and continuous. In in situ carcinoma small discontinuations and changes in linearity were seen; whereas in microinvasive carcinomas erasement of the basal membrane was observed. In well differentiated invasive carcinoma dotted discontinuations of the basal membrane around neoplastic islands were noted. In contrast to moderate and poorly differentiated carcinomas, the decrease and loss of positive stain reaction to laminin and collagen IV was even more progressive. The results of this study show that the distribution pattern of intrinsic components of the basal membrane is proportional to the histologic grade of cervical neoplasia and the invasion ability [23]. Toki et al. investigated laminin and collagen IV spread in the basal membrane of 45 cervical adenocarcinoma cases using the IHC method [23]. They found the staining pattern to be in agreement with the grade of differentiation.
Conclusion

In conclusion IHC expression of topoisomerase II alpha and collagen IV was significantly correlated with defense reaction. A negative and weak IHS to collagen IV was a statistically significant independent predictive variable for lymphovascular invasion, related to metastatic spread in the lymph nodes. The two analyzed IHC markers indicate the existence of factors at the molecular level that might complement the assessment of prognosis of cervical cancer, resulting in appropriate adjustment of the type and extension of cervical cancer treatment.

References


Epigenetic therapy and cisplatin chemoradiation in FIGO Stage IIIB cervical cancer

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L. Taja-Chayeb1, J. Chanona3, D. Arias2, A. Dueñas-González3

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Summary

Introduction: This trial aimed to evaluate the safety and efficacy of epigenetic therapy associated with cisplatin chemoradiation in FIGO Stage IIIB patients. Methods: Hydralazine containing either 182 mg for rapid-, or 83 mg for slow acetylators and magnesium valproate were administered at 30 mg/kg tid. Both drugs were taken until intracavitary therapy was finished. Pelvic external beam radiation and low-dose rate brachytherapy were administered at a total cumulative dose to point A of at least 85 Gy. Weekly cisplatin at 40 mg/m² was delivered for six cycles. Results: Twenty-two patients were included and 18 (82%) patients completed treatment. Mean dose to point A was 84.6 ± 2.2. Median number of cisplatin cycles was 5.5 (range, 1-6). Brachytherapy was delayed for technical reasons; the mean overall treatment time was 11.8 weeks. Grade 3 anemia, leucopenia, neutropenia, and thrombocytopenia were observed in 9%, 45%, 45%, and 9% of patients, respectively. Conclusions: Hydralazine and valproate are well-tolerated and safe when administered with cisplatin chemoradiation. Unfortunately, the suboptimal administration of brachytherapy for technical reasons in this study, precluded assessing the efficacy of epigenetic therapy. However, the tolerability of this regimen administered concurrent to radiation needs to be further tested.

Key words: Epigenetic therapy; Hydralazine; Valproate; FIGO Stage IIIB cervical cancer; Chemoradiation.

Introduction

DNA hypermethylation and histone deacetylation are critical for determining a closed chromatin structure responsible for or related with aberrant gene transcription in malignancies [1]. The strong interplay between DNA hypermethylation and histone deacetylation for silencing the expression of cancer-related genes predicts a synergy in gene expression and antitumor activity of these two agent-types [1, 2]. DNA methylation and histone deacetylase inhibitors (HDAC-I) exert their antitumor effects by inhibiting cell proliferation, metastasis, angiogenesis, and by inducing cell differentiation and/or apoptosis, as well as by increasing chemotherapy cytotoxicity [3, 4]. DNA methylation inhibitors 2-deoxy-5-azacytidine [5] and zebularine [6], as well as HDAC-I trichostatin A, vorinostat, M344, phenylbutyrate, depsipeptide [7-9], and valproic acid [10] are also radiosensitizers and reduce cutaneous radiation toxicity following radiotherapy [11]. Therefore, chemoradiation in combination with DNA demethylating agents and/or HDAC-I is a research avenue to be explored.

Hydralazine has been shown to demethylate and reactivate the expression of several tumor suppressor genes [12-15] and this activity is synergized when used in combination with valproic acid [16-18]. Clinical studies with this combination of epigenetic agents have demonstrated that they are well-tolerated when used with chemotherapy against a number of solid tumors and that they lead to re-expression of hundreds of genes in the primary tumors of cancer patients [19-21].

The DNA demethylating and reactivating activity of hydralazine in tumors was demonstrated in cervical cancer in an oral dose range of 50-150 mg/day [22]. Pharmacokinetic characterization in healthy volunteers of the slow-release hydralazine formulation employed in this study (unpublished) demonstrated under the curve (AUC) concentrations of 6,034 ± 1,899 ng/h/ml and 2,751 ± 954 ng/h/ml for slow- and rapid acetylators, respectively; hence, for further testing, doses of 182 mg/day and 83 mg/day for rapid- and slow acetylators, respectively, were chosen. This dose adjustment leads to similar hydralazine levels in plasma in both types of acetylators (means, 246 and 249 ng/ml, respectively) in patients with breast cancer [20].

Valproic acid causes hyperacetylation of the N-terminal tails of histones H3 and -4 in vitro and in vivo, and inhibits HDAC activity [23, 24]. Its ability to inhibit deacetylase activity in solid tumors has been demonstrated at doses between 20 and 60 mg/kg [25-27]. At doses of 30 mg/kg daily, we found HDAC inhibition in the breast cancer study at a mean plasma concentration of 87.5 g/ml [20]. Hydralazine and valproic acid in combination show inhibitory growth effect in vitro and in vivo, chemosensitization, a synergistic effect on global gene expression...
Patients and Methods

Patient selection. Previously untreated patients with a historical diagnosis of carcinoma of the cervix and International Federation of Gynecology and Obstetrics (FIGO) Stage IIIb were entered into this study. Additional eligibility requirements included the following: Eastern Cooperative Oncology Group (ECOG) performance status, ≤ 2; absolute leukocyte count, ≥ 4,000/mm$^3$; platelets, ≥ 100,000/mm$^3$; hemoglobin, ≥ 9.0 g/dl; total bilirubin, aspartate amino transferase, and alanine amino transferase (< 1.5 the upper normal limit); creatinine, ≤ 1.2 mg/dl; absence of paraaortic lymph node involvement as evaluated by computed tomography (CT) scan, and written informed consent. Patients were excluded from the study if they referred a history of allergy to hydralazine or valproate, past or present condition of rheumatic disease, central nervous system disease, heart failure from aortic stenosis and postural hypotension as diagnosed by a physician, and previous use of the two experimental drugs, as well as if patients were pregnant or breastfeeding.

Study design. This was a MinExpSize 2-stage, pilot, open-label, single-arm study. A sample size of 18 patients was established, estimating a 75% probability of attaining clinical complete response with cisplatin and radiation, and a 95% probability of clinical complete response rate when hydralazine and magnesium valproate were added to this regimen. With these parameters, the true type-I error rate, alpha is 0.047 and power of 0.818%. In the first stage of the study, four patients were evaluated; it was required that at least one of these obtain complete response to continue to the second stage. Sample size was calculated with ExpDesign Studio for Windows version 1.5.0 CtriSoft software (CtriSoft International).

Treatment plan. Patients received a single oral dose of 500 mg of sulphamethazine in the early morning, and urine was collected within the ensuing 6 h for phenotyping of acetylator status.

Afterwards, patients began treatment (day 0) with a daily dose of a slow-release formulation of hydralazine tablets containing either 182 mg for rapid-, or 83 mg for slow acetylators. Magnesium valproate tablets of 700 mg were also administered as a slow-release formulation at a dose of 30 mg/kg tid. Both drugs were administered until intracavitary therapy was completed.

Radiation. Patients received external beam radiation employing Co$^{60}$ or lineal accelerator equipment with a minimum photon-beam energy of 4 MeV at a target or skin source distance of 80 cm to the whole pelvis for a total dose of 50 Gy (5 weeks, 2 Gy fractions from Monday to Friday).

Patients were treated with the 4-field box technique. Brachytherapy was administered using Cesium sources after completion of external radiation, with the goal of maintaining the total treatment duration at fewer than eight weeks when possible. The protocol specified that all patients receive a total cumulative dose to point A of at least 85 Gy.

Chemotherapy. Cisplatin was administered during external radiation beginning on day 1 of radiation by peripheral vein in an out-patient setting as follows: hydration with 1000 ml of normal saline for 1 h followed by cisplatin diluted in 500 ml of normal saline containing 62.5 ml of 20% mannitol for 1 h, followed by 500 ml of normal saline for 30 min. Intravenous 8 mg of dexamethasone and 8 mg of ondansetron were employed as antiemetic prophylaxis.

Toxicity and dosage modification guidelines. Dose modification was not allowed. Cisplatin was withheld in any case of grade 3 toxicity until the toxicity regressed to any grade ≤ 3. In patients with grade 3 toxicity that persisted beyond two weeks, cisplatin was no longer administered. Radiation was only stopped in cases of grade 4 hematologic or non-hematologic toxicity until toxicity resolved to at least grade 3. Every effort was made to administer all six courses of cisplatin with radiation; however, when radiation was delayed < 1 week due to technical reasons the chemotherapy schedule remained unchanged; with longer periods, chemotherapy administration was also delayed. In cases in which radiation ended before chemotherapy was completed, the last dose of chemotherapy was administered as long as the time elapsed after last radiation dose did not exceed one week.

Evaluation of response and toxicity. Baseline work-up included a complete history and physical examination, complete blood cell count, blood chemistry, CT scan of abdomen and pelvis, and chest X-ray. Biopsies from the primary tumor were taken the day that hydralazine-valproate was initiated and at day 8, before starting cisplatin chemoradiation for pathological and molecular analysis. Clinical response was evaluated by bimanual pelvic examination at the end of external radiation, on completing brachytherapy, and at three months after treatment was ended. The evaluation at the third month also comprised cytological examination. A complete response was defined as no evidence of tumor. All other cases (persistence or progression) were registered as no complete response.

Toxicity was evaluated weekly during chemoradiation and thereafter at every visit utilizing National Cancer Institute (NCI) version 2 Common Toxicity Criteria. Laboratory assessment was performed weekly and consisted of complete blood cell count and blood chemistry. Follow-up included pelvic and cytological examination, blood counts, and clinical chemistry every three months on completion of treatment. Imaging studies were performed when clinically indicated.

Drug plasma levels. Valproic acid was measured in plasma employing fluorescence polarization immunoassay technology, as previously described [20], and hydralazine was determined in plasma by high-performance liquid chromatographic (HPLC) assay, as previously described [20].

Statistical analysis. Survival was calculated according to the Kaplan-Meier method. Progression-free survival was registered from date of patient entry into the protocol until date of progres-
sion. Overall survival was registered from date of patient entry into the protocol until death. For patients who were lost of follow-up, the date of death was investigated by phone call to the relatives. Statistical analysis was carried out on SPSS-10 software.

Results

Demographic characteristics. From May 2005 to June 2006 a total of 28 patients signed an informed consent to participate in the trial, however, six patients were ineligible, hence 22 were allocated to intervention. Mean age was 50.4 years (range, 28-69 years). All had a histologically confirmed diagnosis of cervical carcinoma and were in FIGO Stage IIIB. Other relevant characteristics of disease are shown in Table 1. Twelve patients (54.5%) were rapid- and ten (45.5%) were slow acetylators.

Table 1. — Clinical characteristics (22 patients).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (%)</th>
</tr>
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<tr>
<td>Mean age (years)</td>
<td>50.4 (28-69)</td>
</tr>
<tr>
<td>ECOG</td>
<td>3 (16)</td>
</tr>
<tr>
<td>1</td>
<td>19 (86)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
<td>21 (95.4)</td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>1 (4.6)</td>
</tr>
<tr>
<td>Parametrial infiltration</td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>2 (9)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>20 (90)</td>
</tr>
<tr>
<td>Tumor size (median)*</td>
<td>8 (5.9)</td>
</tr>
<tr>
<td>Pelvic lymph nodes**</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6 (27)</td>
</tr>
<tr>
<td>Negative</td>
<td>16 (37)</td>
</tr>
<tr>
<td>Mean basal hemoglobin (g/dl)</td>
<td>11.6 (4.0–15.1)</td>
</tr>
</tbody>
</table>

*Longest diameter in cm; **As evaluated by CT scan.

Table 2.

<table>
<thead>
<tr>
<th>Radiation treatment</th>
<th>18 patients</th>
</tr>
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<tbody>
<tr>
<td>Mean dose external beam radiation (Gy)</td>
<td>50.4(50-56)</td>
</tr>
<tr>
<td>Mean dose of brachotherapy (Gy)</td>
<td>33.36 (18.8-37)</td>
</tr>
<tr>
<td>Mean dose point A (Gy)</td>
<td>85 (68.6-86.8)</td>
</tr>
<tr>
<td>Time for delivering external beam radiation (weeks)</td>
<td>6.4 (5.5-7.1)</td>
</tr>
<tr>
<td>Overall treatment time (weeks)</td>
<td>11.8 (6-17)</td>
</tr>
<tr>
<td>Median # of weekly cisplatin courses</td>
<td>5.5 (4-6)</td>
</tr>
<tr>
<td>6</td>
<td>9 (50%)</td>
</tr>
<tr>
<td>5</td>
<td>6 (33%)</td>
</tr>
<tr>
<td>4</td>
<td>3 (17%)</td>
</tr>
</tbody>
</table>

Treatment. Overall, 18 (82%) patients completed external beam and intracavitary therapy and were evaluated for response and survival. Three patients abandoned treatment during external radiation for reasons other than toxicity and one patient died after the first week of chemotherapy because she coursed with hypercalcemia-granulocytosis syndrome-associated rapidly progressive disease. Mean + standard deviation (SD) dose administered to point A was 84.6 + 2.2 (range, 79.6-87). Median number of cisplatin cycles administered was five (range, 1-6 cycles). Intracavitary treatment was not optimally delivered due to physical decay of Cesium-137 sources at the time this study was performed. Six (33%) patients received a single application, 11 (61%) two, and one patient (5%) had three applications. The patient receiving three applications had uterine perforation during the third application. While external radiation was delivered in a mean of 6.4 weeks (range, 5.5-7.1 weeks), brachytherapy was delayed; thus, mean overall treatment time was 11.8 weeks (range, 6-17 weeks) (Table 2). Hydralazine and valproate were administered until the end of brachytherapy in all patients. Plasma samples at weeks 1, 4, and 7 were available for drug quantitation. Mean plasma concentrations were 66.4, 63.7, and 63.5 g/ml, respectively (overall mean of 64.5 g/ml) for valproic acid. Hydralazine concentrations were 158.4, 185.9, and 126.9 ng/ml. Mean values of plasma hydralazine from all patients (5%) had three applications. The patient receiving three applications had uterine perforation during the third application. While external radiation was delivered in a mean of 6.4 weeks (range, 5.5-7.1 weeks), brachytherapy was delayed; thus, mean overall treatment time was 11.8 weeks (range, 6-17 weeks) (Table 2). Hydralazine and valproate were administered until the end of brachytherapy in all patients. Plasma samples at weeks 1, 4, and 7 were available for drug quantitation. Mean plasma concentrations were 66.4, 63.7, and 63.5 g/ml, respectively (overall mean of 64.5 g/ml) for valproic acid. Hydralazine concentrations were 158.4, 185.9, and 126.9 ng/ml. Mean values of plasma hydralazine from all patients (100%) were rapid- and ten (45.5%) were slow acetylators.

Table 3. — Toxicity (22 patients).

<table>
<thead>
<tr>
<th>Grade</th>
<th>0 (n (%)</th>
<th>1 (n (%))</th>
<th>2 (n (%))</th>
<th>3 (n (%))</th>
<th>4 (n (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>10 (45)</td>
<td>5 (20)</td>
<td>4 (18)</td>
<td>2 (9)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>7 (32)</td>
<td>2 (9)</td>
<td>4 (18)</td>
<td>9 (45)</td>
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<tr>
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<td>1 (5)</td>
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</tr>
<tr>
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<td>4 (18)</td>
<td>1 (5)</td>
<td>2 (9)</td>
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</tr>
<tr>
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<td>5 (23)</td>
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<tr>
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<tr>
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<td>3 (14)</td>
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<td>3 (14)</td>
<td>0</td>
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<tr>
<td>Diarrhea</td>
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<td>6 (27)</td>
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</tr>
<tr>
<td>Constipation</td>
<td>15 (68)</td>
<td>7 (32)</td>
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<td>0</td>
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<tr>
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<td>4 (18)</td>
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<td>9 (41)</td>
<td>10 (45)</td>
<td>3 (14)</td>
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<td>0</td>
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<tr>
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<td>8 (36)</td>
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<td>3 (14)</td>
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<td>0</td>
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<tr>
<td>Dermatitis</td>
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<td>3 (14)</td>
<td>2 (9)</td>
<td>0</td>
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</tr>
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<td>Headache</td>
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</tr>
<tr>
<td>Edema</td>
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<td>3 (24)</td>
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</table>

Progression-free and overall survival for the 18 patients evaluated for efficacy.
between pre- and post-treatment biopsies was performed by hematoxilin and eosin staining and evaluated by two pathologists in a blinded fashion. All cases showed mild, moderate or intense infiltration composed mainly of lymphocytes in tumor and in stroma after treatment exposure (hydralazine and valproate effect as post-treatment biopsies were taken before starting chemoradiation). Also, an increase in connective tissue with fragmentation of solid malignant nests, with a trabecular pattern, was identified.

Toxicity. All 22 patients were evaluated for toxicity. The majority of patients had grades 1 and 2 toxicity (Table 3). Death was registered as not-treatment related. The most important toxicity was hematological: grade 3 anemia, leucopenia, neutropenia, and thrombocytopenia were observed in 9%, 45%, 45%, and 9% of patients, respectively. Among non-hematologic toxicities, grade 3 events were recorded for asthenia (9%), nausea/vomiting (14%), diarrhea (9%), proctitis (9%), hypoalbuminemia (9%), and headache (5%). Grade 1 and 2 somnolence was observed in 73% of patients.

Follow-up. At median follow-up time of 37 months [10-48], 12 (66.6%) patients relapsed. Four local, five systemic and three patients local-systemic for a median PFS of 32 months. Of these, six died of disease; five are alive with disease and one is alive with no evidence of disease.

Regarding overall survival, the estimated 48-month survival rate is 67% (Figure 1).

Discussion

Currently, there is limited information on the efficacy and safety of epigenetic therapy in patients with solid tumors. This partially stems from the fact that regarding the two long-established demethylating agents, 5-azacitidine and 2-deoxy-5-azacytidine, while effective in myelodysplastic syndromes, exhibit poor activity against solid tumors [32] and marked myelosuppression when combined with cisplatin [33, 34]. Likewise, HDAC inhibitors are being assayed at present in solid tumors; to date; only vorinostat has been approved for cutaneous-T-cell lymphoma [35].

The rationale for utilizing DNA methylation and HDAC inhibitors for cancer treatment has relied on the thought that reversing epigenetic aberrations would turn-on tumor suppressor genes and consequently exert antitumor effects. The results of this trial suggest that hydralazine and valproate increase the antitumor effect of external radiation and concurrent cisplatin, as demonstrated by the 100% clinical complete response rate achieved. In comparison, our historical cisplatin-and radiation-treated control achieves a complete response rate of 75% after both external radiation and intracavitary treatment though we acknowledge that the clinical evaluation of response to chemoradiation by pelvic examination and cytology is subjective [36]. It is noteworthy that all responses observed in this study were registered at the end of external therapy, and that the epigenetic treatment alone, as evaluated in biopsies on day 8 led to tumor lymphocytic infiltration and tumor fragmentation, further supporting the suggested increased antitumor effect of chemoradiation when combined with hydralazine and valproate.

Our previous studies in breast cancer and advanced solid tumors using hydralazine and valproate with chemotherapy have shown increased but manageable hematological toxicity [19, 20]. This side-effect also occurred in this study; however, it has no impact on the ability to deliver external radiation and chemotherapy on time. The myelosuppression was clearly higher, with grade 3 anemia, leucopenia, neutropenia, and thrombocytopenia observed in 9%, 45%, 45%, and 9%, respectively. In comparison to our historically treated patients with cisplatin chemoradiation, grade 3 anemia and thrombocytopenia were below 10%, whereas the rate of grade 3 leucopenia and neutropenia was around 30% [36]. On the other hand, as expected, experimental drug-related toxic-
ity was mainly grade 2 and consisted of drowsiness, distal tremor, weight loss, headache, edema, and hypoaalbuminemia; all these may be explained by the known pharmacologic effects of hydralazine and valproate on the cardiovascular and central nervous systems. Regarding the plasma drug levels achieved, at present there is no information on the predictive or prognostic significance of the hydralazine levels however, previous studies have demonstrated the DNA demethylating effects at a dose between 50-150 μg/day [22, 37]. On the other hand, it is now accepted that the target concentration of valproic acid is 50 μg/ml which was achieved [38] in this study. Further, in a separate report (submitted) we show that in the post-treatment primary tumor samples taken on day 8 of starting hydralazine valproate there were more than 900 hundred genes up-regulated with the treatment supporting that the reactivating gene effect searched was accomplished in this study.

Despite cisplatin-based chemoradiation has been widely accepted as the standard of care for patients with cervical cancer whose treatment requires radiation, most individual studies report that the survival effect of this treatment on Stage IIIB is not statistically significantly better as compared to radiation alone [39-41] except for the recently updated GOG 120 study which demonstrated significant overall survival differences also in Stage IIIB patients [42]. In this report, despite all patients having achieved a clinical complete response at the end of external radiation, the relapse rate seems high. At a median follow-up time of 37 months, two-thirds of patients had either local, systemic or local systemic relapse hence the PFS rate was only 32% at a median follow-up time of 37 months (maximum 48 months). Most likely this is the result of the long-time need in this study to deliver intra-cavitary treatment but which due to technical reasons led to delivering treatment in 11.8 weeks (range, 6-17 weeks), very far from the optimum. It is known that one of the most negative prognostic factors of tumor control in cervical cancer is longer treatment time [43-45]. Interestingly, despite the short progression-free survival rate, overall survival was encouraging. In our institution we have found that cisplatin-chemoradiation yields a 60% three-year survival in Stages IIIB-IV [36], results comparable to the updated GOG 120 trial which reports 60% OS at five years for both cisplatin-containing arms [40] and the updated RTOG 9001 with 62% in Stages IIIB and IV A [46]. Thus, it could be suggested that the addition of epigenetic therapy with hydralazine and valproate compensated in some way the negative prognostic significance of the longer treatment time. Supporting this suggestion, it has been reported that the achievement of no gross residual tumor status to external radiation results in a 5-year OS rate of 62.6% versus 33.7% in those who do not [47] and that in those patients treated only with external radiation, the achievement of complete response is the only factor predicting better survival in a multivariate analysis [48].

In conclusion, we demonstrate herein that hydralazine and valproate are well-tolerated and safe when administered with radiation concurrent with cisplatin in locally advanced cervical cancer.

Unfortunately, the suboptimal administration of brachytherapy for technical reasons in this study precluded assessing the efficacy of epigenetic therapy, however, the tolerability of this regimen administered concurrent to radiation needs to be further tested.

Acknowledgements
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References
Epigenetic therapy and cisplatin chemoradiation in FIGO Stage IIB cervical cancer


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Loop electrosurgical excision procedure in Greek patients with vaginal intraepithelial neoplasia

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³ Department of Pathology, St. Savvas Anticancer-Oncologic Hospital, Athens (Greece)

Introduction

Vaginal intraepithelial neoplasia (VAIN) is uncommon, accounting for approximately < 1% of lower genital tract intraepithelial neoplasia [1, 2]. However, VAIN is now being diagnosed in younger women and this rise seems to be associated with the increased prevalence of human papilloma virus (HPV) infections of the lower genital tract [3]. Generally, most patients are asymptomatic. If present, symptoms may include postcoital spotting, vaginal bleeding, unusual vaginal discharge and odor [4, 5]. The majority of lesions located in the upper one-third of vagina and are often multifocal [4, 5].

The natural history of VAIN is thought to be similar to that of cervical intraepithelial neoplasia (CIN), with risk for progression to vaginal cancer [4]. In women with VAIN, 78% may regress, 13% may persist, and 9% may progress to invasive vaginal cancer [4].

The management of women with VAIN remains controversial. Treatment protocols use surgical procedures (local excision, partial vaginectomy, total vaginectomy, loop electrosurgical excision procedure (LEEP), laser CO₂ surgery), topical medical therapy (5% 5-fluorouracil) or radiation therapy [6-11].

The aim of our study was to evaluate the therapeutic effectiveness of the loop electrosurgical excision procedure (LEEP) in Greek patients with vaginal intraepithelial neoplasia (VAIN).

Material and Methods

Between January 2002 and January 2009, 23 women with histologically confirmed VAIN were included in our study. For the LEEP procedure we used a high frequency electrosurgery unit with at least 80 W output. Results: Complete response rate at 12 months of follow-up was 86.96%. Recurrence rate at 12 months of follow-up was 13.04%. Complete response rate at 24 months of follow-up was 75%. Recurrence rate at 24 months of follow-up was 25%. Conclusion: LEEP may constitute a valuable excisional method for the treatment of VAIN. It provides an interpretable specimen of the whole lesion within a few minutes. It needs a short period of training and has low cost.

Key words: Electrosurgery; LEEP; Vaginal intraepithelial neoplasia; VAIN.
Results

The median age at diagnosis of VAIN was 55 years (range 23-80 years). The median follow-up was 34.6 months (range 16-60 months). The demographics of women are shown in Table 1.

The median operating time was 15 min (range 10-20 min) depending on multifocal and extent of the lesion. The median healing time was five weeks (range 4-6 weeks) depending on the extent of the wound. All tissue specimens had free surgical margins. In our study population we had four VAIN 1, nine VAIN 2 and ten VAIN 3.

Complete response rate at 12 months of follow-up was 86.96%. Recurrence rate at 12 months of follow-up was 13.04%. Complete response rate at 24 months of follow-up was 75%. Recurrence rate at 24 months of follow-up was 25%. None of the treated patients progressed to invasive vaginal cancer during a mean follow-up of 34.6 months. These data are shown in Tables 2 and 3.

Table 1. — Women’s demographics (n = 23).

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of patients (n = 23)</th>
<th>Percentage (%)</th>
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</thead>
<tbody>
<tr>
<td>&lt; 40</td>
<td>2</td>
<td>8.69%</td>
</tr>
<tr>
<td>40-60</td>
<td>15</td>
<td>65.22%</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>6</td>
<td>26.09%</td>
</tr>
<tr>
<td>VAIN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAIN 1</td>
<td>4</td>
<td>17.39%</td>
</tr>
<tr>
<td>VAIN 2</td>
<td>9</td>
<td>39.13%</td>
</tr>
<tr>
<td>VAIN 3</td>
<td>10</td>
<td>43.48%</td>
</tr>
<tr>
<td>History of CIN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>39.13%</td>
</tr>
<tr>
<td>No</td>
<td>14</td>
<td>60.87%</td>
</tr>
<tr>
<td>History of cervical cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>13.04%</td>
</tr>
<tr>
<td>No</td>
<td>20</td>
<td>86.96%</td>
</tr>
<tr>
<td>History of radiotherapy</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>No</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>History of immunosuppression</td>
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<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>No</td>
<td>23</td>
<td>100%</td>
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</table>

Table 2. — Response at 12 months of follow-up (n = 23).

<table>
<thead>
<tr>
<th>VAIN</th>
<th>Complete response</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAIN 1</td>
<td>4 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>VAIN 2</td>
<td>8 (88.89%)</td>
<td>1 (11.11%)</td>
</tr>
<tr>
<td>VAIN 3</td>
<td>8 (80%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td>20 (86.96%)</td>
<td>3 (13.04%)</td>
</tr>
</tbody>
</table>

Table 3. — Response at 24 months of follow-up (n = 16).

<table>
<thead>
<tr>
<th>VAIN</th>
<th>Complete response</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAIN 1</td>
<td>2 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>VAIN 2</td>
<td>5 (83.33%)</td>
<td>1 (16.67%)</td>
</tr>
<tr>
<td>VAIN 3</td>
<td>5 (62.5%)</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>12 (75%)</td>
<td>4 (25%)</td>
</tr>
</tbody>
</table>

Discussion

Vaginal intraepithelial neoplasia (VAIN) has histopathology similar to cervical intraepithelial neoplasia (CIN) [12]. VAIN development, following HPV infection, may require a greater period of time and may occur less frequently because of the different type of epithelium from which VAIN arises [13]. The vagina lacks an active transformation zone with immature epithelial cells susceptible to HPV infection. However, HPV entry may result from vaginal mucosal abrasions (from coitus or tampon use) and reparative metaplastic squamous cell activity [12].

VAIN may occur as an isolated lesion or as a lesion on the vaginal vault after hysterectomy for CIN or invasive cervical carcinoma [4, 14]. Most VAIN lesions occur in women with a history of CIN or invasive cervical carcinoma [6, 15]. These lesions may arise at the same time (synchronous lesions) or up to several years after the initial CIN lesion (metachronous lesions) [16]. The time interval from an initial diagnosis of CIN 3 to a current diagnosis of VAIN 3 varies from two to 17 years [17]. In our study 11 patients had an isolated VAIN lesion, nine patients had a VAIN lesion on the vaginal vault after hysterectomy for CIN 3 and three patients had VAIN lesions on the vaginal vault after hysterectomy for microinvasive cervical carcinoma Stage 1a1.

The majority of VAIN lesions occur in the upper one-third of the vagina. The middle and lower thirds of vagina are involved by less than 10% of lesions [6]. The majority of VAIN are also multifocal [4, 6, 17]. In our study, all women had VAIN lesions in the upper one-third of the vagina. Among them, 13 women had unifocal VAIN lesions and ten women had multifocal VAIN lesions.

Risk factors for developing VAIN are low education, low family income, previous abnormal Papanicolaou smear, genital warts, CIN or cervical cancer, immunosupression, radiation therapy and history of diethylstilbestrol exposure [18]. In our study nine patients had been treated for CIN 3 and three patients had been treated for microinvasive cervical carcinoma Stage 1a1. None of the women had any history of immunosupression, radiation therapy or diethylstilbestrol exposure.

Vaginal intraepithelial neoplasia is a rare disorder that, in most instances, will regress after initial treatment. However, patients with VAIN require careful monitoring because of the risk of recurrence and even progression to invasion [4, 5]. Risk factors for recurrence of VAIN include multifocality, association with neoplasia on other anogenital sites, histologic grade, immunosupression and treatment modality [4-6]. In our study three women treated for VAIN 3 and one woman treated for VAIN 2 recurred after initial treatment. None of the women in our study progressed to invasive vaginal cancer during a mean follow-up of 34.6 months.

The choice of treatment modality for patients with VAIN is influenced by the number of lesions, location of lesions, length of vagina, sexual activity, previous radiation therapy, previous VAIN treatment, patient preference and operator experience [6, 19]. Multifocal lesions are more difficult to treat because some lesions could be missed during treatment [6].

LEEP for VAIN lesions has been proposed with excellent results in selected groups of patients [6, 8, 9]. There are potential advantages of LEEP for treating VAIN lesions which include low cost of equipment, avoidance of the operating room, avoidance of general anesthesia, limited tissue damage, provision of a specimen, reduced...
bleeding and reduced discomfort [8, 9]. LEEP may be more accurate than laser CO2 in uncovering foci of early invasion (LEEP uses excision rather than ablation) [9]. In our study all tissue specimens had free surgical margins. The operating time ranged between 10-20 min depending on multifocal and extent of the lesion. We believe that every gynecologist is capable of performing LEEP on VAIN after 10-15 supervised applications with a high index of confidence.

There are potential complications of LEEP for treating VAIN lesions which include bleeding, infection, vaginal perforation, bladder injury, rectal injury, vesicovaginal and rectovaginal fistulae [8, 9, 20, 21]. In our study population, there were no complications. Only in a few cases did spot bleeding occur during surgery. The newly formed vaginal epithelium, after a mean period of five weeks, presents excellent topography. None of the women complained of post-treatment sexual dysfunction.

It is clear that current treatments for VAIN are suboptimal and continue to represent a clinical challenge. The best approach is individualized management based on clinical presentation, extent of disease and patient preference. LEEP may constitute a valuable excisional method for the treatment of VAIN. It provides an interpretable specimen of the whole lesion within a few minutes. It needs a short period of training and has low cost.

References


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Clinical features of 215 Stage I ovarian tumors in Japanese women

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Summary

Purpose: Differences of the clinical features of Stage I borderline ovarian tumors and Stage I ovarian cancer need to be clarified.

Methods: We retrospectively investigated 215 patients with Stage I ovarian tumors (67 with borderline tumors and 148 with ovarian cancer) treated between 1988 and 2001.

Results: Only one patient with a borderline tumor developed recurrence, while recurrence was found in 20 patients with Stage I ovarian cancer. There was a significant difference in the recurrence rate between patients with Stage Ia or Ib ovarian cancer and those with Stage Ic cancer ($p = 0.007$). Clear cell adenocarcinoma showed a higher recurrence rate. Among our patients with recurrence, only five in whom the recurrent tumor could be surgically resected are currently alive and disease-free.

Conclusions: This study confirmed the low aggressiveness of Stage I borderline ovarian tumors and high aggressiveness of Stage Ic ovarian cancer or clear cell adenocarcinoma. In patients with recurrence, surgical resection may improve survival.

Key words: Stage I; Borderline ovarian tumors; Ovarian cancer; Recurrence.

Introduction

Borderline ovarian tumors (BOTs) account for approximately 10-15% of malignant epithelial ovarian tumors [1]. These tumors form a separate entity within the category of ovarian tumors, and methods of treatment show a clear difference between BOTs and ovarian cancer [2]. A wide range of recurrence rates has been reported for BOTs [3-8], which may be attributed to the difficulty of distinguishing between these tumors and ovarian cancer by pathological examination [9-11].

We performed a retrospective study that compared the characteristics of Stage I BOTs, which are most commonly encountered, with those of Stage I ovarian cancer in patients treated at the same institution during the same period (to minimize differences of surgical technique or pathological diagnosis).

Materials and Methods

Between 1988 and 2001, 67 patients with Stage I BOTs and 148 patients with Stage I ovarian cancer were treated at the Cancer Institute Hospital in Japan. Clinical features of the patients with Stage I BOTs or Stage I ovarian cancer are shown in Table 1.

Among the 67 patients with Stage I BOTs, 56 had Stage Ia tumors (83.6%) and 11 had Stage Ic disease (16.4%). The tumor was serous in 18 patients (26.9%) and mucinous in 49 patients (73.1%). Among the 148 patients with Stage I ovarian cancer treated at our hospital during the same period, 57 had Stage Ia tumors (38.5%), six had Stage Ib tumors (4.1%), and 85 had Stage Ic disease (57.4%). Tumor histology was serous in 31 patients (20.9%), mucinous in 37 patients (25.0%), endometrioid in 20 patients (13.5%), clear cell in 49 patients (33.1%), and mixed in 11 patients (7.4%) (Table 1).

The pathological diagnosis of all tumors was confirmed after careful histological examination by an experienced gynecologic pathologist according to the World Health Organization (WHO) classification. BOTs were defined as tumors that showed nuclear atypia, stratification of the epithelium, and microscopic papillary projections without any stromal invasion. The 1987 International Federation of Gynecology and Obstetrics (FIGO) classification was used for surgical staging.

The chi-square test or Student’s t-test was employed for comparison of the two groups. Survival analysis was done by the Kaplan-Meier method, and the log-rank test was used for comparison of survival times. Survival was calculated from the day of the first operation to the last day of review or to the date of death.

Results

Age

Patients with Stage I BOTs ranged in age from 17 to 72 years (mean age: 46.3 years) and patients with Stage I ovarian cancer ranged in age from 17 to 78 years (mean age: 51.4 years) (Table 1). Comparison of the mean age between the two groups showed that the patients with BOTs were significantly younger than those with ovarian cancer ($p = 0.008$).

Initial surgery

Conservative surgery was defined as preservation of the uterus and at least one ovary. Initial surgery was conservative in 32 out of 67 patients with Stage I BOTs (47.8%). Simple cystectomy was performed in one of these 32 patients, but the residual ovary on the diseased side was resected after a diagnosis of BOT was made. In the remaining 35 patients, non-conservative surgery was performed. Among them, six patients (8.9%) also underwent lymphadenectomy because ovarian cancer was strongly suspected prior to surgery (Table 1).
Only 12 out of 148 patients (8.1%) with Stage I ovarian cancer underwent conservative surgery. Non-conservative surgery without lymphadenectomy was performed in 33 out of 148 patients (22.3%), while procedures that included lymphadenectomy were done in 103 patients (69.6%) (Table 1). In the patients with clear cell adenocarcinoma, lymphadenectomy was performed in the majority of them (46/49, 93.9%).

Adjuvant chemotherapy
Only one of the 67 patients (1.5%) with BOTs received adjuvant chemotherapy, while it was performed in 70 out of 148 ovarian cancer patients (47.3%). An average of three courses of platinum-based chemotherapy was given as adjuvant therapy, with a range of one to eight courses (Table 1).

Recurrence of Stage I BOTs
Among patients with Stage I BOTs, the mean follow-up period was 101.8 months (range: 12-183 months). Recurrence was only discovered in one patient with a Stage Ia mucinous tumor that was treated by left salpingo-oophorectomy. In this patient, pulmonary metastasis was found at 14 months after initial surgery, and metastatic BOT was verified by pathological examination of a biopsy specimen obtained from the lung tumor at bronchoscopy. CT scans did not reveal any signs of recurrence in the lymph nodes or other organs.

Recurrence of Stage I ovarian cancer
Among patients with Stage I ovarian cancer, the mean follow-up period was 91.9 months (range: 2 to 185 months), and recurrence was found in 20 patients (Table 2). A significant difference in the recurrence rate was observed between patients with stage Ia or Ib tumors and patients with Stage Ic tumors ($p = 0.007$).

With respect to tumor histology, patients who had clear cell carcinoma showed a high recurrence rate (10/49, 20.4%), despite lymphadenectomy being performed in almost all cases. There was a higher recurrence rate for clear cell carcinoma than the average rate for other types of tumors, but the difference was not significant.

Disease-free survival and overall survival
Disease-free survival and overall survival five years after the initial operation were compared between the patients with BOTs and those with ovarian cancer. The 5-year disease-free survival rate for all patients with Stage I BOTs was 98.2% and their overall survival rate was 98.5%. On the other hand, the 5-year disease-free survival rate for all patients with Stage I ovarian cancer was 85.9% and their 5-year overall survival rate was 89.6%. A significant difference was observed with respect to both 5-year disease-free survival ($p = 0.008$) (Figure 1) and 5-year overall survival ($p = 0.025$) (Figure 2) when patients with Stage I BOTs were compared to patients with stage I ovarian cancer.

Treatment of recurrence
The only BOT patient with metastasis (to the lung) received chemotherapy, but she died approximately 11 months after the detection of recurrence. Twenty ovarian cancer patients developed recurrence; all 20 patients received chemotherapy and eight of them underwent further surgical treatment. Five patients in whom the recurrent tumor could be completely resected are currently alive without further recurrence, but the patients whose lesions could not be removed surgically and the patients who only received chemotherapy all died. A significant difference in the survival rate was observed between patients who had surgery as well as chemotherapy and patients who received chemotherapy alone ($p = 0.009$) (Figure 3).

Discussion
BOTs form a separate entity within the category of ovarian tumors. Because BOTs occur in younger women and are usually diagnosed at an early stage, the prognosis is excellent [12]. However, a wide range of recurrence

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**Table 1. — Clinical features of patients with stage I BOTs or Stage I ovarian cancer.**

<table>
<thead>
<tr>
<th></th>
<th>BOTs</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>67</td>
<td>148</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>17-72</td>
<td>17-78</td>
</tr>
<tr>
<td>Average</td>
<td>46.3</td>
<td>51.4</td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ia</td>
<td>56</td>
<td>57</td>
</tr>
<tr>
<td>Ib</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Ic</td>
<td>11</td>
<td>85</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td>Mucinous</td>
<td>49</td>
<td>37</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Clear cell</td>
<td>0</td>
<td>49</td>
</tr>
<tr>
<td>Mixed</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conservative surgery</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>Non-conservative surgery without LN</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>Non-conservative surgery with PLA</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Non-conservative surgery with PLA+PALA</td>
<td>5</td>
<td>89</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
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<td></td>
</tr>
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<td>70</td>
</tr>
<tr>
<td>No</td>
<td>66</td>
<td>78</td>
</tr>
</tbody>
</table>

LN: lymphadenectomy, PLA: pelvic lymphadenectomy, PALA: paraaortic lymphadenectomy.

**Table 2. — Tumor histology and recurrence rate of Stage I ovarian cancer.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Serous</th>
<th>Mucinous</th>
<th>Endometrioid</th>
<th>Clear cell</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>0/10</td>
<td>0/5</td>
<td>2/16</td>
<td>2/31</td>
<td></td>
</tr>
<tr>
<td>Ib</td>
<td>0/22</td>
<td>0/22</td>
<td>3/15</td>
<td>5/37</td>
<td></td>
</tr>
<tr>
<td>Ic</td>
<td>0/12</td>
<td>0/12</td>
<td>1/3</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3/57</td>
<td>0/6</td>
<td>25/49</td>
<td>12/85</td>
<td></td>
</tr>
</tbody>
</table>

Serous: 0/10 (6.5%) Mucinous: 2/22 (9.1%) Endometrioid: 0/12 (0%) Clear cell: 1/12 (8.3%) Mixed: 0/1 (0%)

$\text{Total: } 45/148 (30.6\%)$
Clinical features of 215 Stage I ovarian tumors in Japanese women

rates have been reported [3-8]. This may be attributable to the fact that it can be difficult to differentiate between BOTs and ovarian cancer by pathological examination [9-11]. In the present study, we compared patients with BOTs to patients who had ovarian cancer. Both groups were treated during the same period at the same institution, thus minimizing any differences related to surgical technique or pathological diagnosis.

Only one patient developed recurrence in our group with BOTs tumors, so there were no differences of the recurrence rate between Stage Ia, Ib, and Ic BOTs, or between the histological types. On the other hand, there was a significant difference of the recurrence rate between patients with Stage Ia or Ib ovarian cancer and patients with Stage Ic cancer (p = 0.007). We also found a higher recurrence rate of clear cell carcinoma compared with the average rate for the other types of ovarian cancer (p = 0.081), although the difference was not statistically significant. Several authors have reported that clear cell tumors have a higher recurrence rate than other histological types of ovarian cancer [13-16]. Indeed, our patients with clear cell carcinoma had a very high recurrence rate (20.4%) even though the majority of them underwent non-conservative surgery with lymphadenectomy. Accordingly, we feel that it is necessary to not only perform surgery but also intensive adjuvant chemotherapy for Stage Ic ovarian cancer or clear cell adenocarcinoma.

Among our patients with recurrence, only five in whom the recurrent tumor could be surgically resected are currently alive and disease-free. There was a significant difference in the survival rate between patients who underwent additional surgery as well as chemotherapy and patients who received chemotherapy alone, suggesting the value of aggressive resection for managing intraperitoneal recurrence of ovarian cancer.

Conclusion

This study confirmed the low aggressiveness of Stage I borderline ovarian tumors and high aggressiveness of Stage Ic ovarian cancer or clear cell adenocarcinoma. Accordingly, we feel that it is necessary to not only perform surgery but also intensive adjuvant chemotherapy for the latter tumors. In patients with recurrence, surgical resection may improve survival.
References


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Complications and obstetric outcomes after laser conization during pregnancy

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Department of Obstetrics and Gynecology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama (Japan)

Summary

Objective: The purpose of the present study was to evaluate the efficacy of diagnostic laser conization and the obstetric outcomes of patients undergoing diagnostic laser conization during pregnancy. Study Design: The study population consisted of a consecutive series of 47 patients who presented with histologically proven carcinoma in situ microinvasive carcinoma and were treated with laser conization during pregnancy. Results: Diagnostic laser conization was performed at 3-28 weeks (median, 13 weeks) of gestation. Intraoperative blood loss of > 500 ml was observed in two cases (4.3%); however, hemotransfusion was not required in either case. In the early postoperative period, two miscarriages due to preterm premature rupture of the membrane were observed. In the late postoperative period, one spontaneous abortion, three preterm deliveries, and one neonatal death were observed. All the poor obstetric outcomes were observed in the case of patients who underwent conization in the first trimester. The pathology report for the laser conization revealed that two patients (4.3%) had invasive carcinoma. Of the 47 patients, 29 (61.7%) had positive cervical margin, and 13 required postpartum surgical intervention. All patients treated were disease-free at the time of the subsequent follow-up. Conclusions: The results of the present study suggest that laser conization in pregnant patients is feasible and is comparable to cold-knife conization and loop electrosurgical excision procedures with regard to the rates of complication and obstetric outcomes. Furthermore, they indicate that the optimal time for conization is probably the second trimester.

Key words: Laser conization; Pregnancy; Complication.

Introduction

Cervical cancer is the most common gynecological cancer associated with pregnancy worldwide [1]. The incidence of cervical cancer has been reported to be increasing among young women, and pregnant women should undergo cervical cancer screening because the gestation period is ideally the appropriate time to undergo screening for cancer. It was reported that the incidence of cervical cancer, including cases of carcinoma in situ (CIS), was 1.5-12 per 100,000 pregnancies [2]. Colposcopy is recommended for pregnant women with high-grade squamous intraepithelial lesions (HSILs). Colposcopically directed biopsy is preferred in the case of lesions suspicious for cervical intraepithelial neoplasia (CIN) 2, 3, or cancer [3]. Diagnostic excision is not recommended unless invasive cancer is suspected on the basis of the referral cytology, colposcopic appearance, or cervical biopsy [4]. Patients with Stage IA1 disease confirmed by conization may be followed-up through the gestation period until term, and these patients may undergo vaginal delivery [5]. However, pregnancy per se does not influence disease progression in cervical cancer.

In previous studies that have reported the risks and benefits of conization during pregnancy, conization was performed with cold-knife and loop electrosurgical excision procedures (LEEPs) [6-11]. A few studies have reported the safety and effectiveness of laser conization during pregnancy [12]. The purpose of the present study was to evaluate the efficacy of diagnostic laser conization and the obstetric outcomes of patients undergoing this procedure during pregnancy at our institution.

Materials and Methods

The study population consisted of a consecutive series of patients treated between April 1990 and September 2006 who had been diagnosed with histologically proven CIS/microinvasive carcinoma on the basis of colposcopically directed biopsy during pregnancy. Informed consent was obtained from all the patients. Each of these patients then underwent diagnostic laser conization at the Okayama University Hospital. Spinal anesthesia was administered. The extent of the excision was then determined by a colposcopic evaluation of the external width of the lesion. The laser instrument used was a KTP/YAG laser, and conization was performed using a KTP laser. After removal of the cone, the YAG laser was used to achieve hemostasis. Prophylactic suturing of the descending branch of the uterine artery is common practice. Cervical cerclage is not routinely included as a part of the procedure. Prophylactic antibiotics were routinely administered, and tocolytics were administered when necessary. The clinical charts of the patients were referred to for information about age, parity, the results of colposcopically directed biopsy, preoperative cervical length, gestation age at the time of surgery, intraoperative complications, height of conization, conization histology, time and modality of delivery, fetal outcome, and postpartum follow-ups.

Results

Forty-seven pregnant women underwent diagnostic laser conization during the study period; their clinical characteristics are summarized in Table 1. Histological
that CIS was present in 31 cases, microinvasive carcinoma in the first trimester. 
Intraoperative blood loss of > 500 ml was observed in 2 cases (4.3%). Previous studies have reported that immediate excessive blood loss and delayed cervical bleeding occurs in about 8% and 3.5% of the patients who undergo conization, respectively [6-11]. The two spontaneous abortions (4.3%) in our study may be directly related to the excision procedure, because chorioamnionitis and/or preterm PROM had occurred a few weeks after laser conization. Furthermore, preterm delivery resulting in neonatal death occurred in the case of one patient. Previous investigations have reported that spontaneous abortion and neonatal deaths occur in around 4% and 2% of patients who undergo conization, respectively [6-12]. In the study by Fambrini et al. laser CO2 conization performed within the 18th week of gestation in 26 pregnant women was found to be safe for both the patient and the fetus [12]. No major intraoperative or postoperative complications occurred in their study, and the median length of gestation was 39.1 weeks (range 35-42 weeks). However, the results of our study indicate that even laser conization poses the risk of hemorrhage and fetal outcome. Because of the high rate of complications attributed to cold-knife conization during pregnancy, analysis of the colposcopically directed biopsy revealed CIS in 39 women and microinvasive carcinoma in eight women. The median age at the time of surgery was 29 years (range 21-39 years). Of the 47 pregnant women, 28 were nulliparous and 19 were multiparous. The gestational age when diagnostic laser conizations were performed was 3-28 weeks (median, 13 weeks). Twenty-five women were examined in the first trimester, and 22 in the second trimester. The median preoperative cervical length was 39 mm (range 27-57 mm). The median height of the cone was 10 mm (range 5-20 mm). Cervical cerclage was performed in four patients. All the laser procedures were conducted successfully. 

In the present study, intraoperative blood loss of > 500 ml was observed in two cases (4.3%). Previous studies have reported that immediate excessive blood loss and delayed cervical bleeding occurs in about 8% and 3.5% of the patients who undergo conization, respectively [6-11]. In the study by Fambrini et al. laser CO2 conization performed within the 18th week of gestation in 26 pregnant women was found to be safe for both the patient and the fetus [12]. No major intraoperative or postoperative complications occurred in their study, and the median length of gestation was 39.1 weeks (range 35-42 weeks). However, the results of our study indicate that even laser conization poses the risk of hemorrhage and fetal outcome. Because of the high rate of complications attributed to cold-knife conization during pregnancy, analysis of the colposcopically directed biopsy revealed CIS in 39 women and microinvasive carcinoma in eight women. The median age at the time of surgery was 29 years (range 21-39 years). Of the 47 pregnant women, 28 were nulliparous and 19 were multiparous. The gestational age when diagnostic laser conizations were performed was 3-28 weeks (median, 13 weeks). Twenty-five women were examined in the first trimester, and 22 in the second trimester. The median preoperative cervical length was 39 mm (range 27-57 mm). The median height of the cone was 10 mm (range 5-20 mm). Cervical cerclage was performed in four patients. All the laser procedures were conducted successfully. 

Intraoperative blood loss of > 500 ml was observed in two cases (4.3%); however, hemotransfusion was not required in either case. With respect to postoperative complications, none of the patients required hemostatic treatment. However, in the early postoperative period, two miscarriages due to preterm premature rupture of membrane (PROM) were observed (1 and 3 weeks after conization). During the late postoperative period, one spontaneous abortion and three preterm deliveries were observed. In one case, preterm delivery at 23 weeks resulted in neonatal death. All the poor obstetric outcomes were observed in patients who underwent conization in the first trimester.

The pathology report of the laser conization revealed that CIS was present in 31 cases, microinvasive carcino-

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Gestational age at conization (weeks)</th>
<th>Cervical length (mm)</th>
<th>Height of cone (mm)</th>
<th>CAM</th>
<th>pPROM</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>CIS 13</td>
<td>13</td>
<td>48</td>
<td>20</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>CIS 13</td>
<td>13</td>
<td>39</td>
<td>10</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>lb1 16</td>
<td>16</td>
<td>43</td>
<td>17</td>
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<td>40</td>
<td>17</td>
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<tr>
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<td>25</td>
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<td>38</td>
<td>15</td>
<td>–</td>
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<td>33</td>
<td>5</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

**Table 1. — Clinical characteristics of the study patients.**

**Table 2. — Cases of abortion and preterm delivery.**

**Discussion**

It has been reported that women who underwent excision procedures, such as cold-knife conization, laser conization, and LEEP, presented with similar pregnancy-related morbidity during subsequent pregnancies [13]. It has also been reported that a short conization-to-pregnancy interval indicates an increased risk of preterm birth [14]. Cervical conization is discouraged for pregnant patients mainly because of the high rates of positive margins and the relatively high rate of complications such as hemorrhage, spontaneous abortion, premature delivery, and infection.

In the present study, intraoperative blood loss of > 500 ml was observed in two cases (4.3%). Previous studies have reported that immediate excessive blood loss and delayed cervical bleeding occurs in about 8% and 3.5% of the patients who undergo conization, respectively [6-11]. The two spontaneous abortions (4.3%) in our study may be directly related to the excision procedure, because chorioamnionitis and/or preterm PROM had occurred a few weeks after laser conization. Furthermore, preterm delivery resulting in neonatal death occurred in the case of one patient. Previous investigations have reported that spontaneous abortion and neonatal deaths occur in around 4% and 2% of patients who undergo conization, respectively [6-12]. In the study by Fambrini et al. laser CO2 conization performed within the 18th week of gestation in 26 pregnant women was found to be safe for both the patient and the fetus [12]. No major intraoperative or postoperative complications occurred in their study, and the median length of gestation was 39.1 weeks (range 35-42 weeks). However, the results of our study indicate that even laser conization poses the risk of hemorrhage and fetal outcome. Because of the high rate of complications attributed to cold-knife conization during pregnancy,
Hunter et al. do not recommend this procedure to rule out invasive disease [2]. Dunn et al. reported that the loop-cone cerclage technique of conization was effective in their small series: there were no intraoperative or late postoperative complications, and all their patients delivered at term [15]. In our experience, cervical cerclage before conization results in only a small amount of blood loss; therefore, this may be a safe and effective conization technique. Conization during pregnancy did not primarily have a therapeutic intent in our study. Therefore, 29 of the 47 patients had a positive cervical margin and 13 required postpartum surgical intervention.

The optimal time for conization has not yet been determined. Some reports have recommended the second trimester, preferably between 14 and 20 weeks of gestation, as the optimal time to undergo conization to minimize fetal and maternal complications [16]. This view is supported by the present study, because all the poor obstetric outcomes were observed in patients who underwent conization in the first trimester. However, Hannigan et al. reported that no spontaneous abortions occurred after first-trimester conization, and no neonatal morbidity occurred after third-trimester conization [11].

The main benefit of performing conization during pregnancy is that it can be used to confirm or refute the diagnosis of invasive cervical carcinoma. The degree of abnormality present during pregnancy may be underestimated if it is determined on the basis of colposcopic examination. Furthermore, it is important to take into consideration that a significant discrepancy was reported between the results of punch and cone biopsies. Moreover, we found that in the case of non-pregnant patients, Stage IA2-IB1 disease was found in five of 272 (1.8%) CIN 3 patients and six of 46 (13.0%) microinvasive carcinoma patients. In the present study, Stage IB1 disease was found in two of eight (25%) patients with microinvasive carcinoma, although none of the CIS patients had Stage IA2-IB1 disease. The total number of patients with microinvasive carcinoma in this series was too low to allow us to come to definitive conclusions; however, we think that conization can still be considered in pregnant patients.

In conclusion, the present study suggests that laser conization in pregnant patients is feasible and is comparable to cold-knife conization and LEEP with respect to the rates of complication and obstetric outcomes. Furthermore, our study supports the previously reported optimal time for conization, i.e., the second trimester.

References


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Anti-tumor activity of histone deacetylase inhibitors and the effect on ATP-binding cassette in ovarian carcinoma cells

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Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, and National Yang-Ming University School of Medicine
Taipei (Taiwan)

Summary

Introduction: Ovarian cancer is of worldwide importance, and has a significantly high mortality rate due to therapy failure. Drug resistance might be one of most importance factors. Histone deacetylase inhibitors (HDACi) have been reported to be a new class of promising anti-tumor agents, thus this study aimed to investigate the effect of HDAC on the chemo-resistance genes of human ovarian carcinoma cell lines. Methods: The expressions of ATP binding cassette (ABC) transporter genes, multidrug-resistant protein (MDR1) and multidrug resistance-associated proteins (MRP1 and 2) of ovarian cancer cell lines OC-109 and SK-OV-3 after HDACi treatment were determined. Results: HDACi, including sodium butyrate (NaB), suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) reduced ovarian cancer cell viability from 4.4% to 68.8%, in both dose- and time-dependent manners. The effect of HDACi on MDR1, MRP1, and MRP2 showed induced expression of MDR1 mRNA, but reduced mRNA expression of MRP1 and MRP2. Conclusions: The effect of HDACi on the reduced viability of ovarian cancer cell lines, concomitant with the induced expression of MDR1 and reduced expression of MRP1 and 2, might provide additional benefits in the management of ovarian cancers in the future.

Key words: Histone deacetylase inhibitors; Multidrug-resistant protein; Multidrug resistance-associated proteins; Ovarian cancer cell line.

Introduction

Ovarian cancer is of worldwide importance, and has a significantly high mortality rate, not only in the US, but also in Taiwan [1, 2]. The well-documented and standard treatment for ovarian cancer is complete surgery followed with combination platinum and paclitaxel chemotherapy. Even though patients with ovarian cancers undergo this active and intensive treatment, more than half of them will die from the disease. Most cancers are intrinsically resistant to chemotherapy or become resistant after an initial partial response [3]; therefore, cancers might escape the cytotoxicity of the chemotherapy. Resistance to chemotherapy is a major obstacle for successful treatment of cancer [4]. Clinical paclitaxel resistance is often associated with ATP binding cassette transporter gene (ABCB1) overexpression, and in vitro paclitaxel resistance typically demonstrates overexpression of the ABCB1 gene [5]. One study reported that patients with primary serous papillary adenocarcinoma of the ovary were tested for mRNA expression of multidrug-resistant protein (MDR1), and multidrug resistance-associated proteins 1, 2, and 3 (MRP1, MRP2, and MRP3) to show the correlation with clinical outcome [6]. The high relative mRNA levels of MRP1 were significantly correlated with a short period of progression-free survival, suggesting that higher levels of MRP1 mRNA expression may be a useful predictor of a shorter period of progression-free survival [6].

In addition, since the cytotoxic effect of chemotherapy does not discriminate between cancer and normal cells, chemotherapy toxicity is unavoidable. Therefore, the ability of differentiating agents to selectively kill cancer cells or transform cancer cells into a non-proliferating or normal phenotype could lead to cell- and tissue-specific drugs with the minimal side-effects of current chemotherapy for the management of cancer. Among these, a new generation of histone deacetylase inhibitors (HDACs) derived from amino acids might be one of the best candidates [7].

Histone modifications mediated by HDACs are now known to be centrally involved in the malignant transformation of cells; these small-molecule inhibitors of HDACs (HDACi) have been reported to show low toxicity toward normal cells, but have the ability to inhibit tumor growth in vivo [8]. Therefore, they may have promise as a new anti-tumor agent. Recently, HDACi have been evaluated in late-phase clinical studies for different types of solid tumors [8,9], and in a broad variety of tumors [8-11], including ovarian cancer [12], and the results favored their effect. Although these HDACi have been demonstrated to have cytotoxic effects on cancer cells, whether used alone or in combination with other chemotherapeutic agents, the mechanism is not fully understood [13].

The aim of this study was to evaluate the changes of ABC transporters (MDR1, MRP1, and MRP2) in ovarian cancer cell lines which were treated by different types of HDACi and to explore the possible therapeutic effects of these compounds on ovarian cancers. The effect of this type of targeted therapy on the development of chemo-resistance might help us in the management of ovarian cancers in the future.
Material and Methods

Cell culture

Cells were grown in DMEM/F-12 medium (Gibco BRL, Grand Island, NY, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone, Logan, UT, USA), penicillin 100 U/ml, streptomycin 100 μg/ml, and amphotericin B 2.5 μg/ml. Cells were incubated at 37°C with 5% CO2, and at more than 95% humidity. Media were changed twice a week, and cells were sub-cultured weekly by detachment with 2.5 mg/ml trypsin/ethylene-diamine-tetra-acetic acid 0.02% in Dulbecco’s phosphate-buffered saline (PBS) solution (Gibco BRL). Cells were maintained and sub-cultured every two to four weeks, split at a 1:2 to 1:4 ratio and prepared for subsequent studies.

HDACi reagents: Sodium butyrate (Sigma) was dissolved in H2O and stored at -20°C. SAHA (Cayman) and TSA (Sigma) were dissolved in DMSO and stored at -20°C.

Cell viability assays

Cell viability assays were examined using the MTS/PMS cell proliferation kit from Promega, according to manufacturer’s instructions. The percentage of cell viability as compared to untreated controls was calculated. Cells (1-2.5 x 10^4/well in 96-well-plates; 100 μl) were plated 24 h before treatment and incubated with HDACi for 24, 48 and 72 hours. At the last 4-hour segment of HDACi treatment, we added 20 μl per well of MTS/PMS mixture, and at the end we read the plate with a microplate reader (Multiscan, Labsystems, Helsinki, Finland) at an optical density (OD) of 490 nm.

Viability to control (%) = Absorbance of treated wells / Absorbance of control wells

RNA purification and checking of the MDR1, MRP1 and MRP2 genes using RT-PCR

We used the TRIzol kit (Gibco BRL) to purify the RNA of ovarian cancer cells. We added 1 ml of TRIzol solution to the drug-treated ovarian cancer cells, after centrifugation at 12,000 g at 4°C for 30 min, collected the upper layer, and added isopropanol, after another centrifugation at 12,000 g at 4°C for 30 min, and then washed the RNA twice with 70% alcohol.

We used the SuperScript First-Strand Synthesis System (Gibco BRL) reverse transcriptase (RT) to reverse transcript RNA to cDNA, following the manufacturer’s protocol, by incubating 1 μg of total RNA with oligo-d(T) 12-18 (Invitrogen, Carlsbad, CA, USA) as the initiation primer in a final reaction volume of 20 μl, as described elsewhere [12-18].

Polymerase chain reaction (PCR) was carried out in a total volume of 25 μl containing 2.0 Master Mix (Ampliqon), dNTPs at a concentration of 200 M, and 0.1 M of each primer. The PCR condition consisted of 10 min at 94°C followed by 40 cycles of 30 sec at 94°C, 30 sec at 55°C and 1 min at 72°C, followed by 72°C for 10 min. PCR products were subjected to electrophoresis in 2% agarose gel. The PCR primer sequences of MDR1 [17], MRPI [18], MRPI [18], and the glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) that was used as an internal control, were as follows: MDR1: CCCCATCGATGCAATGACGAC/GGTCAAAACTCTGCTCTCA (to amplify a 673 bp product) [17], MRPI: GGACCTGTGACCTCGTCTCA/ATGGTTCCGGATGGTGAGACTG (to amplify a 500-bp product) [18], MRPI: CTTGGCTCCAGAGTTCTGCTTG, (to amplify a 600-bp product) [18], and GAPDH: GTCAGTGGTGGACCT GACCT/TTAGCTTGACAAAGTGCTCG (accession number in GenBank 002046) (to amplify a 212-bp product).

Statistical analysis

Statistical analysis of the data was performed with Statistica version 5.1 (StatSoft Holdings Inc, Tulsa, OK, USA) and SPSS version 12.0 (SPSS Inc, Chicago, IL, USA). Every experiment was repeated six times. Experimental data are presented as mean ± standard deviation, using Dunnet’s test. Statistical analysis was performed by repeated analysis of variance (ANOVA), with a post hoc test for between-group analysis. A p value of less than 0.05 was considered statistically significant.

Results

Overall cytotoxic effect of HDACi on ovarian cancer cell lines

Cytotoxic effects of HDACi on the cells were analyzed using the MTS/PMS assay, as described above. Attached cells were incubated with different doses of HDACi for 24, 48 and 72 h. Dose-dependent effects of HDACi at the final concentrations of 1, 2, 5 mM for NaB (Figure 1), and 1, 2, 5 μM for SAHA (Figure 2) and TSA (Figure 3) were observed in both the OC-109-VGH and SK-OV-3-NIH lines. The morphological changes of the cells treated with HDACis are shown in Figures 4 and 5. All HDACi in this study were found to have highly cytotoxic effects on both ovarian cancer cell lines.

The effect of NaB

The dose-response curves and time-dependent manners of the ovarian cancer cell lines OC-109 and SK-OV-3 which were treated with three concentrations (1.0, 2.0 and 5.0 mM) of NaB are shown in Figures 1a and 1b. This data revealed that NaB inhibited the growth of adenocarcinoma cell lines OC-109 and SK-OV-3 dramatically in both dose- and time-dependent manners. The survival rates of OC-109 cells were 68.1 ± 5%, 45.2 ± 8%, and 36.7 ± 5%, respectively, after 48-h 1.0-, 2.0-, and 5.0-mM NaB treatment; and 64.2 ± 7%, 36.1 ± 8%, and 20.9 ± 7%, respectively, after 72-h NaB treatment. A similar dose- and time-dependent cytotoxicity of NaB on the SK-OV-3 cancer cells was seen, with survival rates of OC-109 and SK-OV-3- VGH cells were incubated with different doses of HDACi for 24, 48 and 72 h. Dose-dependent effects of HDACi at the final concentrations of 1, 2, 5 mM for NaB (Figure 1), and 1, 2, 5 μM for SAHA (Figure 2) and TSA (Figure 3) were observed in both the OC-109-VGH and SK-OV-3-NIH lines. The morphological changes of the cells treated with HDACis are shown in Figures 1a and 1b. This data revealed that NaB inhibited the growth of adenocarcinoma cell lines OC-109 and SK-OV-3 dramatically in both dose- and time-dependent manners. The survival rates of OC-109 cells were 68.1 ± 5%, 45.2 ± 8%, and 36.7 ± 5%, respectively, after 48-h 1.0-, 2.0-, and 5.0- mM NaB treatment; and 64.2 ± 7%, 36.1 ± 8%, and 20.9 ± 7%, respectively, after 72-h NaB treatment. A similar dose- and time-dependent cytotoxicity of NaB on the SK-OV-3 cancer cells was seen, with survival rates of 59.9 ± 4%, 48.1 ± 6%, and 37.4 ± 3% after 48-h treatment, and 47.5 ± 5%, 38.6 ± 7%, and 33.0 ± 5% after 72-h treatment, respectively.

The effect of SAHA

The dose-response curves and time-dependent manners of ovarian cancer cell lines OC-109 and SK-OV-3 which were treated with three concentrations (1.0, 2.0 and 5.0 μM) of SAHA are shown in Figures 2a and 2b. Compared with NaB, the cytotoxicity of SAHA on the ovarian cancer cells (OC-109 and SK-OV-3) was more prominent. The survival rates of OC-109 cells were down to
Figure 1. — Effect of sodium butyrate (NaB) on viability of OC-109 and SKOV3 cell lines. The MTS/PMS test was performed after a 24-72-h incubation for OC-109 cells (a) and for SKOV3 cells (b). Results are expressed as percent control and represent means ± SD of three independent experiments.

Figure 2. — Effect of suberoylanilide hydroxamic acid (SAHA) on viability of OC-109 and SKOV3 cell lines. The MTS/PMS test was performed after a 24-72-h incubation for OC-109 cells (a) and for SKOV3 cells (b). Results are expressed as percent control and represent means ± SD of three independent experiments.

Figure 3. — Effect of Trichostatin A (TSA) on viability of OC-109 and SKOV3 cell lines. The MTS/PMS test was performed after a 24-72-h incubation for OC-109 cells (a) and for SKOV3 cells (b). Results are expressed as percent control and represent means ± SD of three independent experiments.
27.2 ± 5%, 16.4 ± 8%, and 12.2 ± 5% after 48-h 1.0-, 2.0, and 5.0-uM SAHA treatment, and 12.3 ± 5%, 6.6 ± 7%, and 5.3 ± 4% after 72-h treatment. A similar strong cytotoxicity of SAHA on SK-OV-3 cancer cells was seen, with survival rates of 21.5 ± 5%, 17.4 ± 7%, and 12.6 ± 4% after 48-h treatment, and 8.5 ± 5%, 6.1 ± 6%, and 4.4 ± 3% after 72-h treatment.

The effect of TSA
The dose-response curves and time-dependent manners of the ovarian cancer cell lines OC-109 and SK-OV-3, which were treated with three concentrations (1.0, 2.0 and 5.0 uM) of TSA are shown in Figures 3a and 3b. The cytotoxicity of TSA on ovarian cancer cells was also prominent, as demonstrated by the survival rate of cancer
Cells after TSA treatment. The survival rates of OC-109 cells were 68.3 ± 5%, 38.5 ± 8%, and 28.3 ± 5% after 48-h 1.0-, 2.0-, and 5.0-uM TSA treatment, and 55.4 ± 5%, 12.3 ± 7%, and 4.4 ± 4% after 72-h treatment. A similarly strong cytotoxicity of TSA on SK-OV-3 cancer cells was also demonstrated, with survival rates of 63.7 ± 5%, 17.6 ± 7%, and 12.3 ± 4% after 48-h treatment, and 60.4 ± 5%, 12.3 ± 7%, and 8.2 ± 5% after 72-h treatment.

Morphological changes
Morphological changes were obvious in both ovarian cancer cell lines treated with HDACi, as shown in Figures 4 and 5.
Morphological change of OC-109 cells

Similar to the cytotoxicity of HDACi (NaB, SAHA, and TSA) on OC-109 cancer cells, the cancer cells treated with 2 mM NaB (row 2), 2uM SAHA (row 3) or 2 uM TSA (row 4) showed aberrations of the general morphology with scanty distribution on the plate, compared to the control (row 1, Figure 4), in time-dependent manners (original magnification, x 200).

Morphological change of SK-OV-3 cells

As with the OC-109 cancer cells, SK-OV-3 ovarian cancer cells treated with 2 mM NaB (row 2), 2 uM SAHA (row 3) or 2 uM TSA (row 4) showed aberrations of general morphology with scanty distribution on the plate, compared to the controls (row 1, Figure 5), in time-dependent manners (original magnification, x 200).

Effect of HDACi on ABC transports

In an evaluation of the expression of ABC transporters of OC-109 cells after NaB treatment, it was found that NaB treatment induced a dose-dependent increase of MDR1 mRNAs in these cancer cells after 48-h incubation. By contrast, NaB treatment resulted in a dose-dependent inhibition of MRP1 and MRP2 mRNAs on OC-109 cells after 48-h incubation of NaB (Figure 6).
Similar effects were reproducible in the other two types of HDACi: SAHA (Figure 7) and TSA (Figure 8), suggesting that HDACi might involve these ABC transport genes.

To test whether the phenomenon of the inducible increase of MDR1 mRNA and inhibition of MRP1 and MRP2 in OC-109 ovarian cancer cells was really present, a similar strategy was used to evaluate the changes in the ABC transport genes of SK-OV-3 ovarian cancer cells. As shown in Figure 9 for NaB, Figure 10 for SAHD, and Figure 11 for TSA, findings similar to those of the study of OC-109 cells were reproducible in SK-OV-3 ovarian cancer cells, suggesting that up-regulation of MDR1 and down-regulation of MRP1 and MRP2 might be present in ovarian cancer cells after HDACi treatment.

Discussion
HDACi have been reported to prevent proliferation in numerous cancer cell lines, including neuroblastoma, erythroleukemia, acute myelogenous leukemia, and carcinomas of the skin, breast, prostate, bladder, lung, colon, and cervix [17]; however, the effect of HDACi on ovarian cancer has not been examined fully, although one report showed that expression of class I histone deacetylases indicated poor prognosis in endometrioid subtypes of
Anti-tumor activity of histone deacetylase inhibitors and the effect on ATP-binding cassette in ovarian carcinoma cells

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ovarian carcinomas [19]. Recently, Yap’s group tested the effect of TSA on SK-OV-3 cancer cells and HEY cancer cells and found that TSA can inhibit ovarian cancer growth [20]. In addition, a novel HDAC6-specific inhibitor, NK84, was found to reduce ovarian cancer cell spreading and migration [21]. Therefore, it was rationale to suspect the inhibitory effects of HDACi on ovarian cancer cell growth. In this study, we rechecked the effects of TSA on the SK-OV-3 and got a similar finding that TSA could inhibit the tumor cell growth. Furthermore, as predicted, we examined the effects of two other types of HDACi, including SAHA and NaB, on two ovarian cancer cell lines (OC-109 and SK-OV-3) and showed that HDACi indeed suppressed the growth of these human ovarian cancer cells. The strong inhibition of HDACi on the cancer cells was also demonstrated in thyroid cancers [22].

Since ovarian cancer treatment failure often results from chemo-resistance, ABC transport genes, such as MDR1, MRP1 and MRP2, might play an essential role during treatment. In addition, HDACi could successfully inhibit tumor growth; therefore, the alteration of these ABC transport genes of tumors after HDACi treatment is more important. Unfortunately, the results on MDR1 mRNA after HDACi are still highly controversial. Up-regulation of MDR1 and induction of doxorubicin resistance by HDACi depsipeptide (FK228) was reported in acute promyelocytic leukemia cells [23]. However, HDACi were reported to have a down [24] or up [25] P-gp regulatory effect on leukemia or colon carcinoma cell lines, respectively. In our study, the expression of MDR1 was increased in both OC-109 and SKOV3 cancer cell lines. This finding was compatible with Satake’s group, who showed significant MDR1 expression in 60% of their cell lines after HDAC1 treatment [26]. Furthermore, the MTS/PMS assay showed that intensive HDACi resistance was associated with higher MDR1 expression.
Moreover MDR1, MRP1 and MRP2 have been demonstrated to confer drug resistance on tumor cells from various tissues through an increased efflux of anticancer drugs [27]. Our results demonstrated the down-effect of HDACi on MRP1 and MRP2 mRNAs, and a rise in MDR1 expression. Against the increasing MDR1 expression of ovarian cancer cells after HDACi treatment in this study, the expression of MRP1 and MRP2 of these two ovarian cancer cell lines was significantly inhibited after HDACi treatment. The conflicting data of the ABC transporter genes in the study of ovarian cancer cell lines treated with HDACi are worthy of our attention, since the net results of the increasing MDR1 and decreasing MRP1 and MRP2 would contribute to the final success or failure when using these compounds (HDACi) in the management of these highly lethal ovarian cancers.

In conclusion, the up-regulation of MDR1, but down-regulation of MRP1 and MRP2 in the HDACi-treated ovarian cancer cells lines might have a critical value when we consider the possibility of the using these drugs either alone or in combination with other anti-neoplastic reagents, for example the combination of HDACi and aspirin [28], in the management of ovarian cancers in the future.

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References


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p16\textsuperscript{INK4a} and low-grade cervical intraepithelial neoplasia. Diagnostic and therapeutic implications

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**Introduction**

A papillomavirus is a small DNA virus belonging to the papovaviridae family. It presents tissue-specificity for squamous epithelium (cutaneous, mucous and metaplastic), and is hence also found in oral and laryngeal mucosa [1]. The viral genome contains coding sequences (open reading frames - ORF) of which ten have been identified; these sequences have been divided into two groups on the basis of their intervention during the viral infection: early ORF and late ORF.

Integration of early ORF, in particular E6 and E7, into the nucleus of the target cell leads to neoplastic transformation of the cell while late ORF codify the structural proteins for the virus capsid for the assembly of new viral particles.

The importance of this infection, medically and socially, has been highlighted by data showing that the number of diagnosed human papillomavirus (HPV) infections is growing [2] and that on a global level, cervical carcinoma is second only to breast cancer for incidence and mortality [3]. The invasive phase of the carcinoma is preceded by intraepithelial lesions (cervical intraepithelial neoplasia - CIN) which in many cases and if diagnosed in time, can be treated successfully.

When ORF E6 and E7 penetrate the target cell, they disrupt the delicate protooncogen-onc suppressor system, inhibiting p53 and pRb (retinoblastoma protein), respectively [4]. The bond between E6 and p53 results in the rapid degradation of p53, thus the loss of its function in repressing and activating transcription and therefore its role in repairing damage to DNA. The binding of E7 to the immuno-onco-suppressor pRb, on the other hand, determines the release of the transcriptional factor E2 (E2F) and thus the continuation of the cycle, associated with uncontrolled cellular division (Figure 1) [5].

This study concentrates on the role of E7 protein as its action appears to responsible for the over-expression of p16\textsuperscript{INK4a}.

The protein p16\textsuperscript{INK4a}, encoded for by the CDKN2A gene, is an inhibitor of cyclin-dependent kinases and its physiological function is to slow down the cellular cycle through inhibition of the kinases that phosphorylate pRb which allows the release of the E2F [6]. In a non-infected cell the p16 protein is regulated by a negative feed-back that involves the pRb/E2F complex [7]. The deactivation of pRb by E7 results in the over-expression of p16 (Figure 2).

Low-risk HPVs have no effect on the p16/cyclin D1/CDK 4/pRb complex because the affinity of their E7 protein for pRb is ten times lower than that of high-risk oncogenes [7].

As the carcinoma is invasive and almost all high-risk squamous intraepithelial lesions (HSILs) are caused by high-risk HPV [8-10], it is in these lesions that the overexpression of the protein is found [11, 12]. Therefore the overexpression of p16\textsuperscript{INK4a} is considered a valid marker for the malignant mutation caused by HPV [4, 13, 14].

**Materials and Methods**

Between January 2007 and October 2009, 245 patients of differing nationalities were enrolled in the study at the Colposcopic and Cervical-vaginal Pathology Centre of the University of L’Aquila Obstetrical and Gynecological Clinic. The median age was 37 years. After colposcopic (Colposcopio plus, Zeiss) and colpophotographic analysis (Contax 35 mm, Rsx), a small cervical fragment from each patient was sent to the Anatomy and Pathological Histology Institute. To identify the antigen...
p16INK4a in CIN 1 (LSIL) samples, the CINtec Histology Kit (mtm laboratories, Heidelberg, Germany) was used. The kit uses a clone of the monoclonal murine antibody E6H4 and relies on a coloration procedure using Autostainer Link 48 (produced by DAKO). The results of the kit were evaluated through a binary system composed of negative and positive evaluations. Samples with widespread coloration of the epithelium and in particular the basal and parabasal layers, were classified as positive, while samples with incomplete or focal coloration were classified as negative. To correlate immunohistochemical positivity to the neoplastic potential of the implicated HPV, in the last phase of our study we typed the viral DNA present in the biopsy samples using polymerase chain reaction-enzyme-linked immunosorbent assay (PCR-ELISA).

Results

The results of the biopsy exam performed under colposcopic control and the subsequent histological exams are shown in Table 1.

Biopsies that were CIN 1 (199) were also tested for the protein p16\(^{\text{INK4a}}\): 22 were positive (Figures 3-6). Only CIN 1 samples were tested for the presence of protein p16\(^{\text{INK4a}}\) as previous studies have shown that HSILs test positive for this protein [6, 8, 11, 15].

PCR-ELISA showed that the infection in these 22 patients was determined by middle to high-risk oncoproteins: HPV16 (14), HPV 31 (3), HPV 33 (2), HPV 43 (1), HPV 45 (1), HPV 18 (1) (Figure 7).

To evaluate the role of the protein p16\(^{\text{INK4a}}\) as a marker for low-grade lesions that may worsen, we performed four- and eight-month follow-ups thus allowing re-evaluation of the patients and monitoring of the development of the lesions over time (Table 2).

Table 1. — Histological results of 245 biopsy exams.

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Classification</th>
<th>%</th>
<th>Colposcopic pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>199</td>
<td>CIN 1</td>
<td>81.2</td>
<td>ANT2 G1: 191</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ANT2 G2: 8</td>
</tr>
<tr>
<td>18</td>
<td>CIN 2/3</td>
<td>7.4</td>
<td>ANT2 G1: 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ANT2 G2: 15</td>
</tr>
<tr>
<td>28</td>
<td>Not pathological*</td>
<td>11.4</td>
<td>ANT2 G1: 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ANT2 G2: 18</td>
</tr>
</tbody>
</table>

*Incomplete squamous metaplasia and/or reactive cellular anomaly.

Table 2. — Four and eight-month follow-up on the 22 patients.

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>No. of patients</th>
<th>Histological report</th>
<th>%</th>
<th>Colposcopic pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>4th month</td>
<td>22</td>
<td>CIN 1: 16</td>
<td>73</td>
<td>ANT2 G1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CIN 1-CIN 2: 6</td>
<td>27</td>
<td>ANT2 G2</td>
</tr>
<tr>
<td>8th month</td>
<td>20</td>
<td>CIN 1: 14</td>
<td>70</td>
<td>ANT2 G1-G2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CIN 1-CIN 2: 6</td>
<td>30</td>
<td>ANT2 G2</td>
</tr>
</tbody>
</table>

* Two patients did not attend follow-up exams for unknown reasons.
Discussion and Conclusion

The correlation between HPV and cervical carcinoma is now universally accepted and the role of this virus in cervical pre-oncogenesis has been well documented [11, 16] and in light of this we carried out a study on low-grade pre-neoplastic lesions.

Considering that the natural course of cervical cancer involves increasing levels of intraepithelial neoplasia, establishing the precise stage of the disease (SIL) is, in our opinion, important for establishing the best therapeutic approach thus avoiding worsening of the lesion both in morphological and clinical terms.

Currently, treatment options vary considerably for CIN 1 patients, and are influenced by varying factors: colposcopic and histological parameters, the patient’s physical, psychological and immune state, as well as the knowledge that lesions can spontaneously regress [8].

Figure 3. — LSIL (CIN 1) with HPV cellular alterations, p16 positive with inflammatory lymphocyte infiltration.

Figure 4. — LSIL (CIN 1) with HPV cellular alterations, p16 positive.

Figure 5. — LSIL (CIN 1) with HPV cellular alterations, p16 negative.

Figure 6. — LSIL (CIN 1) with HPV cellular alterations, specific coloration (focal, discontinuous, overbasal).
To establish a more standard clinical approach, we performed viral typing and carried out immunohistochemical tests for the antigen p16 on biopsy samples with a LSIL.

The overexpression of p16\(^{ink4a}\), generally associated with viruses with middle to high neoplastic potential, should according to our study act as an additional pointer, from a diagnostic point of view, for treatment.

The correlation between overexpression of p16 and the evolution of a cervical lesion therefore represents a parameter that should be taken into consideration given the frequency of colposcopic lesions worsening.

In conclusion, our study underlines the importance of the over-expression of p16 as a parameter to be analyzed in CIN 1 patients, as it allows the differentiation between patients with LSIL who would benefit from immediate therapy and those who can be followed-up at four and eight months.

The results of our study are in agreement with the literature that protein p16\(^{ink4a}\) represents a marker that influences the prognostic-therapeutic combination.

References


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Retrospective analysis of hysteroscopic findings in breast cancer patients having adjuvant tamoxifen treatment

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Summary

Purpose of Investigation: To evaluate the effects of tamoxifen on the endometrium of breast cancer patients by hysteroscopy and endometrial sampling. Methods: Thirty-seven breast cancer patients using tamoxifen underwent hysteroscopy because of postmenopausal endometrial thickening or abnormal uterine bleeding. Hysteroscopic findings were compared with histopathology and ultrasonographic measurement of the endometrium. Results: Nineteen women showed endometrial abnormalities (51%) out of 37 patients. Negative and positive predictive values for hysteroscopy in detecting endometrial abnormalities were 100% and 94%, respectively. Conclusion: Endometrial surveillance is an important part of gynecological follow-up in breast cancer patients using tamoxifen and the liberal use of hysteroscopy aids in the diagnosis of abnormal endometrium.

Key words: Tamoxifen; Breast Cancer; Hysteroscopy; Endometrium.

Introduction

Tamoxifen is a nonsteroid partial estrogen agonist, which has been used in the adjuvant treatment and chemoprevention of disseminated receptor-positive breast cancer. Recent studies have demonstrated a reduction in breast cancer incidence by the prophylactic use of tamoxifen [1]. The estrogenic activity is cell type-specific and although tamoxifen antagonizes estrogen in the breast, it has agonistic activity on the endometrium [2]. Tamoxifen appears to increase the risk of endometrial abnormalities, such as hyperplasia and polyps, as well as endometrial cancer [3].

The thickening of endometrium in women using tamoxifen is caused by subendometrial thickening so that transvaginal ultrasonography (TVS) becomes an unreliable screening tool [4]. In postmenopausal women a double-layer 4 mm endometrial thickness measured by TVS is an acceptable cut-off for excluding endometrial pathologies [5] and a thicker endometrial stripe should lead to further evaluation. Hysteroscopy is the gold standard method of endometrial evaluation and it is more accurate than endometrial curettage [6].

The objective of the present study was to evaluate the effects of tamoxifen on the endometrium of breast cancer patients by hysteroscopic evaluation and endometrial sampling.

Materials and Method

Premenopausal (n = 9) and postmenopausal (n = 28) breast cancer patients using tamoxifen were evaluated in the outpatient clinic of Ege University Hospital between January 2003 and December 2008. These patients underwent hysteroscopy because of postmenopausal endometrial thickening or abnormal uterine bleeding. In the postmenopausal group, a double-layer endometrial thickness of 4 mm or more was accepted as abnormal.

All patients received 20 mg/dl tamoxifen as adjuvant treatment for a duration of a minimum of four months and maximum of 36 months (18.7 ± 11.5 months). The postmenopausal period was defined as 12 months of amenorrhea. The age of patients ranged from 34 to 76 years (52 ± 10.06 years) and mean parity was 2.7 ± 0.9. Patients did not show any pelvic mass and/or cervical cytological abnormality.

Ultrasonography was performed with the Honda HS-2000 convex scanner and a vaginal probe of 5.0 MHz was used. The uterus was scanned longitudinally and transversly to determine regularity of the endometrium. Endometrial thickness was recorded by measuring a double layer at the widest anteroposterior diameter across the uterine cavity [7]. When endometrial fluid was present, the anterior and posterior layers were measured separately and added together. An endometrial polyp was suspected when increased endometrial thickness was focal with distinct margins.

Hysteroscopy was performed in all patients with intravenous general anesthesia. A 5 mm hysteroscope tipped with a 30° lens and a 1.5 mm working channel (Karl Storz, Tuttlingen, Germany) was used. Normal saline distension medium was delivered by a peristaltic irrigating suction device and endouterine pressure was always set below 120 mm Hg. All hysteroscopies were aided by a videocamera and whole uterine inspection and operative procedures were recorded for the patient’s medical documentation. Focal endometrial abnormalities were sampled under vision by using mechanical hysteroscopic scissors for tissue dissection and hysteroscopic grasping forceps for tissue extraction. Endometrial sampling was obtained from all patients. In 12 patients endometrial sampling was scant and did not give any histological diagnosis.

Endometrial hysteroscopic findings were classified as atrophic endometrium, functional endometrium, endometrial hyperplasia, polyp and leiomyoma [8]. TVS findings were classified as polyp and endometrial thickening.
Results

Nineteen women out of 37 patients (51%) showed endometrial pathology. Out of five symptomatic patients three had abnormal findings. Out of 32 asymptomatic patients, 16 had abnormal findings. Hysteroscopic findings have a sensitivity of 100% and specificity of 94%, positive predictive value (PPV) of 94% and negative predictive value (NPV) of 100% to predict endometrial abnormalities. Hysteroscopy and sonography were both positive in 12 patients who had endometrial polyps.

The comparison of hysteroscopic findings with histopathology is summarized in Table 1. In 16 cases of hysteroscopic endometrial atrophy, 11 cases revealed insufficient endometrial material by curettage. Two patients with hysteroscopic imaging of functional endometrium were well correlated with histopathological findings of functional endometrium. Hysteroscopic imaging mostly showed only endometrial polyps and was unable to reveal concomitant endometrial hyperplasia. Five cases, diagnosed with endometrial polyps by hysteroscopy showed endometrial hyperplasia associated with a polyp.

Discussion

There is clear evidence of an increased risk of endometrial cancer in women treated with tamoxifen [9]. Even with this increased incidence, tamoxifen is very beneficial in reducing the number of contralateral breast cancers. Postmenopausal women with uterine bleeding must be promptly investigated whereas the indication for planning regular endometrial surveillance for asymptomatic patients using tamoxifen is a matter of debate. The measurement of endometrial thickness is a poor discriminating feature as endometrial thickness is naturally increased during tamoxifen treatment. In the majority of women with thickened endometrium on TVS, the scan showed cystic changes within the endometrium. It has been suggested that this may be caused by endometrial edema so that TVS cannot identify the real endometrial thickness. Although this can provide an explanation for the edematous hysteroscopic appearance, it is not possible for TVS to predict whether the women had edema or benign endometrial pathology. Neven et al. [10] reported a similar difficulty in distinguishing an endometrial polyp and a sonographic tamoxifen-related thickened cystic endometrium.

Hysteroscopy is a more accurate method in selected patients for further diagnostic testing. The detection rate of endometrial cancer by using hysteroscopy in asymptomatic patients is much higher than the detection rates by blind dilatation and curettage [11]. Blind techniques underestimate the prevalence of pathologic findings in the endometrium under tamoxifen treatment. There is no doubt that hysteroscopy is the reference-test to evaluate the endometrial cavity [12]. Loffer [13] stated that, normal hysteroscopic images should meet the criteria of good visualization of an homogeneously thin and regular endometrium. Cystic changes and irregular lining should be considered as abnormal findings leading to an endometrial sampling.

Several reports indicate that the detection of hyperplasia or carcinoma within a polyp in women under tamoxifen use is a frequent finding [14] as five hyperplasias were confined in a polyp in the present study. Endometrial polyps may have a possible neoplastic potential and they may present as the first step in endometrial carcinogenesis [15]. Polyps, on the other hand, are considered to be an important intermediate stage in tamoxifen-related carcinogenesis, even in the absence of endometrial hyperplasia. We believe that endometrial polyps, probably often go undiagnosed because of inappropriate sonographic cut-off values and blind techniques of tissue sampling.

Kedar et al. [16] found that the PPV of endometrial thickness for the detection of an abnormal pathology rose to 100% with a cut-off limit of 8 mm. As endometrial pathologies have been rarely found in postmenopausal patients with an endometrial thickness of less than 8 mm [17], there is no reason to raise the sonographic cut-off in tamoxifen users.

Conclusion

Endometrial surveillance is an important part of gynecological follow-up in breast cancer patients using tamoxifen and the liberal use of hysteroscopy aids in the diagnosis of abnormal endometrium.

References

Retrospective analysis of hysteroscopic findings in breast cancer patients having adjuvant tamoxifen treatment

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Ultrasound urodynamic study of urinary tract dysfunction after radical hysterectomy and pelvic lymphadenectomy in women with cervical carcinoma

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Summary

Purpose: To investigate the functional changes of the lower urinary tract after radical hysterectomy and pelvic lymphadenectomy node dissection using ultrasound urodynamics. Methods: Fifty-one women with cervical carcinoma underwent pre- and postoperative B-mode ultrasound imaging urodynamics. Results: Twenty-six women needed abdominal straining to void after radical surgery, and the urinary stream was intermittent. The maximum flow rate, average flow rate, and voiding volume significantly decreased, but the post-void residual volume significantly increased. Bladder sensory function deteriorated and maximum detrusor pressure decreased. The functional length decreased, but the maximum urethral pressure and urethral closure pressure did not change. Ultrasound measurement showed no significant changes regarding the depth of the bladder wall or the position of the bladder neck. Conclusions: Lower urinary tract dysfunction after radical hysterectomy is characteristic. Measurement of ultrasound urodynamics may be used as a preliminary screening method to detect voiding dysfunction following surgery.

Key words: Hysterectomy; Lymph node excision; Ultrasonography; Uterine cervical neoplasms.

Introduction

Cervical (CC) is one of the most frequent malignant tumors in the field of gynecology. Currently, surgery is still the primary technique for treatment of early CC. For patients in Stage I-II, radical hysterectomy is always performed in combination with dissection of the lymph nodes in the pelvic cavity. However, dysfunction of the lower urinary tract is commonly observed in these patients. The incidence of lower urinary tract dysfunction after CC surgery ranges between 8-80% depending on the specific report [1]. At present, the discussion regarding the etiology has mainly focused on neurogenic bladder dysfunction which is thought to arise from the injury or to partial severing of the autonomic nerve of the bladder leading to decrease in elastic muscular fibers; however, this is still controversial. The characteristics of surgical injury appear to represent partial rather than complete denervation. Additionally, bladder dysfunction might be caused by the loss of β adrenergic innervation and the subsequent increase of adrenergic innervation or remnant sympathetic innervation in the detrusor muscle [2].

Urodynamics is mainly based on the fundamental principles and methods of fluid mechanics and electrophysiology. It is used to assess the functional status of the bladder, urethra, pelvic floor, and sphincter at stored urine time and emiction time. Ultrasound images can be used to gather information regarding the anatomy and morphological as well as dynamic changes of the bladder and urethra.

Imaging urodynamic technology combined with the morphologic features can provide new clues for diagnosis of lower urinary tract dysfunction. Ultrasound urodynamics was used as a technique to evaluate dysfunction of the lower urinary tract after extensive hysterectomy.

Materials and Methods

Experimental subjects

Fifty-one CC patients who underwent radical hysterectomy and dissection of the pelvic cavity lymph node at the Department of Obstetrics and Gynecology of the Affiliated First Hospital of Chongqing Medical University from March 2006 to March 2008 were selected for the study. All patients were assessed using complete preoperative and postoperative B-mode ultrasound urodynamics. Patients had no correlated lesions such as pelvic surgery, urinary surgery, or infection of the nervous system before the operation and had no auxiliary radiotherapy during the surgical examination period and urodynamics. Prior to surgery, two experienced oncologists performed bimanual physical examination and trimanual gynecological examinations. The final diagnosis was made after reviewing the patient’s history, clinical manifestations, and biopsy, and was staged according to the clinical standards of CC revised by FIGO in 2002 [3]. CC was diagnosed in 15 Stage IB cases and 36 Stage IIA cases with an average age of 46.2 ± 9.3 years and an average follow-up time of 8 ± 1.3 months after surgery. All cases were confirmed by pathologic examination after surgery; 39 cases were squamous carcinoma and 12 cases were adenocarcinoma.

Surgical methods and scope

Surgeries were performed by the same surgeon using unified standard techniques. All patients received radical hysterectomy and dissection of the bilateral pelvic cavity lymph node.
Radical hysterectomy with dissection of the bilateral pelvic cavity lymph node

The uterine lateral incision edge was ≥3 cm away from the focus of infection, including the excision of the cardinal liga-
ment, two-thirds of the sacrouterine ligament, and 3-4 cm of the vagina. The rectum and cystic areola were opened and a tunnel free from the uterine wall and ureter of the cervix was performed straight into the bladder. The uterine artery was ligated free from the uterine wall and ureter of the cervix was per-
formed straight into the bladder. The uterine artery was ligated at the start of the internal iliac artery free from the uterine wall. The internal iliac artery was dually ligated 0.5-1 cm below the bifurcation of the general iliac artery. Avulsion was used to thor-

Catheter retention

The catheter was left in place for two weeks after surgery. The first week consisted of persistent patefaction of the catheter, and antibiotics were given to prevent infection of the urinary system. The second week consisted of interrupted patefaction of the catheter; gymnastic functional exercise was performed for the bladder, and bladder training was used to allow filling of the bladder. Residual urine was tested 10-14 days after surgery and the catheter was removed when residual urine was <100 ml; if the residual urine was >100 ml, retention of the catheter was continued for 3-5 days and acupuncture and oral molubustion were administered to enhance functional recovery. Patients were told to schedule a follow-up visit at the outpatient clinic and to visit the hospital regularly (once a month for one year after sur-
gery, and once every 3 months, 2 years and beyond) for B-mode ultrasound examination to monitor residual urine and the gener-
al condition of the upper urinary tract.

Urodynamic examination

The DUET Logic urodynamic apparatus from Medtronic was used to partly determine free uroflometry, bladder P-V/pressure flow, and quiet urethral pressure. The procedure was performed by an experienced urodynamic technician, according to the method instituted by the International Continence Society (ICS) [4]. Thickness of the bladder wall and location of the bladder neck were simultaneously measured by ultrasound examination with urethral manometry through the perineum vestibule.

Free uroflometry

Patients were told to evacuate and drink 300-500 ml water one hour before the examination. When patients showed intense micturition need, a urinary assembler was used to pass urine and the flow rate curve was automatically recorded by the urody-
namic apparatus which recorded the maximum flow rate, average flow rate, urinary output, and flow time [5].

Bladder P-V/pressure flow determination

A manometric catheter (10 Fr) was placed into the lumen of the rectum with about 10-15 cm and 3-5 ml diffusion. Patients were asked to change their sitting positions, and the superior part of the pubic symphysis was exteriorized in vitro. The duct manometry of he bladder tip and ano-rectal manometry were partly connected with the epitalix interface in the barocceptor of the urodynamic apparatus. Each duct was injected with water and released. After checking for bubbles or intortion, room tem-
perature stroke-physiological saline solution was used to engerge the bladder at a speed of 20-50 ml/min with a booster pump until the patients experienced an intense and intolerable need to urinate. Patients were then told to bring the duct with voluntary micturition into the uroflowmeter, and the detrusor pressure, intravesical pressure, rectum pressure, and urine flow were simultaneously recorded.

Table 1. — Comparison of free uroflometry results before and after surgery.

<table>
<thead>
<tr>
<th>Urodynamic parameter</th>
<th>Before surgery</th>
<th>After surgery</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max urinary flow (ml/sec)</td>
<td>23 ± 5</td>
<td>13 ± 6*</td>
<td>86.603</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Average flow (ml/sec)</td>
<td>14 ± 4.9</td>
<td>6 ± 2.5*</td>
<td>23.094</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary output (ml)</td>
<td>316 ± 41</td>
<td>179 ± 92*</td>
<td>19.136</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uretion time (sec)</td>
<td>26 ± 9.8</td>
<td>28 ± 10.4</td>
<td>-1.718</td>
<td>0.092</td>
</tr>
<tr>
<td>Residual urine (ml)</td>
<td>6 ± 3.2</td>
<td>198 ± 131.9*</td>
<td>-10.659</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Comparison of uroflometry results before and after surgery; p < 0.05.

Table 2. — Bladder pressure and volume change before and after surgery.

<table>
<thead>
<tr>
<th>Urodynamic parameter</th>
<th>Before surgery</th>
<th>After surgery</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder volume (ml)</td>
<td>80 ± 81</td>
<td>359 ± 103*</td>
<td>-6.704</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Max bladder volume (ml)</td>
<td>401 ± 116</td>
<td>658 ± 165*</td>
<td>-8.265</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Max detrusor pressure (cmH2O)</td>
<td>40 ± 12</td>
<td>12 ± 8*</td>
<td>23.020</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bladder compliance</td>
<td>103 ± 50</td>
<td>17 ± 5*</td>
<td>13.541</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Comparison of uroflometry results before and after surgery; p < 0.05.

Table 3. — Comparison of quiet urethra before and after surgery.

<table>
<thead>
<tr>
<th>Urodynamic parameter</th>
<th>Before surgery</th>
<th>After surgery</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max urethral pressure (cmH2O)</td>
<td>102 ± 25.4</td>
<td>103 ± 26</td>
<td>-0.078</td>
<td>0.938</td>
</tr>
<tr>
<td>Max urethral closure pressure (cmH2O)</td>
<td>95 ± 23</td>
<td>93 ± 22</td>
<td>0.364</td>
<td>0.718</td>
</tr>
<tr>
<td>Functional urethral length (mm)</td>
<td>40 ± 6</td>
<td>32 ± 6.2*</td>
<td>7.958</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Comparison of uroflometry results before and after surgery; p < 0.05.

Table 4. — B-mode ultrasound imaging parameters.

<table>
<thead>
<tr>
<th>Ultrasound parameter</th>
<th>Before</th>
<th>After</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of bladder wall (mm)</td>
<td>3.4 ± 0.7</td>
<td>3.5 ± 0.8</td>
<td>-0.470</td>
<td>0.641</td>
</tr>
<tr>
<td>Bladder neck position (mm)</td>
<td>34.2 ± 3.4</td>
<td>2.4</td>
<td>-1.812</td>
<td>0.076</td>
</tr>
</tbody>
</table>

Quiet urethral pressure determination

Patients were in the dorsal position with a 10 Fr manometric duct of the double lumen and choke tube when filling of the bladder reached 200 ml in the metabolic stage. The speed of perfusion was set at 2 ml/min and the rate of retreat at 1 mm/sec.

B-mode ultrasound examination

Sonography (DP-8800) Mindray Co., China was used with an intracavitary vaginal feeler (65EC10EA, 6.5MHz). The thickness of the bladder wall and location of the bladder neck [6] were simultaneously measured by B-mode ultrasound imaging with urethral manometry through the perineum and vestibule.

Statistical methods

Data were analyzed using the SPSS13.0 software package. Parameter results are expressed as the mean ± standard deviation (x ± s). Statistical methods were tested with paired t-tests with significance accepted at p < 0.05.
Results

Urethral symptoms and metergasis after CC surgery

Lower urinary tract symptoms after surgery included increased frequency, urgency, incontinence, continuous urine, and dysuria. Dysuria was observed in 20 cases; urine leakage and incontinence were observed in 12 cases; and increased frequency and continuous urine were observed in eight cases. Only six cases were content with uresiesthesia. Urodynamics revealed that 14 patients had reduced detrusor function of the bladder and 28 patients had detrusor muscle paralysis; completely normal function of the detrusor muscle was observed in four cases.

Comparison of urodynamic parameters before and after surgery

Stage IB-IIA CC patients underwent radical hysterectomy and dissection of the bilateral pelvic cavity lymph node, and the urodynamic parameters were significantly different from before surgery. Prior to the surgery, 26 patients had normal emission mode, but needed the aid of abdominal pressure to pass urine and the curve of flow showed discontinuity (Figure 1). The maximum detrusor pressure of four patients was < 25 cm H₂O before surgery, but the maximum detrusor pressure of 14 patients was < 25 cm H₂O after surgery. Residual urine levels of > 100 ml were observed in 21 cases at follow-up with urodynamics after surgery.

Comparison of free uroflometry results before and after surgery

After radical hysterectomy, the maximum urinary flow, average urinary flow, and urinary output were reduced. Additionally, residual urine volume increased significantly.

Bladder function before and after surgery

A significant increase in bladder volume after surgery with normal micturition need likely represents decreased sensory function of the bladder. In contrast, a significant decrease in maximum detrusor pressure and bladder compliance suggests decreased myofibril contraction.

Quiet urethra before and after surgery

The functional urethral length was significantly shortened after surgery, but the maximum urethral pressure and maximum urethral closure pressure had no significant change.

Ultrasound imaging of CC before and after surgery

There was no significant change in the thickness of the urinary bladder wall after radical hysterectomy. No significant displacement in the bladder neck was observed before or after surgery. In total, four patients had lateral or bilateral hydronephrosis at an average of 11 months after surgery, and the manifestation was lateral or bilateral waist soreness, distension, and pain.

Discussion

Neuroanatomical aspects of radical hysterectomy

Radical hysterectomy and dissection of the bilateral pelvic cavity lymph node for CC can damage the nerve plexus on the pelvic floor [7]. When the sympathetic nervous system is active, urinary accommodation will occur and urinary reflex will be inhibited. The parasympathetic nervous system has the opposite function of the sympathetic nervous system. Bladder sensation is transferred to the central nervous system by two types of afferent nerve fibers, and bladder distension is mediated by proprioceptive sensory nerve endings (collagen bundles). Discomfort, pain and other stimuli are transmitted by free nerve endings into the mucous membrane of the urinary bladder and submucosa. Receptors are in the trigone and the body of the bladder. Urethral sensory information is referred as pain, temperature, urethral sounding, and urinary passage.

Mechanisms of lower urinary tract changes after radical hysterectomy

The pathophysiology of lower urinary tract disturbance after radical hysterectomy is not clear. Possible mechanisms include surgical coup injury, successive fibrosis of the peripheral bladder tissues, afferent sensory nerve dysfunction, interruption of sympathetic and parasympathetic nerve impulses, or injury of the detrusor muscle [8]. Etiological studies of functional urethral changes after surgery have mainly focused on neurogenic bladder dysfunction [9]. However, some investigators have suggested that features of surgical injury involve partial denervation and the loss of β adrenergic innervation, as well as successive increases in adrenergic nerve innervation or effects on the remaining sympathetic innervation [7]. Thus, chronic bladder dysfunction following extensive hysterectomy might be related to the degree of nervous injury after surgery and successive injury caused by bladder hyperdistension.

Lower urinary tract disturbance after radical hysterectomy

According to previous reports, after radical hysterectomy of CC about 10-32% of patients showed severe bladder dysfunction [10]. Most investigators have found lower urethral dysfunction after radical hysterectomy in 29-76% of patients [11]. The present study showed that 12 patients suffered from incontinence and 20 from dysuria; only six patients were content with uresiesthesia after surgery, and only four cases had completely normal emission. These results are inconsistent with the previous literature, possibly due to different diagnostic stages, surgical methods, and curative outcomes, as well as different follow-up times after surgery.

Change of free urinary flow after extensive hysterectomy

Free urinary flow reflects the allomeric function of the bladder and urethra. The maximum flow of normal
Ultrasound urodynamic study of urinary tract dysfunction after radical hysterectomy and pelvic lymphadenectomy in women etc.

Figure 1. — Urodynamic curve before and after surgery.
Figure 2. — Comparison of free uroflometry parameter before and after surgery.
Figure 3. — Comparison of detrusor of bladder function before and after surgery.
Figure 4. — Comparison of quiet urethra before and after surgery.

Figure 5. — Comparison of bladder neck position before and after surgery.

Figure 6. — Hydronephrosis.
females is 20-36 ml/sec and increases linearly (5.6 ml/sec/100 ml) with the increased urinary output [3]. Studies using urodynamics have shown decreases in the maximum flow after extensive hysterectomy [12]. Additionally, flow time increased without significant change in urinary output before or after surgery, but there was a significant increase of residual urine volume after emiction after radical hysterectomy. The flow declined but recovered to baseline as determined by urethral pressure six months after surgery. In the present study, a significant decrease in maximum flow, average flow, and urinary output after extensive hysterectomy was observed in addition to a significant increase in residual urine volume after emiction and flow time. Twenty-six patients with active emiction in the detrusor muscle before surgery required the aid of abdominal pressure for emiction after surgery, and the curve was interrupted. After extensive hysterectomy, emiction damage has been attributed to changes to the pelvic plexus nerve, parasympathetic deficiency, action of detrusor of the bladder, and neurosensory damage.

Change of bladder function after radical hysterectomy

Early on after extensive hysterectomy, provisional changes in bladder function appeared including bladder achesis, underactivity of the detrusor muscle, and bladder hypesthesia, with successive presentation of evacuation disorder, frequency, urgency, incontinence, and decreased bladder compliance [1]. Bladder dysfunction was typically due to reduced bladder sensory function, decreased bladder compliance, and maximum detrusor pressure at eight months follow-up. Chuang et al. [13] found significant increases in maximum filling pressure in the bladder as well as significantly decreased bladder compliance that recovered within six months after surgery.

Dysfunction of the detrusor muscle might be due to both sympathetic and parasympathetic bladder denervation. During extensive hysterectomy, the automatic nerve fibers innervating bladder are disrupted. Thus, postganglionic nerve fibers originating from the pelvic plexus and leading to the bladder might also be disrupted. Such damage would compromise the vesicoureterine ligament and lead to various bladder dysfunctions such as detrusor muscle dysfunction and incontinence. In terms of urinary tract function, parasympathetic damage would result in reduced contraction of the bladder or abnormal bladder contractions with reduced bladder sensory function, and damage to the sympathetic system would result in reduced bladder compliance and increased storage pressure in the bladder [14]. However, some investigators have suggested that simple nerve injury cannot explain the retention of urine after surgery [15].

Incontinence after extensive hysterectomy

The incidence of incontinence after extensive hysterectomy was 23.6% in the present study. There was no obvious change in the position of the bladder neck, thus displacement of the bladder neck cannot explain incontinence after surgery. When exeresis of the superior extremity in the vaginal wall was performed, the supporting anatomy of the bladder and junction between the bladder and urethra showed only a temporary change. Etiological factors can include preoperative weakness of the wall of the bladder neck, postoperative posterior displacement in the bladder, and decreased urethral pressure.

No significant change was found in maximum urethral pressure or maximum urethral closure pressure, but a significant decrease in functional urethral length was observed. Villena-Heinsen et al. [16] suggested that radical hysterectomy did not significantly change urethral resistivity, pressure conduction, functional urethral length, or urethral pressure. During the entire process, the maximum urethral closure pressure showed a declining trend that recovered within six months after surgery. After extensive hysterectomy, sympathetic innervation as well as loss of coordination of the urethral peripheral muscle could cause inadequate closing of the bladder neck and incontinence.

Effects on upper urinary tract function after extensive hysterectomy

Normal bladder function was measured in terms of emiction and storing time. Four patients had hydronephrosis 11 months after surgery, on average. In a study where renal impairment was caused by lower urinary dysfunction, the most significant abnormal change was long-term neurogenic bladder dysfunction. The first stage involved high storage pressure of the bladder generating back flow of the metanephric duct during filling. The second stage involved gradual reduction of bladder compliance and fibrosis of the detrusor muscle, where most of the muscle was replaced by collagen. This can cause sharp elevations in vesical pressure at minimum bladder volume during filling. It was reported that when increasing pressure in the bladder the filling pressure was > 40 cmH2O because if the urine simultaneously flowed back, renal blood flow and function might have been affected [17].

After extensive hysterectomy following CC, patients are encouraged to perform postoperative early abdominal pressure emiction, Crede’s action emiction, and intermittent catheter dissection to avoid dysuria and urinary tract infection. Periodic inspection, free urinary flow, and ultrasound imaging are preliminary screening methods used to test emiction disorders after surgery. Additionally, complete imaging urodynamic examination has been used to spot early urethral functional disturbances which could permit early precaution and treatment for upper urethral functional lesions.

References


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The prevalence of *Leptotrichia amnionii* in cervical swabs of HPV positive and negative women

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Summary

The aim of the present study was to determine the prevalence of *Leptotrichia amnionii* in cervical swabs of women and its possible correlation with HPV infection and the stage of cervical cancer. **Material and Methods:** A total of 139 cervical swabs from healthy women with normal cytology, with dysplastic changes and with cervical cancer were tested for the presence of *L. amnionii* and high-risk HPV DNA by PCR methods. **Results:** *L. amnionii* was found in normal vaginal flora and in women with bacterial vaginosis (BV), which suggests that it may be opportunistic pathogen. *L. amnionii* infection was diagnosed in 13.7% (19/139). Statistical analysis showed that there was positive association (*p < 0.01*) between the presence of *L. amnionii* in women with cervical cancer (38.5%) and its presence in women without cancer (11.1%). On the other hand, there was no statistically significant association between *L. amnionii* and HPV infections. **Conclusion:** The data presented in this study show for the first time the prevalence of *L. amnionii* infection in cervical specimens collected from 2004-2006 in Poznan and Lublin, Poland, and its association with HPV infection and the stage of carcinogenesis of the cervix.

**Key words:** *Leptotrichia amnionii*; Cervical cancer; HPV; Bacterial vaginosis.

Introduction

Although the cervix is not normally colonized with bacteria, they are present in the vaginal epithelium. Predominant bacterial genus in vaginal smears is *Lactobacillus* sp. [1]. A common condition affecting millions of women annually is bacterial vaginosis (BV), which is connected with loss of vaginal lactobacilli and concomitant overgrowth of anaerobic and facultative bacteria [2]. It was found that bacterial vaginosis (BV) and cervical inflammation may be a cofactor for high-grade cervical lesions in women infected with oncogenic HPV [3].

The human papillomaviruses (HPV) are simple, nonenveloped, double-stranded DNA viruses, which are associated with cervical cancerogenesis [4]. Oncogenic HPV types 16 and 18 are responsible for a majority of cervical cancers and can also cause low- and high-grade cervical lesions (CIN 1, 2, 3). Nononcogenic types HPV 6 and 11 also contribute to the overall burden of HPV disease, giving rise to CIN 1, anogenital warts, cutaneous lesions, and respiratory papillomatosis [5-8].

*Leptotrichia* as a cause of infection in the female urogenital tract was first described in 2002 [9], when a novel species, *L. amnionii*, was described for the first time. It was isolated from the amniotic fluid of a woman after intrauterine fetal demise. Since then, several cases of *L. amnionii* infection have been reported, which were associated with the female genital tract [2, 10, 11], caused illness during pregnancy and in the postpartum period [12, 13] and also arthritis [14]. *Leptotrichia* sp., an anaerobic gram-negative, fastidious, nonmotile bacteria with large fusiform rods, belongs to the family *Fusobacteriaceae* and is a part of the normal oral [15] and vaginal flora [16, 17]. Species included in the genus are *L. buccalis*, *L. trevisanii*, *L. sanguinegens*, and *L. amnionii* [9, 18]. To date there have been no data about its presence in the cervix. *L. amnionii* was found in normal vaginal flora [16] and in women with bacterial vaginosis (BV) [19], which suggests that it may be opportunistic pathogen.

*L. amnionii*, like other *Leptotrichia* species, is extremely fastidious and cannot be grown easily on conventional microbiologic media or by conventional methods. Its detection was enabled by the application of bacterial 16S rRNA gene amplification and sequencing technique [2, 9, 20], which has emerged lately as one of the most successful culture-independent methods for identification of bacteria. The large rRNA sequence databases (e.g. GenBank) allow a quick comparison of 16SrDNA sequences and accurate identification of bacteria [21, 22]. Using amplification and sequencing of part of the 16SrRNA gene, we have recently identified *Leptotrichia amnionii* in DNA isolated from cervical swabs of pregnant women [23].

The aim of the present study was to determine the prevalence of *Leptotrichia amnionii* in cervical swabs of women and its possible correlation with HPV infection and the stage of cervical cancer.
Material and Methods

Samples

The women in this study were aged 30-50 (mean age 42), who were examined gynaecologically for three consecutive years from 2004-2006 in the Department of Gynaecology and Obstetrics, Poznan University of Medical Sciences and from the Department of Obstetrics and Pathology of Pregnancy, Medical University of Lublin. A Pap smear was done for cytological evaluation and for HPV DNA isolation for further PCR. A total of 139 cervical swabs were divided into three groups according to cervical cancer development stage: group I (43 swabs) from healthy women with normal cytology (Papa I-II), group II (83 swabs) with dysplastic changes (Papa III – CIN1-CIN3) and group III (13 swabs) from cervical cancer (Papa IV-V, CA) (Table 1).

DNA isolation and PCR amplification

Swabs were collected and stored at -20°C for DNA isolation. The DNA was extracted using the QIAamp DNA Midi Kit (Qiagen) and tested for the presence of Leptotrichia amnionii and HPV DNA by PCR methods.

For identification of Leptotrichia amnionii primers complementary to the part of bacterial 16SrRNA gene were designed. The forward and reverse primer sequences were: 5’ G C A G C A T T G G G A AT AT T G G C A AT G - GAGGGAACTCTG-3’ and 5’TAGGCCGGTAATTTCAGGTG-3’, respectively. Amplification was performed with a 0.2 mM concentration of each primer and 0.4 U of Taq DNA polymerase (DyNAzyme II, Finnzymes).

For human papilloma virus (HPV) identification, the AMPLICOR HPV PCR test (Roche) was used according to the manufacturer’s instructions [24]. Primers in the test were designed to amplify HPV DNA from 13 high risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). PCR product length was 165 bp. Polymerase chain reactions were performed in an UNO II thermocycler (Biometra, Germany).

Ligation, transformation and sequencing

Purified PCR products of L. amnionii amplification were ligated into pGEM-T Easy vector (Promega). Competent Escherichia coli DH5alfa cells were transformed with ligation products. Plasmids with inserts were extracted from white transformed colonies with a QIAprep Plasmid Kit (Qiagen). Inserts and PCR products were sequenced with an automated 3130x Genetic Analyzer (Applied Biosystems) in the Faculty of Biology, Adam Mickiewicz University in Poznan, Poland. Sequences obtained were analysed and compared to those available in GenBank database (NCBI, USA) using BLAST search.

Statistical analysis

Statistical analysis of the results involved the test of difference between parameters in the nominal scale (i.e., diagnosis, presence of infection). Therefore the chi-square test was used to analyse groups counting at least 5, and Fisher’s exact test if one of the groups was smaller than 5. Significance level was taken as p < 0.05. Analyses were carried out using STATISTICA software (StatSoft, ver. 7.0).

Results

Prevalence of L. amnionii infection in cervical swabs

PCR products of L. amnionii amplification were initially sequenced to confirm that they in fact contained the 16S rRNA sequence of L. amnionii by database search (BLAST, NCBI, USA).

The 16S rRNA gene amplification and sequencing determined a 1493 nucleotide sequence. This sequence had 99.7% nucleotide similarity to the NCBI (National Centre for Biotechnology Information) database sequence for Leptotrichia amnionii (GenBank acc.no. AY489565; AY078425).

After DNA isolation and 16S rRNA gene amplification of 139 women, L. amnionii infection was diagnosed in 13.7% (19/139) (Table 1). The group of 139 women was divided according to their cytological evaluation into three groups: the first group with normal cytology, the second with dysplastic changes (CIN I-CIN III), the third with cervical cancer (CA). Among 13 women with cervical cancer (group III), L. amnionii was diagnosed in 38.5% (5/13).

Table 1. — Number of women with L.amnionii infection, grouped according to diagnosed cervical dysplastic changes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diagnosis</th>
<th>Number of patients</th>
<th>L.amnionii+ n/%</th>
<th>L.amnionii- n/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>–</td>
<td>139</td>
<td>19/13.7</td>
<td>120/86.3</td>
</tr>
<tr>
<td>Group I</td>
<td>Papa I-II (normal cytology)</td>
<td>43</td>
<td>4/9.3</td>
<td>39/90.7</td>
</tr>
<tr>
<td>Group II</td>
<td>Papa III - CIN1-CIN3 (dysplastic changes)</td>
<td>83</td>
<td>10/12.1</td>
<td>73/87.9</td>
</tr>
<tr>
<td>Group I+II</td>
<td>“lack of cancer”</td>
<td>126</td>
<td>14/11.1</td>
<td>112/88.9</td>
</tr>
<tr>
<td>Group III</td>
<td>Papa IV-V, CA (cervical cancer)</td>
<td>13</td>
<td>5/38.5</td>
<td>8/61.5</td>
</tr>
</tbody>
</table>

n = number of swabs.

Prevalence of HPV infection in cervical swabs

All the 139 women were screened for the presence of high-risk HPV infection by means of viral L1 gene PCR amplification using the AMPLICOR HPV PCR test (Roche). High-risk HPV infection was diagnosed in 48.2% (67/139) of women (Table 2). Among the group of women with recognised cervical cancer (group III), HPV infection was diagnosed in 92.3% (12/13) and in the group with normal cytology (group I) only in 4.7% (2/43).

Table 2. — Number of women with HPV infection, grouped according to diagnosed cervical dysplastic changes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diagnosis</th>
<th>Number of patients</th>
<th>HPV+ n/%</th>
<th>HPV- n/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>–</td>
<td>139</td>
<td>67/48.2</td>
<td>72/51.8</td>
</tr>
<tr>
<td>Group I</td>
<td>Papa I-II (normal cytology)</td>
<td>43</td>
<td>2/4.7</td>
<td>41/95.3</td>
</tr>
<tr>
<td>Group II</td>
<td>Papa III - CIN1-CIN3 (dysplastic changes)</td>
<td>83</td>
<td>53/63.9</td>
<td>30/36.1</td>
</tr>
<tr>
<td>Group I+II</td>
<td>“lack of cancer”</td>
<td>126</td>
<td>55/43.7</td>
<td>71/56.3</td>
</tr>
<tr>
<td>Group III</td>
<td>Papa IV-V, CA (cervical cancer)</td>
<td>13</td>
<td>12/92.3</td>
<td>1/7.7</td>
</tr>
</tbody>
</table>

n = number of swabs.
Statistical correlation studies

We tested whether there was any association between the infection with L. amnionii and clinical diagnosis. The analysis showed that there was positive association of statistical significance ($p < 0.01$) between the presence of L. amnionii in women with cervical cancer (group III - 38.5%; 5/13) and its presence in women without cancer (groups I and II – normal and dysplastic cytology - 11.1%; 14/126) (Table 1). The measure of this correlation in the nominal scale is relative risk. In this case it is the rate of probability of L. amnionii infection in women with cervical cancer (group III) to the probability of the same infection in the group without cancer (groups I and II). In this case relative risk of L. amnionii infection in the group with cervical cancer to the groups without cancer was 3.46 (Table 3).

Table 3. — Correlation between L. amnionii positive/negative women and women with cervical cancer (group III) or lack of cancer (groups I and II). L. amnionii relative risk is the rate of probability of L. amnionii infection in women with cervical cancer to the probability of the same infection in group without cancer.

<table>
<thead>
<tr>
<th>L. amnionii</th>
<th>Cancer n = 13</th>
<th>No cancer n = 26</th>
<th>$\chi^2$ test</th>
<th>Relative risk L. amnionii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>n = 19</td>
<td>5</td>
<td>14</td>
<td>$p = 0.0063$</td>
</tr>
<tr>
<td>Negative</td>
<td>n = 120</td>
<td>8</td>
<td>112</td>
<td></td>
</tr>
</tbody>
</table>

According to these data, similar relation of statistical significance ($p < 0.05$) between the presence of L. amnionii in women with cervical cancer (group III - 38.5%; 5/13) and its presence in women with normal cytology (group I - 9.3%; 4/43) was also shown (Table 1). In this case relative risk of L. amnionii infection in the group with cervical cancer to the group with normal cytology is 4.13 (Table 4).

Table 4. — Correlation between L. amnionii positive/negative women with women with cervical cancer (group III) or normal cytology (group I). L. amnionii relative risk is the rate of probability of L. amnionii infection in women with cervical cancer to the probability of the same infection in group with normal cytology.

<table>
<thead>
<tr>
<th>L. amnionii</th>
<th>Cancer n = 13</th>
<th>Normal cytology n = 43</th>
<th>Precise fisher test</th>
<th>Relative risk L. amnionii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>n = 19</td>
<td>5</td>
<td>4</td>
<td>$p = 0.0240$</td>
</tr>
<tr>
<td>Negative</td>
<td>n = 120</td>
<td>8</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>

As infection with the HPV virus is a known etiological factor in cervical cancer development, we subsequently tested whether there was any association between L. amnionii and HPV infection. However, statistical analysis showed that there was no statistically significant association between L. amnionii and HPV infections. In the L. amnionii positive group, 52.6% were also HPV positive and 47.4% were HPV negative. In the L. amnionii negative group 43.3% were HPV+ (Table 5).

Table 5. — Analysis of correlation (chi-square test) between L. amnionii and HPV infection.

<table>
<thead>
<tr>
<th>L. amnionii</th>
<th>HPV+</th>
<th>HPV-</th>
<th>$\chi^2$ test</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV+</td>
<td>10 (52.6%)</td>
<td>9 (47.4%)</td>
<td>$p = 0.4487$</td>
</tr>
<tr>
<td>HPV-</td>
<td>52 (43.3%)</td>
<td>68 (56.7%)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The data presented in this study show for the first time the prevalence of L. amnionii infection in cervical specimens collected from women aged 30-50, who were examined gynaecologically from 2004-2006 in Polish medical centres of Pozna and Lublin. L. amnionii infection was diagnosed in 13.7%. It is consistent with our previous research when cervical swabs of pregnant women were screened for the presence of L. amnionii. In the group of 69 pregnant patients L. amnionii sp. nov. was identified in 11.6% [23].

Additionally, among group of women with recognised cervical cancer, HPV infection was diagnosed in 92.3% and in the group with normal cytology only in 4.7%. This result is consistent with other research which showed the connection between HPV infection of the cervix and the development of cervical cancer [5, 25].

HPV infection is a major risk factor for the development of cervical cancer. HPV involvement in the etiology of some major human cancers is of particular interest: specific oncogenic types (HPV 16, 18 and several others) have been identified as causative agents of at least 90% of cancers of the cervix and are also linked to more than 50% of other anogenital cancers [5, 8]. HPV DNA can be detected in most cervical cancers [25]. However, few HPV infections progress to cervical cancer; the vast majority cause no or mild cytological abnormalities. The factors that determine whether an HPV infection will resolve to normalcy or progress to high-grade lesions is not completely understood [3]. Additionally there is also interest in bacterial infections as possible factors elevating the risk of cancer development.

Obtained data show that there was a positive association of statistical significance ($p < 0.01$) between the presence of L. amnionii in women with cervical cancer and its presence in women without cancer. A similar relation of statistical significance ($p < 0.05$) between the presence of L. amnionii in women with cervical cancer and its presence in women with normal cytology was also shown. Different bacterial species colonize cancer tissue with increased intensity than others during cervical cancer development. Similarly, it happens in bacterial vaginosis (BV), when the normal balance of bacteria in the vagina is disrupted and replaced by an overgrowth of anaerobic and facultative bacterial species [2]. It is suggested that dysplastic changes in the cervix could contribute to the creation of optimal conditions for development of different bacteria, especially anaerobae. For example, G. vaginalis was detected at a high incidence in patients with uterine cervical cancer, suggesting that the lesions of uter-
The association of inflammation with many cancer types suggests that it might be a universal risk factor in oncogenesis [27, 28], especially in cervical cancer [3]. Increasing evidence indicates that the inflammation may result from persistent mucosal or epithelial cell colonisation by microorganisms. Clinical and epidemiologic studies have suggested an association between infectious agents, chronic inflammation and cancer [27, 28].

PCR methods for identification of the bacterial species used in this study are very sensitive, fast and reliable. These results suggest that culture-independent methods can provide new insights into the diversity of bacterial species found in the human cervix.

To sum up, our results show that there was no correlation between L. amnionii and HPV infections, but there could be a positive association between the presence of L. amnionii and cervical cancer. Further research would be helpful to confirm if there is any correlation between L. amnionii and the stage of carcinogenesis of the cervix.

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References

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Centralization of ovarian cancer surgery: Do patients benefit?

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A. Mousavi1

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2Gynecology Unit, Mashhad University of Medical Sciences
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Summary

Background: The aim of the study was to compare the effect of surgery in patients with ovarian cancer by gynecologist-oncologists and general gynecologists on overall survival. Material and Methods: In a retrospective study all patients diagnosed with primary ovarian cancer at Vali-e-Asr Hospital (Tehran, Iran) between April 1999 and January 2005 were eligible for enrollment. A total 157 consecutive patients with ovarian cancer were available for analysis. There were no significant differences between the two groups with respect the stage, age and histological type of tumor. Of 157 patients, 60 were treated by gynecologist-oncologists and 95 by general gynecologists, and two patients were treated by general surgeons. Results: The number of patients who had optimal cytoreductive surgery performed was higher in the gynecologist-oncologist group than the number in the general gynecologist group (p < 0.001). The majority of patients in the general gynecologist group needed a second operation while only a few in the gynecologist-oncologist group needed one (p < 0.001). The interval between initial surgery and beginning of chemotherapy was significantly longer in the gynecologist-oncologist group as compared to the general oncologist group (p = 0.001). Overall survival and disease-free survival were considerably better in the gynecologist-oncologist group but the difference was not significant. Conclusion: Patients with ovarian cancer should be referred to Gynecology-Oncology Departments for optimal treatment.

Key words: Ovarian cancer; Cytoreductive surgery; Overall survival; Disease-free survival; Prognosis of ovarian cancer.

Introduction

Ovarian cancer accounts for only 5% of all cancers in women, however, it is one of the most common causes of female genital tract cancers [1]. Around 50% of patients with ovarian cancer die of their disease [2]. The standard therapy for ovarian cancer is cytoreductive surgery followed by chemotherapy. Initial surgery with staging and whole tumor resection is one of the most important factors affecting survival in these patients [3-5].

There is evidence based on the considerable improvement of patients with bowel or breast cancer who had been operated on by specialized surgeons in the respective field [6-8].

In patients with ovarian cancer, a complete surgical operation is important, especially in progressive disease. Many studies showed that survival is better when the operation is performed by a gynecologist-oncologist rather than a general gynecologist, which may be attributed to the fact that gynecologist-oncologists are capable of excising more tumoral mass [9]. Patients with complete tumor debulking show better survival than those with incomplete tumor resection [10, 12]. Recent studies showed that all clinically early-stage ovarian cancer patients should be considered for comprehensive staging surgery prior to further treatment recommendations [13, 14]. Moreover patients with early-stage ovarian cancer who have not been properly staged stand a significant risk of recurrent disease despite more frequent use of chemotherapy [15].

A number of studies report better survival in cases where surgery is performed by a gynecologist-oncologist [16-19]. The Clinical Resource and Audit Group recommends that patients who are suspected of having ovarian cancer should be first referred to a gynecologist-oncologist who is specialized in the field [20]. The Royal College of Gynecologists and Obstetricians recommends that women with ovarian cancer should be operated on by gynecologists who specifically work in this field [21]. The aim of the present study was to compare the role of specialized surgeons in the survival of patients with ovarian cancer who undergo surgery so as to provide a solution to improve survival of this disease.

Materials and Methods

This retrospective study was performed from March 1998 through February 2004 on a total of 157 patients. The study included all patients who had been operated on for ovarian cancer or intended to complete their post-operation treatment course in the Gynecology-Oncology Ward of Vali-e-Asr Hospital. Sixty patients had been operated on by gynecologist-oncologists, 95 patients by general gynecologists and two patients had been operated on by general surgeons. The median time of follow-up in patients was 32 months and survival analysis was done. Patients were divided in two groups:

Group 1 - Patients whose first surgery was performed by gynecologist oncologists and Group 2 - Patients whose first surgery was performed in non-specialized centers by other surgeons.
Detailed information on type of surgery, size of residual disease at the end of surgery, histologic type and grade, type and number of courses of first-line chemotherapy, hospital stay, and postoperative mortality was abstracted from the medical records of each hospital. Uniform determination of FIGO stage was based on the outcome of preoperative diagnostic procedures, the operation notes, and the pathology report. All histologic slides were reviewed by a single pathologist and classified according to the World Health Organization guidelines by histologic type and differentiation. Volume of residual disease after primary surgery, as documented in the operative notes, was dichotomized into the largest diameter of residual tumor less than 1 cm or 1 cm or greater.

Patients with Stage > IA epithelial ovarian tumors received a chemotherapy regimen of cisplatin in combination with paclitaxel; those with malignant ovarian germ cell tumors (MOGCTs) were treated with a BEP regimen (cisplatin, etoposide, bleomycin). Also all patients with MOGCTs and immature teratoma grade > 2 received the BEP chemotherapy regimen. This regimen was also administered to patients with Stage > IA sex cord stromal tumors (SCSTs). Only patients with Stage I Sertoli-Leydig cell tumors that were poorly differentiated or that contained heterologous elements were treated with chemotherapy. The number of cycles depended on surgical staging, the patient’s tolerance to chemotherapy and the objective response of any measurable disease. The different regimens were usually administered for four-six courses.

Statistical analysis was done using SPSS-11 software to compare the two groups for completeness of the first operation in the different stages of disease, reason for incomplete primary operation, rate of secondary operations and their cause, period of recurrence, interval between first surgery and chemotherapy and 2-year survival rates. The Student’s t-test was used to compare quantitative variables between the two groups and in cases where this test could not be used the Mann Whitney U-test was employed instead. The chi-square test was used to compare qualitative variables. Kaplan Meier’s test was used in survival graphs which were compared using the Log rank test. By analyzing single variables, factors related to duration of survival and relapse-free interval or overall duration of survival were determined; p values less than 0.05 were considered significant for all statistical tests. To determine independent variables and the overall duration of recurrence-free interval, Cox’s proportional hazard model should have been used. However, considering the rule of thumb and that the number of deaths or cases with recurrent disease were low with respect to the total number of related significant variables, this test could not be performed.

**Results**

A total of 157 consecutive women with ovarian cancer were available for analysis. The mean (± SD) age of the cases was 36.3 ± 16 years (range: 12–76; median 34 years). Patient characteristics are shown in Table 1.

Of 157 patients, 60 (38.2%) were treated by gynecologist-oncologists (GO), 95 (60.5%) by general gynecologists (GG) and two (1.3%) by general surgeons (GS).

Since patients in the GS group were few in number, only the GO and GG groups were compared.

The patients treated by GO were older than those in the GG group by a median of 12 years and the difference was statistically significant (p = 0.021) (Table 2).

There were no significant differences between the two groups with respect to stage, age and histological type of tumor. An equal number of patients received chemotherapy in each group (Table 2). We record all the information of all histological types of ovarian cancer because in patients with epithelial ovarian cancer every effort should be made to attain as complete cytoreduction as possible. However in 30% of patients with early-stage epithelial ovarian cancer who did not have surgical staging, the stages of disease were increased in the second surgery [15]. Moreover in patients with MOGCTs or SCSTs who were treated by gynecologist-oncologists, chemotherapy was induced as soon as diagnosis was ascertained.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GO (n=95)</th>
<th>GG (n=60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 45</td>
<td>43 (45.3)</td>
<td>103 (65.6)</td>
<td>0.021</td>
</tr>
<tr>
<td>≥ 45</td>
<td>52 (54.7)</td>
<td>54 (34.4)</td>
<td></td>
</tr>
</tbody>
</table>

**Pathology**

<table>
<thead>
<tr>
<th>Type of tumor</th>
<th>GO (n=95)</th>
<th>GG (n=60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial cell carcinoma</td>
<td>74 (48.4)</td>
<td>76 (48.4)</td>
<td>0.087</td>
</tr>
<tr>
<td>Non epithelial tumor</td>
<td>81 (51.6)</td>
<td>83 (51.6)</td>
<td></td>
</tr>
</tbody>
</table>

**Stage of disease**

<table>
<thead>
<tr>
<th>Stage</th>
<th>GO (n=95)</th>
<th>GG (n=60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-II</td>
<td>83 (53.2)</td>
<td>83 (53.2)</td>
<td>0.981</td>
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<tr>
<td>III-IV</td>
<td>74 (46.8)</td>
<td>74 (46.8)</td>
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</table>

**Operator**

<table>
<thead>
<tr>
<th>Operator</th>
<th>GO (n=95)</th>
<th>GG (n=60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>General gynecologist</td>
<td>95 (60.5)</td>
<td>95 (60.5)</td>
<td>1.000</td>
</tr>
<tr>
<td>Gynecologist oncologist</td>
<td>60 (38.2)</td>
<td>60 (38.2)</td>
<td></td>
</tr>
<tr>
<td>General surgeon</td>
<td>2 (1.3)</td>
<td>2 (1.3)</td>
<td></td>
</tr>
</tbody>
</table>

**Operation**

<table>
<thead>
<tr>
<th>Type of operation</th>
<th>GO (n=95)</th>
<th>GG (n=60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>104 (66.2)</td>
<td>104 (66.2)</td>
<td>1.000</td>
</tr>
<tr>
<td>Suboptimal</td>
<td>53 (33.8)</td>
<td>53 (33.8)</td>
<td></td>
</tr>
</tbody>
</table>

**Second operation**

<table>
<thead>
<tr>
<th>Required</th>
<th>GO (n=95)</th>
<th>GG (n=60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Required</td>
<td>68 (43.3)</td>
<td>68 (43.3)</td>
<td>1.000</td>
</tr>
<tr>
<td>Not required</td>
<td>89 (56.7)</td>
<td>89 (56.7)</td>
<td></td>
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</tbody>
</table>

**Chemotherapy**

<table>
<thead>
<tr>
<th>Performed</th>
<th>GO (n=95)</th>
<th>GG (n=60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performed</td>
<td>121 (77.1)</td>
<td>121 (77.1)</td>
<td>1.000</td>
</tr>
<tr>
<td>Not performed</td>
<td>36 (22.9)</td>
<td>36 (22.9)</td>
<td></td>
</tr>
</tbody>
</table>
Centralization of ovarian cancer surgery: Do patients benefit?

Table 2. — Characteristics of patients categorized by specialized of surgeon.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GO</th>
<th>GG</th>
<th>p value</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>45 (16-76)</td>
<td>33 (12-70)</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>&lt; 45</td>
<td>29 (48.3)</td>
<td>73 (76.8)</td>
<td>&lt; 0.001</td>
<td>1 (50)</td>
</tr>
<tr>
<td>≥ 45</td>
<td>31 (51.7)</td>
<td>22 (23.2)</td>
<td></td>
<td>1 (50)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td>0.484</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>33 (55.9)</td>
<td>48 (50.5)</td>
<td></td>
<td>2 (100)</td>
</tr>
<tr>
<td>3-4</td>
<td>26 (44.1)</td>
<td>47 (49.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
<td></td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Epithelial cell</td>
<td>26 (43.3)</td>
<td>50 (52.6)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Nonepithelial</td>
<td>34 (7.56)</td>
<td>47 (47.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td>0.949</td>
<td></td>
</tr>
<tr>
<td>Performed</td>
<td>13 (21.7)</td>
<td>21 (22.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not performed</td>
<td>47 (78.3)</td>
<td>74 (77.9)</td>
<td></td>
<td>2 (100)</td>
</tr>
<tr>
<td>Operation</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Optimal</td>
<td>53 (88.3)</td>
<td>49 (51.6)</td>
<td></td>
<td>2 (100)</td>
</tr>
<tr>
<td>Suboptimal</td>
<td>7 (11.7)</td>
<td>46 (48.4)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Second operation</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Required</td>
<td>5 (8.3)</td>
<td>61 (64.2)</td>
<td></td>
<td>2 (100)</td>
</tr>
<tr>
<td>Not required</td>
<td>55 (91.7)</td>
<td>34 (35.8)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Recurrence</td>
<td></td>
<td></td>
<td>0.0108</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>17 (28.3)</td>
<td>39 (41.1)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Absent</td>
<td>43 (71.7)</td>
<td>56 (58.9)</td>
<td></td>
<td>2 (100)</td>
</tr>
</tbody>
</table>

GO: gynecologist-oncologist; S: surgeon; GG: general gynecologist.

Of patients in the GO group 88.3% had undergone optimal cytoreductive surgery compared with 51.6% in the GG group (p < 0.001).

The majority of patients (64.2%) in the GG group versus only 8.3% in the GO group were re-operated (p < 0.001).

The interval between the first operation and the start of chemotherapy was significantly longer in the GG group as compared with the GO group (median of 3 and 1 months, respectively, p < 0.001).

Although the recurrence rate in the GG group was higher than that of the GO group, this difference was not statistically significant (41.1% vs 28.3%, respectively, p = 0.108).

With a median follow-up of 32 months (range: 2-190 months), 27 (28.4%) patients in the GG group and ten (16.7%) patients in the GO group died of disease. This difference was not statistically significant.

Survival Analysis

The median duration of follow-up in the GG group was 28 months (range = 2-102) which was not significantly different from the median of 30.5 months (range = 4-6190, p = 0.4, Mann-Whitney U test) in the GO group.

Median disease-free survival (DFS) for patients in the GG group was 44 months and in the GO group (Log-rank test: p = 0.094) (Figure 1).

Overall survival duration was considerably greater in the GO group (median has not yet been reached) than in the GG group (median survival 56 months) but this difference did not reach a significant level (Log-rank test: p = 0.062).

Discussion

This study showed that 88.3% of patients who had been operated by GOs and 51.6% of patients who had been operated by GGs received optimal cytoreductive surgery. The specialization of the surgeon played a significant role in optimal cytoreductive surgery. The most important factors affecting optimal surgery are the stage of disease and histological type of tumor [13, 14]. In this study these factors were not significantly different between these two groups.

Steinberg et al. [22] studied 109 omenta in 159 patients with ovarian cancers; 22% of the normal looking omenta did carry macroscopic tumor deposits with a mean tumor diameter of 6.7 mm. This emphasizes the importance of histologic evaluation of the omentum, particularly in patients with early-stage ovarian cancer to rule out occult tumor spread. Occult retroperitoneal nodal involvement is also frequent in early-stage ovarian cancer with a possible link to poorer survival if missed. Dexeus et al. [23] documented 15% of positive pelvic nodes and 5.5% of isolated paraaortic nodal involvement in 68 patients with early-stage ovarian cancer who underwent retroperitoneal lymphadenectomy. Similarly Lang [24] studied 116 cases of Stage I ovarian cancer and of these 82 patients had complete lymphadenectomy as part of their surgical treatment. The incidence of microscopic lymphatic metastasis was 10.3%.

Eisenkop and Spirto [25] studied the time during surgical cytoreductions when macroscopic nodes were detected. Even in a study patient group having advanced stage disease, only 31% of the macroscopically enlarged nodes were detected by palpation alone, underscoring the inaccuracy in using palpation to determine nodal status. A number of reports have called for accurate surgical staging assessments prior to deciding on further treatment.

We reviewed patients with epithelial ovarian cancer that had been treated by GOs for complete surgical staging; in these patients without surgical staging and comprehensive cytoreductive surgery, the overall survival and disease-free survival were decreased [1, 15].

Tingulstad et al., reported that centralization of operations on the ovaries by GOs not only improves survival but also improves the quality of life of patients with advanced ovarian cancer [26]. The effect of factors such as age or stage of disease, tumor histology, and suboptimal surgery on survival is well known [27]. Also, in this study, age over 45 years, Stage III-IV disease and suboptimal surgery were associated with lower survival rates.

In a two-year study on ovarian cancer, Ohaitan et al. concluded that the rate of complete cytoreductive surgeries performed by GOs was 2.06 times more than for GGs. They concluded that ovarian cancer surgery should be limited to specialized surgeons so as to increase the number of optimal cytoreductive surgeries [28]. The need for a second operation was significantly higher in patients who had been operated on by GGs than for those oper-
ated on by GOs. The most frequent reason for performing a second operation was incomplete cytoreductive surgery and incorrect staging during the first operation. The rate of second operations was significantly more frequent in subjects who had been operated on by GGs.

Elit et al. reported that there is a clear relationship between surgeon specialization and the rate of second operation within a period of less than three months from the initial operation and they showed that the duration between initial operation and re-operation was shorter in subjects who had been operated on by GGs [29].

In this study, overall survival and recurrence-free survival rates were significantly higher among patients who had been operated on by GOs than those who had been operated on by GGs especially when the survival graph was compared according to age groups. The centralization of ovarian cancer surgeries to specific centers and their treatment by experienced surgeons both help improve therapeutic results [26]. The cost effectiveness of this measure has been approved by many studies [30]. Treatment by inexperienced surgeons and failure to perform complete tumor resection results in higher mortality and recurrence rates [31].

In this study, optimal surgery, starting chemotherapy within less than three months from the primary operation, and Stage I or II disease, are factors which were associated with higher survival rates. Chemotherapy, if required, was started earlier for most patients who had been operated on by GOs while this period was longer in cases that had been treated by GGs (mostly after a period of three months from the first operation). Thus the period between surgery and start of chemotherapy is a factor related to survival. It is concluded that survival rates are higher when the operation is performed by gynecologist-oncologists.

In one study, in patients in whom accurate surgical staging had not been performed, the 5-year survival rate was around 60% for epithelial cancers, which were apparently in Stage 1, whereas survival rate was around 90%-100% in patients in whom accurate surgical staging showed Stage IA to Stage IB disease. Out of every ten patients with ovarian epithelial cancer which is apparently limited to the pelvis, around three will have undetected metastasis in the upper abdomen and retroperitoneal lymph nodes [31].

**Conclusion**

Optimal cytoreductive surgery in patients with advanced epithelial ovarian cancer is prognostic factor. Also optimal cytoreductive surgery in advanced ovarian cancer patients and surgical staging in early stage of disease is necessary for planning of adjuvant chemotherapy and omitted second operation. Therefore, we suggest these patients should be referred to Gynecology-Oncology Departments for treatment.

**References**


Centralization of ovarian cancer surgery: Do patients benefit?


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Twenty-five years of medical colposcopic rural tours in the Republic of Panama: commitment and integration in screening 1983-2008

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Summary

Between the years 1983 and 2008, colposcopic rural tours were carried out which covered six provinces of the Republic of Panama, in which women in those areas were offered the opportunity of a preventive cervical-uterine cancer screening, applying cytology, colposcopy and directed biopsies (in those cases requiring it), as well as immediate treatment. The years of follow-up, especially in the community of Pocri de Los Santos, confirmed that application of the diagnostic methodology was successful, as an initial diagnosis, evolution and a closing prognosis were achieved. The prognosis is of utmost importance because it illuminated the response to the applied conservative treatments and moreover it pointed out zero evolutionary cancer.

Key words: Colposcopic tours; Diagnosis; Treatment; Evolution.

Introduction

The preventive uterine cancer tours started in the year 1983 in Isla Grande, Province of Colon, where 54 women were examined, 17% of which had oncogenic risk (OR). Successively 14 communities of six Republic of Panama provinces were covered. Screening of 2,902 women was performed in whom 5,179 colposcopies were carried out together with the respective cyto-histologic exams.

The analysis of the groups of patients considered as OR integrally was 48.8%, compared to those found in the community of Pocri de Los Santos of 34.5%; thus this study was dedicated to that population [1-3].

The results, based on the evidence of the follow-ups to the year 2008, shed light on the success reached with the application of local treatments which were mainly conservative in 95% of the cases.

Through an optimal physician-patient relationship and the consciousness and perseverance of the population, the scope of these preventive tours has been transformed in an absolute way (1983) to complacency (2008).

Material and Methods

In the 25 years of rural colposcopic medical tours, a total of 65 tours were carried out in which 2,902 women were examined and 5,179 colposcopic studies were performed.

Special attention was given to the population of Pocri de Los Santos where 802 women were examined in whom 2,549 colposcopies, 2,509 cytologies and 269 contemporarily directed biopsies (if necessary) were carried out. Cryosurgery was the preferred treatment.

The population studied is located 320 kilometres from the capital city, where our headquarters are.

Of the women, 274 were selected after presenting atypical colposcopic lesions, with results of cytology and histology in agreement or not. Moreover, women with negative colposcopy studies and/or pathological cytology and/or pathological histological study were included.

Almost all these patients came for periodic health controls as a result of their own initiative, without any referral reason. Both colposcopy and cytology, carried out contemporarily, immediately shed light on the degree of the lesions due to the cytologist and his/her immediate report. In contrast the biopsy was included in the study with a diagnosis at a later date.

When results were obtained, the analytical diagnosis was formulated providing an initial diagnosis, an evolutionary diagnosis and a final diagnosis [1-5].

Results

Of the patients, 274 had OR, equivalent to 34.1% of the patients studied, which in comparison with 53% of the rest of the studies in Panama, demonstrates the low incidence of cancer risk in this population.

The three preventive methods were evaluated and agreement and disagreement were found which were overcome with the simultaneous application of these methods.

Cytology reported the “pure” HPV infection as the most common pathology; i.e., without dysplastic or neoplastic lesions in 36% of the cases, whereas false negatives reached 56.5% and in these cases inflammatory alterations prevailed (Table 2).

Colposcopy revealed the atypical transformation zone (ATZ) as the most common diagnosis, followed by leukoplakia, (L) and condyloma images (HPV) in 23.4%, 18.6% and 18.9%, respectively. False negatives reached 21% (Table 3).

There were 111 biopsies and the most common pathology found was HPV (42.3%) followed by mild dysplasias
associated with HPV (18%). In these cases false negatives reached 32.4% (Table 4).

HPV was found to correspond to 211 cases, equivalent to 26.3% of the total population treated, in comparison with the general HPV findings of 44% for the country of Panama [5].

Analyzing the ages of the patients with HPV alone or associated with dysplasias or cancer, it was found that the majority were between the ages of 25 and 44 (63%) and with extremes at 20 and 79 years of age (Table 5).

Cure or elimination of the lesions was accomplished in 82.7% of the patients with OR who were followed for up to 23 years; there was persistence in 6.7%, improvement in 5.6%, progression in 2.8, and recurrence 1.7%.

With relation to the therapeutic methods applied, cryosurgery – considered as the method of choice – was carried out in 159 cases of the 187 patients treated (85%).

Of the patients with cervical-uterine cancer, the diagnosis for two was made by colposcopic referral. Both patients were cured at the National Oncologic Institute, although one arrived at the study with invasive cancer.

Table 4. — Evaluation of directed biopsies in the group with oncogenic risk.

<table>
<thead>
<tr>
<th>Report</th>
<th>No.</th>
<th>Patients</th>
<th>%</th>
<th>False neg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>6</td>
<td></td>
<td>5.4%</td>
<td></td>
</tr>
<tr>
<td>Cervicitis</td>
<td>30</td>
<td></td>
<td>27%</td>
<td>32.4%</td>
</tr>
<tr>
<td>HPV alone</td>
<td>47</td>
<td></td>
<td>42.3%</td>
<td></td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td>4</td>
<td></td>
<td>3.6%</td>
<td></td>
</tr>
<tr>
<td>Mild dysplasia/HPV</td>
<td>20</td>
<td></td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>1</td>
<td></td>
<td>0.9%</td>
<td></td>
</tr>
<tr>
<td>Sev. dysplasia/HPV</td>
<td>2</td>
<td></td>
<td>1.8%</td>
<td></td>
</tr>
<tr>
<td>Invasive cancer/HPV</td>
<td>1</td>
<td></td>
<td>0.9%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td></td>
<td>99.9%</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. — Ages of patients considered with oncogenic risk (OR).

<table>
<thead>
<tr>
<th>Ages (0-54)</th>
<th>OR (no.:)</th>
<th>HPV (no.:)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>20-24</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>25-29</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>30-34</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>35-39</td>
<td>41</td>
<td>38</td>
</tr>
<tr>
<td>40-44</td>
<td>53</td>
<td>39</td>
</tr>
<tr>
<td>45-49</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>50-54</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>55±</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>274</td>
<td>211</td>
</tr>
</tbody>
</table>

Discussion

Condyloma virus at the cervical level in its various forms not only permitted learning about the incidence but also to do a comparison with other communities, and in addition to learn about its natural evolution or response induced by cryosurgery, with the following questions undoubtedly remaining: Where did it go? Was it really there? [5, 6].

It may be like the herpes virus found by US physicians at Monagrillo, with a published study concluding that this virus was found among this population in a remarkable way, but 15 years later no cases could be found. Could it be the same effect? Only time will shed light on this [7].

Another question remaining is the fact that this was a population with a low sensitivity to HPV infection, consequently with what seems to be a natural resistance to it. How and when was this resistance obtained? A finding
was facilitated based on the samples obtained and the fact that there was an excellent response to the risk diagnosis through conservative treatments [8, 9].

Conclusions

Up to now, it has been demonstrated that perseverance, education and screening for women who for some reason may never have received the benefits of diagnostic methods as those applied up to now, which may not be so modern but still valid, are the only and real tools to achieve the goal. “Evolution to cancer: no cases” – the objective was accomplished [10-12].

Table 6. — Medical tours from 1983-2008.

<table>
<thead>
<tr>
<th>Community</th>
<th>Prov.</th>
<th>No. tours</th>
<th>No. pts.</th>
<th>OR</th>
<th>Colposcopy OR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isla Grande</td>
<td>3</td>
<td>1</td>
<td>54</td>
<td>9</td>
<td>54</td>
</tr>
<tr>
<td>Pocri de Los Santos</td>
<td>7</td>
<td>27</td>
<td>802</td>
<td>279</td>
<td>2549 34.5</td>
</tr>
<tr>
<td>Llano Largo, Ocu</td>
<td>6</td>
<td>1</td>
<td>54</td>
<td>13</td>
<td>54 24.1</td>
</tr>
<tr>
<td>Cerro Campana</td>
<td>8</td>
<td>1</td>
<td>25</td>
<td>3</td>
<td>25 12.0</td>
</tr>
<tr>
<td>Chorrera</td>
<td>8</td>
<td>15</td>
<td>410</td>
<td>267</td>
<td>471 65.1</td>
</tr>
<tr>
<td>Las Cumbres</td>
<td>8</td>
<td>1</td>
<td>17</td>
<td>7</td>
<td>481 41.2</td>
</tr>
<tr>
<td>San Miguelito</td>
<td>8</td>
<td>4</td>
<td>719</td>
<td>478</td>
<td>719 66.5</td>
</tr>
<tr>
<td>El Valle de Anton</td>
<td>2</td>
<td>2</td>
<td>88</td>
<td>22</td>
<td>88 25.0</td>
</tr>
<tr>
<td>San Miguel, Pacora</td>
<td>8</td>
<td>2</td>
<td>58</td>
<td>26</td>
<td>58 48.8</td>
</tr>
<tr>
<td>Cerro Azul</td>
<td>8</td>
<td>2</td>
<td>55</td>
<td>11</td>
<td>55 20.0</td>
</tr>
<tr>
<td>Monagrillo</td>
<td>6</td>
<td>2</td>
<td>330</td>
<td>186</td>
<td>354 56.4</td>
</tr>
<tr>
<td>Salitre, Herrera</td>
<td>6</td>
<td>1</td>
<td>40</td>
<td>14</td>
<td>40 35.0</td>
</tr>
<tr>
<td>El Cope, Cocle</td>
<td>2</td>
<td>1</td>
<td>39</td>
<td>12</td>
<td>39 30.8</td>
</tr>
<tr>
<td>Meteti, Darien</td>
<td>5</td>
<td>1</td>
<td>82</td>
<td>51</td>
<td>52 62.2</td>
</tr>
<tr>
<td>Women’s Penitentiary</td>
<td>8</td>
<td>4</td>
<td>129</td>
<td>36</td>
<td>140 27.9</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>65</td>
<td>2,902</td>
<td>1,414</td>
<td>5,179 48.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25.9% 22.2%</td>
</tr>
</tbody>
</table>

Total 1983-2008: 11,191 23,327

Acknowledgment

Many thanks to everyone who supported and participated in this study, especially the population of Pocri de Los Santos, all of whom patiently waited every year for the gynecologic visit, often for many hours.

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References


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Clinical usefulness of evaluation of quality parameters of blood flow: pulsation index and resistance index in the uterine arteries in the initial differential diagnostics of pathology within the endometrium

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¹Department of Medical Education, ²Clinic of Gynecological Surgery, ³Cathedral and Clinic of Mother’s and Child’s Health, Poznan University of Medical Sciences (Poland)

Summary

Detection of neoplastic changes in the pelvis minor in early stages is a critical prognostic factor for patients suffering from uterine carcinoma. Due to the problem presented, the evaluation of clinical usefulness of ultrasonographic examination including color Doppler in the process of detecting pathologic changes within the endometrium was the main aim of this paper. Quality parameters of blood flow in the uterine arteries, such as the pulsation index (PI) and resistance index (RI) were evaluated. A group of 100 female patients diagnosed with and treated for abnormal genital bleeding in the Clinic of Gynecological Surgery, Gynecological-Obstetrics Clinical Hospital, Poznań University of Medical Sciences from December 2005 until January 2007 constituted the subjects for this work. Analyzing patients with pathology of the endometrium, we obtained lower values of quality parameters of blood flow in the uterine arteries in comparison to the control group. Reduction of the resistance and pulsation indexes of the uterine arteries may be useful elements in the process of differentiating pathological changes within the endometrium.

Key words: Endometrial carcinoma; Proliferation of the endometrium; Pulsation index; Resistance index.

Introduction

Detection of neoplastic changes in the pelvis minor in early stage is a critical prognostic factor for patients suffering from uterine carcinoma. An increasing number of these neoplasms that often result in mortality make this issue very important. Consequently, it is very important to choose the most effective diagnostic method which would be the least invasive, while at the same time taking into account above all the age of patients and their general health condition. Transvaginal ultrasonography (TVS) has been the basic diagnostic method in detecting changes in the uterus for a long time. Ultrasonographic diagnostics with gray scale, enabling the initial differentiation of proliferative changes in the uterus, may be expanded with an analysis of blood flow in the uterine arteries with the use of the color Doppler. The Doppler spectrum of a malignant tumor usually shows decreased resistance (RI) and pulsation (PI) indexes [1-7].

Material and Methods

One hundred female patients were diagnosed and treated for abnormal bleeding of the genitals in the Clinic of Gynecological Surgery of the Gynecological-Obstetrics Clinical Hospital of Poznan University of Medical Sciences from December 2005 until January 2007.

Statistical analysis

The results obtained were submitted to a statistical analysis. Measurable parameters, namely PI and RI, in the group of carcinomas and proliferations of the endometrium and in the
control group were defined, with the aid of arithmetical mean and standard deviation, median and minimum and maximum value as well as 25th and 75th percentiles. Compatibility of the above-mentioned parameters with normal distribution by means of the Shapiro-Wilk test was checked. Analysis of variance (ANOVA) with the post-hoc Newman-Keuls test or the non-parametric Kruskal-Wallis test with multiple comparisons (Dunn’s test) were used for trials compliant with normal distribution for comparison between the three groups.

Statistical assumptions were verified with a level of significance of \( p \leq 0.05 \).

Calculations were done with the aid of the statistical package STATISTICA v 7.1 (StatSoft Inc. 2005), package StatXACT v. 5.0.3, Cytel Software Corp. and Analyse-it Software v. 1.68.

Results

Table 1 shows values of the PI analyzed in the uterine arteries in all three examined groups. Due to the skew distribution of the measurement results of the analyzed feature, the data in the table are characterized with median, minimum and maximum as well as 25th and 75th percentile values.

Table 1. — Comparison of PI values in the group of cancers and proliferations as well as in the control group.

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>Average</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
<th>25th percentile</th>
<th>75th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>46</td>
<td>1.11</td>
<td>0.59</td>
<td>1.74</td>
<td>1.13</td>
<td>0.96</td>
<td>1.24</td>
</tr>
<tr>
<td>Proliferation</td>
<td>11</td>
<td>1.32</td>
<td>1.08</td>
<td>1.63</td>
<td>1.32</td>
<td>1.12</td>
<td>1.48</td>
</tr>
<tr>
<td>Control group</td>
<td>46</td>
<td>1.47</td>
<td>0.65</td>
<td>2.46</td>
<td>1.53</td>
<td>1.21</td>
<td>1.73</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>1.30</td>
<td>0.59</td>
<td>2.46</td>
<td>1.23</td>
<td>1.07</td>
<td>1.56</td>
</tr>
</tbody>
</table>

The median value of the PI in the group of endometrial carcinomas was 1.13; in the group of proliferations 1.32; and in women without any pathology within the endometrium 1.53.

Average values of the PI were statistically significant lower in the group of carcinomas of the endometrium (PI = 1.11), in comparison to the control group (PI = 1.47). During the analysis of the patients with proliferation of the endometrium it turned out that average values of the PI in this group (PI = 1.32) were lower in comparison to the control group as well (PI = 1.47) and were borderline statistically significant.

Table 2 presents values of the RI analysed in the uterine arteries in the groups of carcinomas, proliferations and the control group. Due to the skew distribution of the results of the analyzed feature, the data in the table are characterized with median, minimum and maximum as well as 25th and 75th percentile values.

The lowest values of the RI were observed in women with endometrial carcinoma, higher in the group of proliferations of the endometrium, and the highest in the control group, respectively, 0.65, 0.73 and 0.74.

Average values of the RI in the group of carcinomas (RI = 0.65) were statistically significantly lower than in the control group (RI = 0.75), whereas with respect to proliferations of the endometrium (RI = 0.73) the values were borderline statistically significant. No statistically significant differences were observed when comparing average values of the RI in the group of proliferations of the endometrium (RI = 0.73) and in the control group (RI = 0.75).

Discussion

Kurjak et al. [8] stress the value of Doppler TVS as a non-invasive method useful in the process of preoperative diagnosis of changes within the endometrium. Bourne et al. [9] draw attention to the fact that average values of the PI in uterine arteries in women after menopause were significantly lower in bleeding patients with endometrial carcinoma in comparison to a group of patients in whom the cause of bleeding was not related to neoplastic processes. Average values of the PI in women with carcinoma amounted to 0.91 (range 0.31-1.49), whereas they were 3.83 (range 1.95-6.40) in case of no pathology. Moreover, in one case of carcinoma, despite no bleeding from the genitals, the PI was low (1.10). Values of PI and RI in patients with carcinoma in Szabo et al.’s [10] study were also low and amounted to 1.69 ± 0.56 and 0.78 ± 0.10, respectively. The same parameters were as follows in case of no pathology: high PI - 3.06 ± 1.04 and high RI - 0.93 ± 0.08.

Analyzing our own material, we obtained results similar to those in the literature [9, 10]. Average values of the PI and RI were the lowest in women with endometrial carcinoma and slightly higher in women with proliferations of the endometrium, and the highest in cases of no pathology.

However, Vuento et al. [11] claim that Doppler diagnostics are not more useful for differentiating malignant and non-malignant changes of the endometrium in comparison to gray scale TVS because only Doppler sensitivity is high while its specificity is low. On the other hand, Flam et al. [12] reported total uselessness of the Doppler technique with evaluation of the PI in differentiating malignant and non-malignant endometrial changes due to the lack of any measurable index differences.

Conclusions

Reduction in resistance and pulsation indexes of the uterine arteries may be useful elements in differentiating pathological changes within the endometrium. Ultrasonography using color Doppler should be
regarded as a significant element of preoperative analysis of pathology within the endometrium.

References


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Epithelioid leiomyosarcoma of the uterus: computed tomography findings

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Summary

Uterine epithelioid leiomyosarcoma is a rare neoplasm. There have been no previous reports describing computed tomography (CT) findings for this tumor. A 31-year-old woman presented with a heterogeneous enhancing mass, with internal septa, in the uterus, which was shown on CT images. Histological diagnosis was compatible with epithelioid leiomyosarcoma.

Key words: Computed tomography (CT), Leiomyosarcoma, Sarcoma, Uterus.

Introduction

Epithelioid leiomyosarcoma arising in the uterus is extremely rare [1-5]. No computed tomography (CT) images of this tumor have been reported before. Since CT findings for this tumor may resemble those of other uterine malignancy, we report a case with histologically confirmed epithelioid leiomyosarcoma in the uterus, with characteristic findings on CT imaging.

Case Report

A 31-year-old woman presented with a palpable growing mass over the right lower quadrant of her abdomen for two months. She was also bothered by increased menstrual flow. Physical examination revealed a palpable tumor in her lower abdomen. Blood analysis showed anemia (hemoglobin, 6.8 g/dl) and normal CA 125 (12.9 U/ml). Ultrasound (US) revealed a huge cystic mass with septa measuring 14.8 × 10.1 × 14.9 cm at the uterus with endometrial hyperplasia. Doppler US indicated hypervascularity of the tumor. CT scan showed a huge heterogeneous enhancing cystic mass with internal septa and thickened walls (Figure 1). No detectable enlarged lymph nodes in the obturator or internal iliac chain were noted. Our initial diagnosis was mucinous or serous cyst adenocarcinoma of the uterus.

The patient underwent total hysterectomy and left salpingo-oophorectomy. At surgery, multiple myoma nodules and adenomyosis of the uterus with surrounding bloody ascites were seen. Grossly, the uterus had a poorly defined margin with tan-colored and marked cystic degeneration filled with serous and bloody fluid. On serial sections, focal myxoid change was seen. Microscopy showed an epithelioid and spindle cell tumor of the uterine corpus with multifocal cysts and myxoid degeneration. These epithelioid tumor cells had eccentric cytoplasm with eosinophilic and inclusion-like cytoplasm. The nuclei were vesicular and had prominent nucleoli. In some areas, spotty eosinophilic changes with shrinkage of nuclei were also seen. Marked nuclear atypia, characterized by pleomorphic and hyperchromatic nuclei with some abnormal mitosis features was noted. No frank coagulation necrosis was identified. In the more cellular proliferative area, at least ten mitosis features (some abnormal) per 10 high-power field were noted. The diagnosis was compatible with epithelioid leiomyosarcoma. The tumor was confined to the uterine corpus, free of the serosal surface; the endometrium was not involved by the tumor.

The patient did not receive adjuvant therapy after surgery. Regular follow-up US and serum CA 125 revealed no evidence of recurrent disease over two years.

Discussion

Leiomyosarcomas are the most common type of uterine sarcoma, but variants of these tumors, such as epithelioid leiomyosarcomas, are extremely rare. Only a few cases of these tumors arising from the uterus have been reported before [1-5]. They can easily mimic leiomyomas of the uterus on clinical diagnosis [6].

Kato et al. [7] reported two cases of epithelioid sarcoma in the thighs and calf, respectively, with obviously elevated serum CA 125 levels. However, as in our case, epithelioid leiomyosarcoma in the uterus revealed a normal CA 125 level.

Epithelioid leiomyosarcoma occurs in the third to seventh decades, and ranges in size from 2.7 cm to 30 cm [1-5]. Patients often complain of a palpable growing mass in the lower abdomen, with lower abdominal discomfort, menorrhea and bloody (or non-bloody) vaginal discharge. Our patient had a similar clinical manifestation.

Epithelioid leiomyosarcoma is a subtype of leiomyosarcoma and is defined by histological findings of rounded to polygonal cells with abundant eosinophilic cytoplasm in more than 50% of the tumor [1, 8-10]. On microscopic examination, the epithelioid cells have round nuclei and are eosinophilic in approximately 75% of cases and, less...
frequently, vacuolated or with clear cytoplasm [1, 8-10]. Nuclear atypia, 5 or more mitoses per 10 high-power fields, tumor cell necrosis, and tumor size together constitute the best criteria to predict malignancy [2, 9, 11]. In our case, the high mitotic index, nuclear atypia, and large tumor size all indicated malignancy.

To the best of our knowledge, there have been no previous reports describing radiological findings of epithelioid leiomyosarcoma in the uterus. In our case, US disclosed an echogenic mass with multiple cystic components in the uterus. CT scan showed a uterine tumor with solid and cystic components, consistent with cystic and myxoid degeneration of the tumor on histology. On CT imaging, the tumor also showed internal septa and heterogeneous enhancement, without hemorrhage or necrosis. Histology also showed no calcification or necrosis.

CT findings of a uterine tumor with cystic and solid components may include epithelioid trophoblastic tumor and uterine adenocarcinoma. Coulson et al. [11] reported a 9.8 cm cystic midline mass contiguous with the uterus, with an elevated serum beta-human chorionic gonadotropin (β-hCG) level, later proved to be an epithelioid trophoblastic tumor. Nalaboff et al. [12] showed one case with a heterogeneous enhancing mass with irregular endometrial thickening of the uterus, resembling our case, on CT images. However, uterine epithelioid leiomyosarcoma lacks the abnormal serum β-hCG level and presents with internal septa on CT images, as in our case.

Uterine epithelioid leiomyosarcoma is commonly treated by total abdominal hysterectomy with bilateral salpingo-oophorectomy [2]. Chemotherapy and hormonal therapy have been used in a few patients with
tumor recurrence or metastasis [1, 2]. Distinct metastasis is not common [2, 13]. Miyajima et al. [10] reported that the size and staging of the tumor were the most important predictors of a patient’s prognosis. Other important prognostic factors included the mitosis rate and coagulative tumor cell necrosis in uterine sarcomas [10, 14].

In summary, we have reported the CT findings of an epithelioid leiomyosarcoma in the uterus in a 31-year-old woman. The diagnosis of this rare tumor should be considered in the case of a heterogeneous enhancing mass with internal septa on CT imaging.

References

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Multiple epitheloid plexiform tumourlet leiomyoma of the uterus with focal vascular invasion

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Summary

We report the first case of multiple plexiform tumourlets showing focal vascular invasion suggesting that, unlike what the common and accepted opinion would advocate, these tumours may have a more aggressive behaviour. However, the prognosis of this variant of epitheloid leiomyoma remains unknown, due to its rarity. The main differential diagnosis of this entity are discussed, including endometrial stromal sarcoma exhibiting epitheloid cytomorphology, intravascular leiomyomatosis of epitheloid variant.

Key words: Epitheloid leiomyoma; Plexiform tumourlets; Myometrium; Uterus; Myoma; Vascular invasion; Endometrial stromal sarcoma; Intravascular leiomyomatosis.

Introduction

Small uterine plexiform leiomyomas which are generally detected only through microscopic examination are referred to as plexiform tumourlets. Plexiform tumourlets is a rare subtype of epitheloid leiomyoma characterised by multiple intrauterine small leiomyomas with nuclei arranged in a palisading pattern. These tumours are a variant of epitheloid leiomyomas and are considered as invariably benign and hardly ever have an infiltrative pattern.

Case Report

A 39-year-old white nulliparous woman looked for assistance at the gynaecological consultation for recurrent vaginal bleeding and chronic anaemia. She presented a medical history of infertility. The clinical examination showed an enlarged uterus and no adnexal mass. The ultrasound revealed a submucosal multilobulated lesion 50 x 30 mm in size. The haemoglobin level was 9.6 g/dl since six months. Subsequently, the patient went through hysteroscopic resection of the mass. The main purpose at the beginning of the hysteroscopy was to extract the mass in total. Even though the resection could not be completed due to the large intramural portion of the mass, more than 50% of the tumour could still be resected. The pathological diagnosis highlighted the diagnosis of multiple focally coalescent epitheloid leiomyomas without any criteria of malignant transformation. The patient was then prescribed a 3-month treatment by GnRH agonists and scheduled for an ultrasound and a second-look hysteroscopy three months later. The ultrasound, performed six weeks after the first hysteroscopy, revealed a recurrence of the myometrial lesion. Confronted with such an early recurrence with rapid growth and after having discussed the issue with the patient, we decided to perform a total hysterectomy with preservation of the ovaries, as requested by the patient. The patient was disease-free after 14 months of follow-up.

Pathologic features

The nulliparous uterus measured 8 x 5 x 3.5 cm. On gross examination we noticed a multitude of small worm-like nodules gray to focally yellow with a soft consistency. No visible areas of haemorrhage or necrosis were observed. These nodules occurred in every part of the uterus including the submucosa and subserosa and their diameter varied from 2-13 mm (Figure 1).

On microscopic examination, the nodules were composed of serpiginous cords of epitheloid, uniform and round smooth muscle cells. The nuclei were round, relatively large and centrally positioned and also arranged in a palisading pattern (Figure 2). An extensive hyalinization was focally noted. The mitotic activity was low (1 mitotic figure per 10 high power fields) and no cytological atypia or tumour cell necrosis was noticed.

Some of the tumours showed expansive nests and irregular tongue which infiltrated the adjacent myometrium. Invasion of lymphatic and vascular channels was also focally observed (Figure 3). Immunohistochemistry highlighted cells revealed diffuse and strong positivity with vimentin, desmin, actin, H-caldesmon, estrogen and progesterone receptors. Conversely, they were negative for CD 10, epithelial membrane antigen, Ber-EP4, AE1/AE3, cytokeratin 7, α-inhibin, calretinin melan A and HMB-45 (Figure 6). The Ki-67 index was low (less than 5%).

Discussion

Epitheloid leiomyoma represents a smooth muscle tumour that is predominantly composed of epithelial-like cells instead of more usual spindle cells which can be found in classic leiomyoma [1, 2]. These tumours are uncommon and may occur at any age starting with the third decade. Three subtypes of epitheloid leiomyoma are usually described: leiomyoblastoma, clear cell leiomyoma and plexiform leiomyoma [1-4]. Microscopically, plexiform leiomyomas are characterized by cords or nests of true epitheloid round cells with a scanty or moderate amount of cytoplasm. These should not be confused with a pseudo-epitheloid appearance due to matrix deposition in common leiomyoma [4]. Plexiform tumourlets are generally unique. Those may however be multiple as well, appearing only as microscopic foci in which case they are so-called multiple
plexiform tumourlets [5]. Our case showed multiple tumours of small size with a diameter variation between 2-13 mm. They occurred in every part of the uterus including the submucosa and subserosa. Typically plexiform tumourlets are considered as benign, with no local extension and no vascular invasion. However, a paper by Seidman and Thomas described some of these tumours with an infiltrative pattern as encountered in endometrial stromal sarcoma [5].

In addition to data provided by Seidman et al. we found the presence of focal vascular space invasion as classically encountered in low-grade endometrial stromal sarcoma (ESS). Interestingly ESS can exhibit muscular or epitheloid differentiation and constitutes one of the main differential diagnoses [6]. Nevertheless, these tumours are invariably CD10 positive in opposition to the data observed in this case. The second differential diagnosis is probably the rare variant intravascular leiomyomatosis (IVL) with epitheloid features as described in four cases by Clement et al. In the description of Clement et al. extensive growth of the smooth muscle component within the venous channels was observed in contrast to this case where vascular involvement was only focal [7].

The literature on the subject claims that plexiform tumourlets are as invariably benign [4].

Given the uncertainty and rarity of the case described here, we should discourage dogmatism in predicting the clinical course [8]. Indeed, intravascular invasion of plexiform tumourlets has never been reported in the literature and our case is the first to sustain that this benign pathology might present vascular involvement.

Finally, two studies have indicated that the major criteria of malignancy in epitheloid leiomyomas generally included: significant nuclear pleomorphism, mitotic index more than three figures per ten HPF, necrosis, and vascular invasion. If one of these criteria was present malignant behaviour was observed in 42% of the patients [9, 10].

Our case is too fresh and the follow-up if unremarkable is short-dated (14 months) to offer specific management but the risks of vascular invasion in plexiform tumourlets of the uterus require a close follow-up in order to diagnose early local or distant recurrences.

References
Multiple epitheloid plexiform tumourlet leiomyoma of the uterus with focal vascular invasion


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Myxoid leiomyosarcoma of the uterus: a diagnostic challenge

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Summary

Myxoid leiomyosarcoma is an extremely rare variant of leiomyosarcoma, masquerading almost to perfection as a benign lesion. For, indeed, the tumor lacks the defining features of high mitotic activity, cellular atypia or necrosis, and the microscopic picture is dominated by abundant myxoid stroma containing sparse spindle cells. We report here such a case occurring in the uterus and discuss the differential diagnosis. The relevant literature is reviewed.

Key words: Minimal criteria; Myxoid leiomyosarcoma; Uterus.

Introduction

Uterine myxoid leiomyosarcoma is a rare tumor, with only 33 cases having been reported in the English literature to date. King, et al. in 1982 were the first to draw attention to this lesion describing six cases, distinct from uterine leiomyomas with extensive myxoid change [1]. This lesion is unique because – despite its highly malignant course – it shows a “benign” morphological picture, which is dominated by myxoid stroma with scarce neoplastic cells; the latter may exhibit minimal atypia and little or no mitotic activity.

We report an additional case of uterine myxoid leiomyosarcoma in a young woman and point out the minimal criteria for diagnosing the malignant nature of the tumor.

Case Report

A 30-year-old nulliparous woman presented with a 2-year history of menorrhagia. Clinical examination and ultrasonography revealed the presence of a submucosal mass protruding into the endometrial cavity. No other significant findings were disclosed and the tumor was excised.

Macroscopic examination of the resected specimen revealed a well-circumscribed ovoid mass, 7 x 6 x 5 cm, with a translucent gelatinous cut surface, showing no areas of hemorrhage or necrosis. The microscopic picture was composed of scarce spindle-shaped or stellate cells embedded in abundant myxoid stroma (Figure 1). This was weakly positive for periodic acid-Schiff reaction and strongly positive for Alcian blue staining. The neoplastic cells, having oval or elongated nuclei and scant cytoplasm, were arranged singly and in interlacing bundles. The low cellularity of the tumor was accompanied by minimal atypia and a low mitotic index (1 mitosis per 10 high power fields), which was confined to the most cellular areas. Mitoses were totally absent from the myxoid areas. Despite its gross appearance, the tumor infiltrated the surrounding tissues, namely the myometrium and the endometrial stroma (Figure 1), but there were no areas of necrosis, hemorrhage or vascular invasion.

Immunohistochemical investigation revealed an antigenic profile supporting smooth muscle differentiation with the spindle-shaped cells expressing smooth muscle actin-α, desmin, and vimentin. The diagnosis of uterine myxoid leiomyosarcoma was entertained and the patient was treated thereafter with total hysterectomy. Further examination of the excised specimen was negative for residual disease and the only pathological finding noted was the presence of two usual type leiomyomas. No adjuvant treatment was given, and the patient has been free of disease for two years after the operation.

Discussion

Uterine leiomyosarcoma, a malignant neoplasm composed of cells showing smooth muscle differentiation, accounts for approximately 1.3% of all uterine malignancies [2]. Myxoid leiomyosarcoma of the uterus is an extremely rare and rather aggressive variant of uterine leiomyosarcoma, which often defies recognition because of its innocuous morphological features.

Thus, the tumors, usually large, are characterized by a widespread gelatinous appearance on the naked eye, which, microscopically, corresponds to an abundant myxomatous stroma and remarkably low cellularity [1, 3, 4]. Furthermore, in most cases myxoid leiomyosarcomas lack the three widely accepted criteria for diagnosing leiomyosarcoma, namely high mitotic activity, nuclear atypia, and tumor cell necrosis [5-7].

Our case of uterine myxoid leiomyosarcoma was typical in many respects. The patient presented with abnormal vaginal bleeding [1, 4, 8, 9] which, together with the presence of a pelvic mass [1, 10] and lower abdominal pain [4, 8, 10], make the most common presenting symptoms. The age of the patient (30 years) was within the expected range (from 20 to 74 years) [3, 10, 11], although below the average age of 55. The tumor appeared to have originated from the smooth muscle wall of the body or possibly of its blood vessel walls [1], rather than the cervix [4] or the broad ligaments [1, 12, 13]. It was large (7 cm in the greatest diameter) falling within the defined range of 5 to 45 cm [1, 10], and its cut surface showed the typical gelatinous consistency and well circumscribed borders [1, 4, 12]. Furthermore, it protruded into the uterine cavity [1, 9].
Microscopically, the lesion was of lowcellularity composed of, almost entirely, a myxomatous matrix which was weakly positive for periodic acid-Schiff and strongly positive for Alcian blue staining. As most myxoid leiomyosarcomas, it showed little or no cellular atypia [1, 3, 4, 11, 12] and low mitotic activity: 1 mitosis per 10 HPF in the most cellular areas [1, 3, 10, 12]. There have been occasional cases, however, with unusually high number of mitoses, ranging from 30 [8] and 50 to 100/10 HPF [9], and highly atypical cells [8, 9]. Interestingly, some investigators have described focal areas of necrosis [9, 10] but this was not detected in our material. Contrast to its gross appearance, the tumor had an infiltrating margin, as has been previously noted by many authors [1, 4, 8, 10-12]. Yet, vascular invasion, an equally useful diagnostic, but less common, feature [1, 2, 13] was not seen.

With regard to immunohistochemistry, it is not, at present, possible to unveil the malignant nature of the tumor. However for the positivity of actin-, desmin, and vimentin it is certainly useful to establish the myogenic differentiation of the neoplastic cells [4, 10]. There are authors suggesting that a positive reactivity for Ki-67 [10, 12] is a useful indicator of malignancy.

This deceptive microscopic picture makes the distinction from other myxoid tumors of the uterus important, for indeed many cases of uterine myxoid leiomyosarcomas described in the literature were diagnosed in retrospect, following a poor clinical outcome [14]. No doubt, the main diagnostic challenge is differentiating a myxoid leiomyosarcoma from a leiomyoma with myxoid change [1, 15] - a form of degeneration occurring in approximately 3.5% of uterine leiomyomas [16]. A diagnosis of myxoid leiomyosarcoma requires no more than 0-2 mitoses per 10 HPF [1, 2, 15], although unusually higher counts [8, 9] have been sporadically reported. Areas of cellular atypia may be encountered in parts of myxoid leiomyosarcoma [2], but the presence of infiltrative margins are, by far, a more reliable feature [15].

Myxoid leiomyosarcoma should also be distinguished from leiomyoma with hydropic degeneration – a lesion which represents a focal hydropic change, rather than a true myxoid matrix. It is characterized by frequent hyalinization and a dominance of large thick-walled vessels [15].

The differential diagnosis should also include the inflammatory myofibroblastic tumor with prominent myxoid change. This tumor can be distinguished from myxoid leiomyosarcoma by the absence of infiltrating margins and the presence of chronic inflammatory cells [17].

Another tumor that must be always kept in mind is myxoid endometrial stromal sarcoma, also known as endometrial stromal sarcoma with myxoid change – a neoplasm composed of cells resembling those of the proliferative endometrial stroma and a rich network of thin-walled arterioles [2].

The treatment of myxoid leiomyosarcoma is, by and large, total abdominal hysterectomy with [1, 10, 12] or without salpingo-oophorectomy [13], usually followed by adjuvant therapy (chemotherapy [8, 10, 13] or external irradiation [4, 13], although there have been cases treated with simple tumorectomy [3, 13]. Yet, even after complete excision of the tumor, the majority of cases recur, with the time of recurrence varying from two months [10] to ten years [1].

In summary, there is at present no general consensus as to the minimum criteria for recognizing uterine myxoid leiomyosarcomas, but in all tumors studied there were, at least focialy, smooth muscle cell differentiation and infiltrating tumor margins. This brings to mind Rollason’s and Wilkinson’s words that “any myxoid tumor more than a few centimeters in size, with any mitotic activity or with an infiltrative margin is best regarded as potentially aggressive” [15, 18]. Vascular invasion is equally useful but less common. Moreover, a high index of suspicion is absolutely necessary for diagnosing this entity.

References


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“Can laparoscopy really complete full surgical staging?”
A case of early recurrence and malignant transformation of borderline ovarian tumor

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Summary
Ovarian borderline tumor (BOT) with noninvasive implants traditionally is considered to be non-aggressive. Recurrences are delayed and transformations to high-grade carcinoma are rarely documented. We report on a patient with BOT with early recurrence and high-grade carcinoma transformation in a short interval after complete laparoscopic staging. A 27-year-old unmarried woman presented with a 26 cm in size ruptured left ovarian mass. Laparoscopic left salpingo-oophorectomy with right ovarian biopsies, multiple peritoneal biopsies, omental biopsy and washing cytology were performed. FIGO Stage I ovarian serous borderline tumor with microinvasion was confirmed. About ten months later, a 15 cm in size left BOT recurred and was resected by laparoscopic cystectomy including staging surgery. Seven months after the second surgery, we found a pelvic mass by sonogram and elevated CA125. A third diagnostic laparoscopy revealed invasive serous carcinoma with multiple peritoneal implants. In spite of radical surgery and adjuvant chemotherapy, the patient died of a progressive metastatic liver tumor. A case of early recurrence with malignant transformation of BOT is presented together with a brief review.

Key words: Ovarian borderline tumor; Early recurrence; Malignant transformation.

Introduction
Borderline ovarian tumor (BOT) is characterized by earlier stage and younger age at diagnosis, more indolent behavior, longer survival, and later recurrence than seen in cases of invasive ovarian cancer [1]. Recurrence is delayed and transformation to high-grade carcinoma is rarely documented. The clinical guidelines for the surgical treatment of BOT are still similar to the treatment guidelines for epithelial ovarian cancer, and include total abdominal hysterectomy, bilateral salpingo-oophorectomy, and staging procedures [1]. However they frequently affect young women in which conservative and minimally invasive surgery is required to preserve child-bearing potential. Conservative surgery is defined as surgery with complete staging, but with preservation of the uterus and at least part of one ovary to preserve fertility [2]. A careful inspection of the peritoneum with resection of macroscopic suspected lesions, multiple peritoneal biopsies, and infracolic omentectomy are necessary for thorough staging [2].

We report a case of BOT with early recurrence and high-grade carcinoma transformation in a short interval after complete laparoscopic staging by the guidelines of fertility-sparing surgery.

Case Report
A 27-year-old unmarried nulliparous patient was referred to our department with abdominal discomfort. On abdominal examination, there was a large mass arising from the pelvis reaching up to the upper abdomen. Ultrasound (US) showed a large adnexal mass with internal echoes and multiple septa, and profuse ascites. Pelvic computed tomography (CT) scan revealed a 26 cm sized left adnexal mass with profuse perihepatic and perisplenic ascitic fluid collection (Figure 1A). Laboratory investigations showed a mildly elevated serum CA 125 at 142 IU/l (normal < 35 IU/l). The patient underwent laparoscopy and a left adnexal mass was removed and sent for frozen section. Pathology reported a borderline tumor of the ovary; a left salpingo-oophorectomy, multiple peritoneal biopsies, omentectomy and staging procedures [1]. However they frequently affect young women in which conservative and minimally invasive surgery is required to preserve child-bearing potential. Conservative surgery is defined as surgery with complete staging, but with preservation of the uterus and at least part of one ovary to preserve fertility [2]. A careful inspection of the peritoneum with resection of macroscopic suspected lesions, multiple peritoneal biopsies, and infracolic omentectomy are necessary for thorough staging [2].

We report a case of BOT with early recurrence and high-grade carcinoma transformation in a short interval after complete laparoscopic staging by the guidelines of fertility-sparing surgery.

A 27-year-old unmarried nulliparous patient was referred to our department with abdominal discomfort. On abdominal examination, there was a large mass arising from the pelvis.
including total hysterectomy and right salpingo-oophorectomy, and appendectomy were performed. A diagnosis of peritoneal adenocarcinomatosis with invasive implants involving the peritoneum, omentum, and appendix was rendered. Cytologic assessment of the peritoneal fluid revealed malignant cells (Figure 1D). The patient was subsequently treated with two cycles of carboplatin-taxol chemotherapy.

The patient succumbed to disease 23 months after the initial diagnosis of the serous BOT. After a short period of disease stabilization she developed liver metastases and died five months after the diagnosis of disease relapse.

Discussion

The Gynaecologic Oncology Group (GOG) studied 21 women with Stage I serous BOTs who underwent unilateral salpingo-oophorectomy [3]. There was no recurrence of the disease in the patients with the fertility preserving surgery. However later studies have shown that fertility preserving treatment had a higher recurrence rate than radical treatment [1]. A reason for the difference in recurrence rate between the two studies may be that all the cases in the GOG study were operated by laparotomy,
either as the initial operation or as a second surgery after a laparoscopic procedure.

In our case, the patient had a Stage IC serous BOT with focal microinvasion. Whether the presence of microinvasion in serous BOTs imparts any additional prognostic significance is not clear at this time.

A review of 20 cases of serous BOTs with evidence of microinvasion of the stroma suggested that the presence of microinvasion was associated with a similar prognosis to common serous BOTs [4].

Serous BOTs have a very favorable prognosis, but complete surgical staging and prolonged follow-up are advised because pelvic recurrence and occasionally transformation to invasive carcinoma may occur [5]. Currently, the laparoscopic treatment of low malignant potential tumors has also been reported [1]. However it is still under debate whether conservative laparoscopic surgery can replace laparotomy. In our case, laparoscopy was not a complete surgical staging tool.

References


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Difficulties in the diagnosis of ovarian carcinoma: case report of squamous cell ovarian carcinoma in a 26-year-old woman

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Introduction

Ovarian carcinoma is the fourth most frequent neoplasm among women in Poland, constituting 6% of all neoplasms diagnosed in women. In recent years, a consistent increase in the incidence of this neoplasm has been observed [1, 2].

Ovarian carcinoma primarily affects women of peri- and postmenopausal ages. Two peaks in the incidence of this neoplasm can be observed, the first in women aged 45-49 and the other in women aged 65-69 [2, 3].

The etiology of ovarian carcinomas is not fully known. Pregnancy is thought to be a factor that protects against this neoplasm, as is hormonal contraception before the age of 45. It is estimated that approximately 5-10% of ovarian carcinomas have a hereditary component and are linked to mutations in the BRCA1/BRCA2 genes. The risk of this neoplasm is three times higher in people whose first-line and second-line relatives have suffered from ovarian carcinoma [2, 4-6].

Case Report

Hospitalization 1

A patient, aged 26, was admitted to the Gynecology Department in Kalisz on March 28, 2006 with a referral and a diagnosis of abdominal pain.

Medical history: Her last menstruation was on March 23, 2006. She was age 12 at first menstruation and had had regular menstruations every 28-29 days, lasting for three to five days. She typically experienced moderate pain at the beginning of her cycles. She had had one previous pregnancy which ended with a physiological birth in 2004. There was no report of chronic diseases but there was a previous hospitalization due to enlarged neck lymph nodes. Previous treatment occurred two years prior to hospitalization due to an enlarged thyroid gland. She was currently euthyroid and had had no previous operations. The patient also mentioned her grandmother had had a neoplasm, although she did not remember what kind of neoplasm it was.

Physical examination: The patient was in good condition, with efficient breathing and circulation (T – 37.2ºC, RR 120/80, HR 94/min). Her abdomen was soft to the touch and sensitive, with no peritoneal symptoms.

Gynecological examination: In a bimanual examination, the body of the uterus was found to be anteflexed, of normal size, motile and painless, and the right appendages had no pathological resistance, and an examinable, hardly motile change of 4-5 cm in diameter on the left side in the projection of the ovary was found.

TVS examination: The body of the uterus was 43 x 33 mm in size, and the endometrium was 4 mm wide. The right ovary was 46 x 27 mm in size with rising follicles and a dominating follicle of 22 x 16 mm. A cystic, multi-chamber, thick-walled, partly hyper-, partly hypoechogetic change of the left ovary was observed, which was 55 x 51 mm in size.

During the TVS-Doppler examination, a single vessel in the tumor capsule with a resistance index (RI) of 0.65, a pulsation index (PI) of 0.96, and a ratio of systolic to diastolic velocity of blood flow (S/D) of 2.85 was found.

Laboratory examination: Pregnancy was excluded with the aid of an HCG test. Results of additional tests that were run included the following: urine – general analysis (normal); morphology (HCT 38.9%, HGB 12.6 g/dl, RBC 4.6 M/ml, WBC 14.8 K/ml, PLT 274 K/ml); OB - 63; and neoplastic markers: CA-125 - 6.2

Summary

A 26-year-old woman who was admitted to the Gynecology Department with abdominal pain was later diagnosed with a multichamber tumor in the left ovary. Neoplastic markers were within normal limits. It was proposed that the patient should be operated on in order to remove the tumor, and a left salpingo-oophorectomy was performed. During the intraoperative histopathological examination, the tumor was described as being benign. However, in the final histopathological examination, a malignant neoplasm, a squamous cell carcinoma (G-2) of the ovary (pT1a), was found. It was decided that a hysterectomy and a right salpingo-oophorectomy should be performed. No other neoplastic foci were found in the postoperative material. The patient is currently undergoing periodic control examinations.

Key words: Antigen CA-125; Histopathological examination; Ovarian tumor; Squamous cell ovarian carcinoma; Transvaginal ultrasoundography (TVS).
U/mL. CEA, AFP and LDH markers were not analyzed in the hospital and are recommended for patients under 25. Anti-inflammatory and diastolic drugs were prescribed.

In the 24 hours after being admitted to the hospital, the patient was discharged in good general condition, with no pain and a recommendation to agree on the date of admission to the hospital for the planned operation after taking the hepatitis B vaccine (at the patient’s request).

Hospitalization 2

The patient returned to the hospital two weeks after having the hepatitis B vaccine (25/05/2006) with no abdominal pain. Unfortunately, due to an infection of the upper airways she could not be anesthetized and thus the operation was not performed.

In a control gynecological examination, the examinable tumor of the left ovary was 5 cm in diameter (54 x 58 mm in the TVS examination).

The patient was discharged from the hospital in a generally good condition and was advised to return to the hospital after curing the infection.

Hospitalization 3

The next admission to the hospital took place on June 14, 2006.

Gynecological and TVS examination: In a bimanual and TVS examination the tumor of the left ovary was found to have grown in comparison to the examinations from March and May 2006. At this point, the tumor was found to have increased in size to 6 cm in diameter (58 x 61 mm in the TVS examination).

TVS-Doppler analysis was repeated. In the walls and septums of the ovarian tumor arterial and vein vessels were visualized. The RI values in the arterial vessels were 0.85; 0.87, while the PI values were 1.46; 2.55, and for S/D, 6.39; 7.90, respectively.

Additional analysis: The concentration of antigen CA-125 was marked again and was found to be 6.30 U/mL. The results of additional preoperative examinations were normal.

The patient was qualified for the operation, which aimed to remove the tumor of the left ovary by means of laparotomy with an intraoperative examination. Written consent was obtained from the patient for the operation and also to remove the uterus and the appendages if a malignant lesion in the above mentioned intraoperative examination was observed.

Course of the operation: After opening the abdominal cavity with a transverse cut, the body of the uterus was observed to have a proper shape and size, while the right appendages showed no macroscopic changes and the left appendages had a cystic and solid tumor with a smooth external capsule, 6 cm x 6, 5 cm x 4 cm in size. Since it was not possible to leave normal ovarian tissue, a decision was made to perform the left salpingo-oophorectomy. An initial result of an intraoperative analysis, obtained from macroscopic examination, showed a benign change. For this reason, the operation was ended after the hemostasis procedure.

Postoperative course with no complications: The patient was discharged from the hospital on the sixth day after the operation in generally good condition and was advised to undergo a gynecological examination seven days later, and to call for the final results of the histopathological analysis two weeks later.

Postoperative histopathological analysis: Nine days after the operation the final results of the histopathological analysis were obtained: cells of a malignant neoplasm, a squamous cell ovarian carcinoma (G-2) (pT1a), were found.

The tumor sample was delivered to the laboratory in a 4% solution of neutralized formalin. After macroscopic estimation the segments were submitted to histopathological examination. After preparing, paraffin sections were stained with hematoxylin and eosin.

Results of the histopathological examination T 2941-44/06

Left ovary: squamous cell carcinoma with keratosis (G-2) and focal necrosis (pT1a). The tumor (6.5 x 6.5 x 3 cm) was a cystic lesion with proliferation of neoplastic cells in the inner layer. There were resorptive granulomas (as a reaction to keratosis masses) and aggregations of foam macrophages surrounding the tumor. The teratoma tissues were not revealed. In the part of the ovary that is free from neoplasm invasion, simple small cysts were found. In the ovarian stroma, macrophages with hemosiderin were also observed. The external surface of the ovary was covered with normotypical mesothelium and was free from cancer cell infiltration (Figure 1).

Left oviduct: Normal histological structure

The patient was urgently called to the hospital. Her case was presented to the provincial consultant in the field of obstetrics and gynecology and, due to the diagnosis, the decision was made to perform a laparotomy and remove the uterus together with the right appendages and a fragment of the greater omentum despite the young age of the patient.

Hospitalization 4

The operation was performed on July 10, 2006. No carcinoma cells were found in the final histopathological analysis of the removed tissues or in an uptake peritoneum swab.

Results of the histopathological examination T 3284-92/06

Right ovary: Follicular cysts, simple cysts, cystic corpus luteum and albican corpus of the ovary.

Right oviduct: Normal histological structure.


Follicular endometrium: Normal myometrium.

Cytological smear from peritoneum: Numerous reactive mesothelial cells.

The final diagnosis was squamous cell ovarian carcinoma Stage Ia.

The patient was discharged from the hospital in a generally...
good condition during the seventh day after the operation and was advised to undergo further treatment in the oncological clinic.

The patient currently undergoes periodic control examinations in a different clinic. Based on her medical records, she does not require any further treatment and her health state is described as good.

Discussion

The most frequently occurring ovarian carcinoma is serous carcinoma (40-50%), followed by endometrioid carcinoma (15-30%), mucinous carcinoma (5-15%), clear cell carcinoma (5-10%), and undifferentiated cell carcinoma (5-15%) [2]. Squamous cell ovarian carcinoma occurs extremely rarely. In the literature there are data related to its occurrence together with mature cystic teratoma only [7, 8].

Ovarian carcinomas usually develop asymptptomatically. For this reason, for a significant number of women, the disease is already advanced when they report to doctors with abdominal pain. Often the disease has progressed to the point that the patients show an increased circumference in their abdomen caused by the size of the tumor or by effusion fluid or when there are symptoms as a result of the neoplasm pressing on adjacent structures [4]. Despite the fact that these ailments are non-specific for ovarian carcinoma, precise examinations should be performed to rule out the possibility of a neoplastic process.

Gynecological examinations performed in all women of reproductive age once a year and once every half-year in women in the peri- and menopause age group is currently the only method capable of providing early detection of irregularities within the ovaries [9, 10].

Features that may imply changes of a malignant character that can be observed during a gynecological examination include: a limited motility of a change; very compact regions (a solid change); two-sided locations; and seizure of the Douglas sinus [2].

In all women, a gynecological examination should be supplemented with ultrasonographic analysis. Emphasis should also be placed on the importance of taking accurate medical records related to symptoms reported by the patient and on histories of ovarian carcinoma, breast carcinoma and alimentary canal carcinoma in the family due to the possibility of mutations in the BRCA1/BRCA2 gene as well as incidences of Lynch syndromes I and II [6]. Whenever an ovarian neoplasm is detected, the concentration of antigen CA-125 should be marked and an X-ray examination of the chest should be taken [2, 9].

During the first hospitalization of the patient in this study (March 2006), the gynecological examination could not confirm a malignant neoplastic change unambiguously (a one-sided, cystic change, or a free Douglas sinus). Abdominal pain that regressed after conservative therapy and blood sedimentation rate were indicators of the presence of an inflammatory tumor. The age of the patient supported a mild change, and the patient’s medical history did not unambiguously confirm familial incidences of genital neoplasm or alimentary canal neoplasm. For these reasons, we consented to the patient’s request to postpone the operation so that she could take a hepatitis B vaccine.

Image diagnostics

Ultrasoundographic examination performed with the aid of a vaginal probe is currently a diagnostic standard as far as any suspicion of changes within the ovary is concerned.

Features implying a malignant character include: a change of a solid or cystic-solid character, a thickened capsule, wide septums inside the change, proliferation of a papillious character (specific for mucinous and serous carcinoma), low-resistance (PI < 1.0; RI < 0.4) or high-resistance (RI > 0.8) vessels, and low values of the maximum contractile speed and average maximum speed [2, 11-13]. Computed tomography and/or magnetic resonance may contribute significantly to the diagnostic process, especially in the assessment of the remaining structures of the pelvis minor.

In the first TVS examination performed on the patient (in March 2006), the location and features of the tumor implied a mild character. Examination of blood flow within the tumor (Color Doppler/Power Doppler) showed a single vessel with a regular RI value (0.65) and a PI value (0.97) indicative of an inflammatory tumor of the appendages. These results allowed for a planned operation rather than an emergency procedure.

Laboratory diagnostics

Since the beginning of the 1980s, the determination of antigen CA-125 has been a diagnostic standard in terms of changes within ovaries [3, 4, 9, 14]. Unfortunately, this antigen is not specific for ovarian tissues and thus a higher concentration of CA-125 can be observed in cases of oviduct, pancreas and endometrium carcinoma as well as in pathological states with no malignant character such as endometriosis, uterine myoma, non-malignant cysts, liver diseases, inflammatory states and in the first term of pregnancy [15]. The concentration of CA-125 is regarded as being high at values of > 35 U/ml, which is observed in approximately 80% of patients with diagnosed ovarian carcinoma. An increased level of CA-125 occurs in only 50% of all cases of ovarian carcinoma in the first advancement stage according to FIGO (the International Federation of Gynecology and Obstetrics). A negative result using CA-125 does not exclude a malignant process while an increased level of CA-125 does not necessarily indicate an ovarian carcinoma [2, 3, 4, 16]. According to the recommendations of ASCO (American Society of Clinical Oncology), ACS (American Cancer Society), ACOG (American College of Obstetricians and Gynecologists), ESMO (European Society for Medical Oncology) and the Polish Gynecological Society, concentrations of markers AFP, LDH and beta-hCG should
be used when neoplastic processes are suspected in patients of less than 25 years in order to exclude the possibility of germ cell tumors [3, 4, 9, 10, 14, 17-20].

In the case presented, marker CA-125 was analyzed twice and results were found to be normal. This allowed us to assume once more that the examined tumor had a mild character and thus qualified the patient for an operation in a planned procedure.

**Microscope diagnostics**

Microscope diagnostics comprise the most basic and the only method of detecting changes of a malignant character. In case of ovarian carcinomas, the final diagnosis is only possible after a laparotomy and a histopathological examination of the tissues [2, 3]. Due to the young age of the patient, the procedures in this case were aimed at saving her uterus. Following the oncological rules of conduct, the tumor was removed and was submitted to an intraoperative analysis whose initial result, obtained from macroscopic examination, showed a mild change.

Squamous cell carcinoma cells were only found in the final histopathological analysis of paraffin sections.

**Conclusions**

The case presented in this paper demonstrates that early detection of ovarian carcinoma is still very difficult, despite the use of many diagnostic methods, and shows that there is no particular examination with a sufficiently high sensitivity and specificity that is also easily accessible. This reinforces the importance of regular gynecological controls and physical examinations, together with the TVS examinations.

**References**


Uterine tumors resembling ovarian sex cord tumors.  
A case report

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Summary

Uterine tumors resembling ovarian sex cord tumors (UTROSCT) are rare, usually benign, polypoid or nodular neoplasms which generally arise in the fourth to sixth decade of life. We report a case of a 74-year-old woman who presented with vaginal bleeding and remarkable uterine enlargement. Abdominal hysterectomy with bilateral salpingo-oophorectomy was performed and a diagnosis of UTROSCT was made. Immunohistochemistry is mandatory for a correct diagnosis and a panel of at least two markers of sex cord differentiation is recommended. Differential diagnoses include leiomyosarcoma, UTROSCT and ESTSCLE, mixed müllerian tumor and metastatic ovarian sex cord tumor.

Key words: UTROSCT; Ovarian sex cord tumors; Inhibitin; Calretinin.

Introduction

Uterine tumors resembling ovarian sex cord tumors (UTROSCT) are very rare neoplasms, first described in 1976 by Clement and Scully [1]. To date, only 77 cases have been reported in the literature. Although UTROSCT are usually benign, a case with metastasis was described by Biermann et al. in 2008 [2] thus, at present the definition of tumor with uncertain behavior is more accepted.

Frequently, UTROSCT are polypoid or nodular masses located in the uterine fundus. They usually occur in reproductive age, although some cases arise in postmenopause. These tumors are divided into two groups based on the relative proportion of sex cord-like elements and endometrial stromal cells: endometrial stromal tumors with sex-cord-like elements (ESTSCLE; Group I), characterized by sex-cord-like areas in a leiomyomatous background; uterine tumors resembling ovarian sex cord tumors (UTROSCT; Group II), mainly or exclusively composed of cells with a proliferative pattern resembling ovarian sex-cord tumors. Group II tumors show a benign biological behaviour, whereas Group I tumors stand out due to a high risk of recurrence and metastasis [3].

Clinically, abnormal vaginal bleeding is the most common presenting symptom, though these tumors occasionally show hormone-secreting activity [4]. In most cases the diagnosis is incidental, following immunohistochemical and ultrastructural studies on surgical specimens. A total abdominal hysterectomy with bilateral salpingo-oophorectomy or a simple hysterectomy seems to be the gold-standard treatment, even though successful medical treatment with anastrazole has been reported [5]. In young patients fertility-sparing surgery with a close long-term follow-up is acceptable [6-8].

Case Report

A 74-year-old caucasian woman presented at the Department of Obstetrics and Gynecology of San Salvatore Hospital (University of L’Aquila, Italy) with postmenopausal vaginal bleeding. On bimanual examination a hard mass occupying all the pelvic cavity and extending as far as 1 cm beyond the transversal umbilical line was detected. Pelvic ultrasound (US) and computed tomography (CT) scan showed an enlarged uterus with a 16 x 12 cm fundic, dishomogenous, ill-defined mass. Serous tumoural markers (CA 15-3; CA 19-9; CA-125) were negative. A standard total abdominal hysterectomy and bilateral salpingo-oophorectomy was performed.

The uterus weighed 835 g and was deformed by a nodular intramural lesion of 17 cm in diameter. It was fixed in 10% buffered formalin over a 48-hour period and processed for routine light-microscopic examination. Specimens were embed-
Uterine tumors resembling ovarian sex cord tumors. A case report

Immunohistochemistry was performed with the avidin-biotin-peroxidase complex technique. Heat-induced antigen retrieval was conducted by immersion of the sections in sodium citrate buffer (0.01M sodium-citrate monohydrate, pH 6.0). Sections previously formalin-fixed and paraffin-embedded were stained for calretinin, CD10, CD99, inhibin, actin, CAM 5.2, and estrogen receptors. The results were divided in negative, focally positive, positive and strongly positive. The tumor cells stained strongly positive for CD99, calretinin, CAM 5.2, estrogen receptors and weakly for inhibin; no reaction for actin or CD10 was observed.

Finally, a diagnosis of uterine tumor resembling ovarian sex cord tumors (UTROSCT) was made.

Discussion

Morphologic and immunohistochemical findings suggest that UTROSCT arise from pluripotential uterine mesenchymal cells, which mainly differentiate into sex cord cells. Focal smooth muscle and endometrial stromal cell differentiation can also occur [3]. Because of the difficulties in recognising these structures on hematoxylin-eosin stain, immunohistochemistry is necessary for a correct diagnosis [9].

In the present case, several differential diagnoses had to be considered, such as any kind of polypoid lesion clinically, as well as leiomyosarcoma, UTROSCT and ESTS克莱, mixed mullerian tumor and metastatic ovarian sex cord tumor histopathologically [10, 11]. All UTROSCT stained positive for at least two sex cord differentiation markers, often with co-expression of cytokeratin, CD10, vimentin, estrogen receptor and progesterone receptor; desmin immunoreactivity, if present, is restricted to smooth muscle areas [3]. Inhibin is the most specific marker for these cells [12], even though some studies have shown that calretinin may be more sensitive

Figure 2. — Immunohistochemical positive stainings: cytokeratin AE1/AE3 positivity of the gland-like structures (20 x magnification) (2A); calretinin positivity of the gland-like structures (20 x magnification) (2B); CD99 positivity of the gland-like structures (40 x magnification) (2C); alpha-inhibin positivity of the gland-like structures (40 x magnification) (2D).
than inhibin, thus being useful in inhibin-negative ovarian sex cord stromal tumors [13]. However, calretinin is less specific than inhibin: in a study by Movahedi et al., they demonstrated positivity in approximately one-quarter of ovarian surface epithelial carcinomas tested, compared with only 2% showing inhibin positivity [13]. Inhibin is a peptide hormone normally produced by ovarian granulosa cells, that inhibits FSH production and GnRH release. The mechanism of action of inhibin is still unknown, but may involve competing with activin for binding to activin receptors and/or binding to inhibin-specific receptors [14].

Recently, Pusiol et al. identified a new pathologic entity, defined as “uterine leiomyoma with tubules”. This lesion is characterized by sweeping and intersecting fascicles of smooth muscle cells surrounding a diffuse proliferation of tubular and gland-like structures, lined by plump cells with indistinct cytoplasm. The “uterine leiomyoma with tubules” shows UTROSCT-like histological features, but different immunohistochemical stainings. Thus, if no positivity for at least two sex cord differentiation markers is observed, a diagnosis of “leiomyoma with tubules” should be made [15].

Generally, UTROSCT show benign histological features (i.e., well-circumscribed borders, absence of vascular invasion) and a benign biological behaviour. Rarely, are infiltrative borders and focal vascular invasion [3] observed, usually in association with a malignant biological behavior [2].

Risk factors for this lesion are still unknown. Cases of UTROSCT arising in a patient with Mazabraud’s syndrome [16] and, more frequently, in patients in treatment with tamoxifen [17, 11] have been reported. Since some endometrial stromal sarcomas with low malignant potential have been described in patients treated with tamoxifen, we could hypothesize that this neoplasm would occur more frequently in the future [18].

Conclusions
Since UTROSCT may be confused with several uterine adenocarcinomas or metastasis, immunohistochemistry is mandatory for a correct and accurate diagnosis with the aim of avoiding overtreatment [9]. Thus, we suggest the use of an appropriate immunohistochemical panel to facilitate this diagnosis, including at least two sex cord differentiation markers (calretinin and one of either melan A, CD99, or inhibin) [3]. Immunohistochemical stains may show positivity for vimentin, cytokeratin, actin and desmin in variable proportions. Inhibin is a more specific marker for these cells [12]. Further features, such as including necrosis, vascular invasion, mitotic index and tumour borders are relevant for the outcome of patients.

References

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Immunohistochemical expression of granzyme B and vascular endothelial growth factor (VEGF) in normal uterine cervices and low and high grade squamous intraepithelial lesions

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Summary

Purpose: This study aimed to evaluate the immunoexpression of granzyme B and vascular endothelial growth factor (VEGF) in the variants of cervical squamous intraepithelial neoplasia. Methods: Granzyme B immunohistochemical expression was studied in the epithelium, stroma and in both the epithelium + stroma of 142 fragments of uterine cervix; there were 34 grade 1 cervical intraepithelial neoplasias (CIN 1), 36 grade 2 cervical intraepithelial neoplasias (CIN 2), 33 grade 3 cervical intraepithelial neoplasias (CIN 3) and 39 uterine cervix fragments without abnormalities - control group. Immunoeexpression of VEGF was studied in 160 uterine cervix fragments, with 43 grade 1 cervical intraepithelial neoplasias (CIN 1), 33 grade 2 cervical intraepithelial neoplasias (CIN 2), 31 grade 3 cervical intraepithelial neoplasias (CIN 3) and 53 uterine cervix fragments without abnormalities - control group. Results: In the stroma, immunoexpression of granzyme B in grade 1 cervical intraepithelial neoplasias was smaller than in grade 3 cervical intraepithelial neoplasias. High VEGF immunoeexpression was found in grade 3 cervical intraepithelial neoplasias while it was low in grade 1 cervical intraepithelial neoplasias and in the control group. Conclusion: The higher the severity of the cervical intraepithelial lesion, the higher the immunoexpression of granzyme B. A progressive increase in VEGF immunoeexpression was found in the intense grade, according to the severity of the cervical intraepithelial neoplasia.

Key words: Granzyme B; VEGF; CIN 1; CIN 2; CIN 3.

Introduction

Human papillomavirus (HPV) is highly prevalent in sexually active women. The persistent infection by oncogenic HPV is essential for the progression of the injury from minor to high grade intraepithelial neoplasia and then to an invasive lesion [1].

The host immune response, particularly the cellular response, plays an important role in eliminating HPV infections. The injuries may persist and subsequently progress due to the failure of this local response. The cytotoxic T lymphocytes (CTLs) and natural killer cells (NKs) are the main effector cells in the eradication process of viruses infected cells and tumor cells [2].

The granzyme family consists of a special group of serine proteases, which are stored, fully processed and in an activated form in cytoplasmic granules of cytolytic T lymphocytes and natural killer cells. Granzyme B is abundantly present in T lymphocytes and is linked to induction of cytotoxicity and proteolytic events in the recognition of target cells [2-4].

Angiogenesis is the process by which new blood vessels are formed. It is associated with the development of high-grade cervical intraepithelial neoplasia and invasive carcinoma of the uterine cervix. One of the most potent and specific angiogenic factors is the vascular endothelial growth factor (VEGF) [5, 6].

Immunohistochemical studies have shown progression of cervical intraepithelial neoplasia associated with increased microvessel density and the expression of vascular endothelial growth factor [7-9].

The aim of this study was to evaluate the immunohistochemical expression of granzyme B and VEGF with variants of squamous cervical intraepithelial neoplasia.

Materials and Methods

A series of 196 women underwent treatment at the outpatient clinic for lower genital tract diseases of the Department of Gynecology, Federal University of São Paulo - Escola Paulista de Medicina/UNIFESP-EPM from 2002 to 2005.

As criteria for inclusion, the patients had to be in menarche and suffer from grade 1, 2 or 3 cervical intraepithelial neoplasias, defined by histopathological examination. For the control group tissue fragments of the uterine cervix without cervical intraepithelial neoplasias were used as defined by histopathological examination.

The study did not include pregnant women and nursing mothers, women with previous history of vaginal intraepithelial neoplasias or cervical treatment at any grade, women with a history of uterine cervix surgery or cauterizations and patients with acquired, infectious or iatrogenic immunosuppression. The project was approved by the Ethical Committee of the Institution.

Based on the histopathological diagnosis, the study group was composed of CIN 1 - 54 patients, CIN 2 - 40 patients, and CIN 3 - 38 patients totaling 132 patients.

The control group comprised 64 women without cervical intraepithelial neoplasia.
Due to problems occurring in fragments of the histopathology and immunohistochemistry, the study group was divided to assess immunohistochemistry of granzyme B and VEGF.

Of the total study group, 103 cases were referred for evaluation of immunoexpression of granzyme B. The control group contributed with 39 cases.

For the study of immunoexpression of VEGF 107 cases of the intraepithelial neoplasia group were used (grade 1, 2 and 3) and 53 of the control group.

Results

The immunohistochemical expression of granzyme B was studied in the epithelium, stroma and in the epithelium + stroma of 142 fragments of the uterine cervix; there were 34 grade 1 cervical intraepithelial neoplasias, 36 grade 2 cervical intraepithelial neoplasias, 33 grade 3 cervical intraepithelial neoplasias and 39 with absence of cervical intraepithelial neoplasia, that is, fragments of the uterine cervix without atypia - control group.

The analysis of the slides submitted to immunohistochemical reaction for granzyme B showed positivity of activated lymphocytes in the epithelium and stroma in 29 out of 39 cases analyzed in the control group. In grade 1 intraepithelial neoplasia, positivity of lymphocytes was observed in 28 out of 34 cases, in grade 2 intraepithelial neoplasia in 34 out of 36 cases, and in grade 3 intraepithelial neoplasia the positivity of activated lymphocytes was not observed in only one out of 33 cases (Table 1).

Table 1. — Distribution of the 142 cases of cervical fragments according to the positivity of the immunohistochemical expression of granzyme B, the different pre-neoplastic lesions, and the control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Descriptive</th>
<th>Epithelium</th>
<th>Stroma</th>
<th>Epithelium + stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N</td>
<td>39</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>average</td>
<td>4.0</td>
<td>4.6</td>
<td>8.6</td>
</tr>
<tr>
<td>CIN 1</td>
<td>N</td>
<td>34</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>average</td>
<td>4.3</td>
<td>2.8</td>
<td>7.1</td>
</tr>
<tr>
<td>CIN 2</td>
<td>N</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>average</td>
<td>3.3</td>
<td>4.9</td>
<td>8.1</td>
</tr>
<tr>
<td>CIN 3</td>
<td>N</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>average</td>
<td>4.4</td>
<td>6.3</td>
<td>10.7</td>
</tr>
</tbody>
</table>

When comparing the groups for granzyme B, the Kruskal-Wallis test showed no significant difference between groups (epithelium and epithelium + stroma) and we verified the values of x² 3.321 NS (p = 0.045) for the epithelium and x² 5.477 NS (p = 0.140) for the epithelium and stroma.

On the other hand, considering the count held in the stroma, the Kruskal-Wallis test showed a statistically significant difference between the groups, being x² 11.023 (p = 0.012). The test for multiple comparisons showed a statistically significant difference between the values of group CIN 1 in relation to group CIN 3, with absolute value of the calculated difference 31.79 for the critical value (25.62) while in comparisons between CIN 1 and CIN 2 the value of the calculated statistic (20.45) was close to the critical value (25.95), suggesting a possible difference from CIN 1 to CIN 2, shown by the average 2.8 and 4.9 for CIN 1 and CIN 2, respectively.

For the variable VEGF, immunoexpression in 160 fragments of the uterine cervix were studied with 43 grade 1 cervical intraepithelial neoplasias, 33 grade 2 cervical intraepithelial neoplasias, 31 grade 3 cervical intraepithelial neoplasias and 53 with absence of cervical intraepithelial neoplasia, that is, fragments of the uterine cervix without atypia - control group (Table 3).

Table 3. — 160 cases evaluated for VEGF following subgroups, number of cases, expression and statistical evaluation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Absence/Mild</th>
<th>Moderate</th>
<th>Intense</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>n</td>
<td>31</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>CIN 1</td>
<td>n</td>
<td>14</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>CIN 2</td>
<td>n</td>
<td>14</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>CIN 3</td>
<td>n</td>
<td>3</td>
<td>14</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>n</td>
<td>62</td>
<td>72</td>
<td>160</td>
</tr>
</tbody>
</table>

In the comparison between the groups, previously set and intensity of the color - absent or mild, moderate or intense, in the contingency table we have observed statis-
tically significant differences between the groups and the intensity of color $x^2$ calc = 44.154 ($p = 0.001$).

In grade 1 cervical intraepithelial neoplasia, 14 (32.6%) fragments of the uterine cervix with absence or mild VEGF expression were observed, 28 (65.1%) with moderate expression and one (2.3%) fragment of the uterine cervix with intense VEGF expression.

In grade 2 cervical intraepithelial neoplasia 14 (42.4%) fragments of the uterine cervix with absence or mild VEGF expression were observed, 11 (33.3%) with moderate expression and eight (24.3%) fragments of the uterine cervix with intense VEGF expression.

In grade 3 cervical intraepithelial neoplasia, we found three (9.6%) fragments of the uterine cervix with absence or mild VEGF expression, 14 (45.2%) of cases with moderate expression and another 14 (45.2%) fragments of the uterine cervix with intense VEGF expression.

In the control group, i.e., stratified squamous epithelium without atypia, 31 (58.5%) fragments of the uterine cervix with absence or mild VEGF expression were observed, 19 (35.8%) with moderate expression and three (5.7%) fragments of the uterine cervix with intense VEGF expression (Table 4); the prevalence of cases had absence or mild VEGF expression.

In grade 2 cervical intraepithelial neoplasia 14 (42.4%) fragments of the uterine cervix with absence or mild VEGF expression were observed, 11 (33.3%) with moderate expression and eight (24.3%) fragments of the uterine cervix with intense VEGF expression.

In grade 3 cervical intraepithelial neoplasia, we found three (9.6%) fragments of the uterine cervix with absence or mild VEGF expression, 14 (45.2%) of cases with moderate expression and another 14 (45.2%) fragments of the uterine cervix with intense VEGF expression.

In the control group, i.e., stratified squamous epithelium without atypia, 31 (58.5%) fragments of the uterine cervix with absence or mild VEGF expression were observed, 19 (35.8%) with moderate expression and three (5.7%) fragments of the uterine cervix with intense VEGF expression (Table 4); the prevalence of cases had absence or mild VEGF expression.

Discussion

Since cervical cancer is considered to be a HPV-associated disease and its clinical course of immune response is dependent on the host organism, there have been more and more researches on the biomolecular and immunogenetic aspects.

Viral infection detection comes from the appearance of antigens produced by the expression of viral proteins and generates a stimulus to the immune responses. Around the affected area there is mononuclear cell infiltration composed by T lymphocytes, macrophages and NKs. As the cellular modifications involve changes in premalignant tissues, there is the activation via the immune system. Granzyme B plays a crucial role in CTLs and NKs causing apoptosis of target cells [10, 11].

Angiogenesis is the formation of new capillaries from pre-existing vessels. The number of vessels is known to be the reflection of angiogenesis activity, and increased vascular density showed a relationship with high incidence of metastasis and worse prognosis in different tumors [12].

The immunohistochemical expression of granzyme B refers to the obtained results, to the need of the organism to fight against the most severe high-grade intraepithelial lesions. Similarly, in these cases, there is a greater significance of immunohistochemical VEGF expression as an important factor to access cellular elements of defense.

Although both studied factors are directly related to the defensive process, it is indeed remarkable that the high-grade intraepithelial lesions are those which most often develop into invasive cancer. Thus, in spite of the obtained results and the statistical significance, other functional changes may be occurring to justify the cases that evolve to invasion or post-treatment recurrence.

The methodological limitations of this study can not define what these changes are. Therefore, it is necessary to revise and extend these results from a functional point of view.

We observed that the immunoexpression of granzyme B and VEGF increased in parallel to the severity of the cervical intraepithelial lesion, confirming the hypothesis that granzyme B and VEGF can be used as prognostic markers of remission and persistence or development of pre-neoplastic lesions, and that they are important indicators of survival and therapeutic response.

References


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Immunohistochemical expression of granzyme B and vascular endothelial growth factor (VEGF) in normal uterine cervicles and etc.
Malignant mixed mullerian tumor of the cervix including components of a rhabdomyosarcoma: case report and literature review


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Introduction

Approximately 70% to 80% of cervical malignancies are squamous cell carcinomas, 20% to 25% are adenoscarcinomas, and the other histologic types include adenosquamous carcinomas, clear cell carcinomas, small cell carcinomas, sarcomas, melanomas, lymphomas, and metastatic tumors [1]. Malignant mixed mesodermal tumors (MMMTs) are composed of carcinomatous and sarcomatous components and have an aggressive metastatic potential, resulting in a poor prognosis. MMMTs of gynecologic origin typically arise from either the ovary or the uterus, and MMMTs of the cervix are extremely rare. Due to the rarity of MMMTs arising from the cervix, there is no consensus regarding treatment, prognosis, and outcome; however, aggressive surgical cytoreduction, combined with adjuvant platinum-based chemotherapy and/or radiotherapy, is recommended as the treatment of choice for MMMTs of the cervix. Cervical MMMTs are more often confined to the uterus at the time of diagnosis and frequently have non-glandular epithelial components. For these reasons, MMMTs of the cervix may have a better prognosis compared to the uterine counterparts. A case of an immunohistochemically confirmed primary MMMT of the cervix, including components of a rhabdomyosarcoma, is reported.

Case Report

The patient was a 47-year-old gravida 2, para 2, who was referred to our institution with a suspected submucosal myoma. Her main complaint was vaginal bleeding during the previous five days. She denied a history of medication use, other medical problems or surgeries, except a cesarean section, and the family history was also unremarkable. Her menstruation had been profuse and at irregular intervals for ten days, without dysmenorrhea. On pelvic examination, an egg-sized mass with poor mobility was palpable on the cervix and the lower segment of the uterus. A large ulcerated mass was noted, which had projected onto the exocervix after the Pap smear (Figure 1), and a cervical biopsy was performed. Vaginal ultrasound revealed a 4.7 cm solid mass with mixed internal components on the cervix. Magnetic resonance imaging (MRI) showed the presence of a pear-shaped mass (5 x 4 x 5 cm) that was widening the endocervical canal, and a normal-sized uterus and adnexa (Figure 2). There was no abnormal fluid collection or lymph node enlargement in the bilateral pelvic sidewalls and retroperitoneum. The pathologic results of the Pap smear demonstrated some atypical cells suggestive of a malignancy and the biopsy specimen revealed a MMMT.

Chest X-ray, intravenous pyelogram, mammography, duodenoscopy, and colonoscopy were performed and the findings were normal. Tumor markers were within normal limits; serum CA125 was 12.28 IU/ml, carcinoembryogenic antigen was 1.38 ng/ml, and CA19-9 was 16.92 IU/ml. Other hematologic data were also within normal limits.

Based on these results, the patient underwent a radical hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymph node dissection. At the time of surgery, a 5 cm tumor mass arising from the endocervix and confined to the endocervical canal was identified. The body of the uterus, the bilateral ovaries, fallopian tubes, and other abdominalpelvic organs showed normal gross findings. Microscopically, the tumor consisted of endocervical adenocarcinoma admixed with the stromal (sarcomatous)
Malignant mixed mullerian tumor of the cervix including components of a rhabdomyosarcoma: case report and literature review

The epithelial adenocarcinoma components were positive to cytokeratin (Figure 3B) and vimentin (Figure 3C). The mesenchymal stromal components were composed of spindle cells showing reactivity to vimentin and smooth muscle actin (Figure 3D). Some cells showing rhabdoid features with reactivity to desmin (Figure 3E) suggested rhabdomyosarcoma components. The mass was diagnosed as MMMT with focal rhabdomyosarcoma components. The depth of invasion of the tumor was 7 mm and there was no malignant cell infiltration of the parametrium, paracolpium, resected margins, and pelvic lymph nodes.

The chemotherapeutic protocol consisted of five days of ifosfamide (1.5 g/m² intravenously over 1 h) and cisplatin (20 mg/m² over 15 min), followed by mesna (120 mg/m² by intravenous bolus, then 1.5 g/m²/24 h as a continuous infusion). Chemotherapy was repeated three times at three to four week intervals. The patient tolerated the treatment well with only grade 1 or 2 toxicities (alopecia, nausea, and vomiting) without a severe toxic effect (grade 3-4 neutropenia, anemia, thrombocytopenia, severe nausea, and vomiting). She then received external beam radiation therapy (5040 cGY in fractions) and there was no evidence of any side-effects to radiation. She is currently being followed as an outpatient without evidence of a relapse for 20 months.

Figure 2. — MRI T2W enhanced, sagittal (A) and coronal (B) views of a pelvic mass (arrow) filling the cervical cavity. The uterus is seen as a normal finding.

Discussion

Female genital tract carcinosarcomas, otherwise known as MMMTs, are highly aggressive biphasic neoplasms that exhibit a malignant epithelial component (carcinoma) in conjunction with a malignant stromal component (sarcoma). The sarcomatous element is classified as a homologous tumor, which originates from homogeneous tissue, and the heterologous tumor is composed of foreign materials, such as cartilage, bone, or striated muscle, if it is a uterine MMMT. It is named rhabdomyosarcoma, osteosarcoma, or chondrosarcoma, respectively.

MMMTs have been reported in a variety of anatomic sites. The most common site of occurrence in the female genital tract is the uterine corpus, and primary MMMTs of the uterus account for approximately 2% of all uterine cancers. Ovarian MMMTs are also rare, accounting for approximately 1% of all ovarian cancers [8]. Cervical MMMTs are extremely unusual, so it is difficult to determine the proportion of cervical origin. In a review of the literature, Clement and co-workers [5] reported cervical MMMTs account for < 3% of uterine origin.

In fact, cervical and uterine MMMTs do not differ significantly in their gross appearance (polypoid mass, hemorrhage, necrosis, and invasion). Sometimes, cervical MMMT, are confused with more common uterine...
MMMTs. However, they have striking differences with respect to the carcinomatous component. The carcinomatous component of the cervix includes squamous cell carcinoma, at least focally, but that of the uterus is predominantly a glandular type (endometrioid, serous, or clear cell). Otherwise, the sarcomatous elements resemble each other. In 1998, Clement and co-workers [5] reviewed 30 reported cases of cervical carcinosarcomas and added nine cases of their own. They identified several key features in a large study; specifically, cervical MMMTs are more often confined to the uterus at the time of diagnosis and frequently has a non-glandular epithelial component. For these reasons, cervical MMMTs may have a better prognosis compared to their uterine counterparts [5, 9].

The commonest clinical features are vaginal bleeding, an abnormal Pap smear, and a cervical mass [3, 5, 10, 11]. In our case, the patient sought evaluation at the hospital due to persistent vaginal bleeding. She also had an abnormal Pap cytology and cervical mass, although it was initially thought to be a submucosal myoma. In fact, most patients with cervical cancer visit the hospital with symptoms like postcoital vaginal bleeding. Younger patients tend to manifest symptoms more rapidly than older patients, and receive gynecologic examinations in a more timely fashion in response to abnormal bleeding.

To date, MMMTs within the female reproductive system have been managed with a variety of treatments including local excision, total hysterectomy, pelvic lymph node dissection, radiation, and chemotherapy. In several previous reports, MMMTs of gynecologic origin were treated with cisplatin-based chemotherapy, such as a cisplatin-ifosfamide combination, yielding a high response rate [6, 8, 9, 12]. And some authors have reported the benefits of adjuvant radiotherapy in the treatment of uterine sarcoma, thereby decreasing the pelvic recurrence rate [13]. The origin of the tumor does not influence the clinical course, and there is no evidence to suggest that adjuvant treatment should depend on the primary site (uterus, ovary, and cervix) [14]. For these reasons, treatment of cervical MMMT has been tried with that of the uterus.

Table 1 shows the variable treatments of cervical MMMT and the results. Patients were treated with surgery, local excision, total hysterectomy, radical hysterectomy, pelvic exenteration, and pelvic lymph node dissection, radiation, and chemotherapy according to their condition. Surgery was the initial treatment for most of the patients with early-stage (I or II) disease followed by adjuvant therapy. Patients with advanced stage disease received radiation therapy or chemotherapy as a first-line therapy.

In conclusion, MMMT of the cervix is a rare disease and is associated with a poor prognosis, although it is mostly detected in early stages. Aggressive primary therapy can offer the best chance of cure in patients with early-stage disease.
The patient had an 8 cm in size cervical mass.

RH: Radical hysterectomy; BSO: Both salpingo-oophorectomy; PLND: Pelvic lymph node dissection.

Table 1. — Overview of all reported cases of cervical carcinosarcoma.

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of patient</th>
<th>Stage</th>
<th>Initial Tx</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abadi (2008)</td>
<td>1</td>
<td>I</td>
<td>RH+BA+ PLND+RTx</td>
<td>NED for 21 months</td>
<td>11</td>
</tr>
<tr>
<td>Rosa Laterza (2007)</td>
<td>2</td>
<td>I</td>
<td>TAH+BSO+PLND+CTx</td>
<td>NED for 48 months</td>
<td>15</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td>RH+BSO+PLND+RTx</td>
<td>DOD after 2 months (Systemic recurrence)</td>
<td>15</td>
</tr>
<tr>
<td>Maheshwari (2006)</td>
<td>1</td>
<td>I</td>
<td>TAH + BSO</td>
<td>Recur after 2 months (vagina)</td>
<td>2</td>
</tr>
<tr>
<td>Sharma (2005)</td>
<td>5</td>
<td>I</td>
<td>RH +PLND+RTx</td>
<td>NED for 35 months</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td>RH+ PLND</td>
<td>NED for 42 months</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td>RH+ PLND</td>
<td>NED for 65 months</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td>RTx</td>
<td>NED for 28 months</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td>RTx</td>
<td>DOD after 5 months</td>
<td></td>
</tr>
<tr>
<td>Wright (2005)</td>
<td>5</td>
<td>I</td>
<td>TAH+BSO+PLND</td>
<td>NED</td>
<td>10</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td>RH+BSO+PLND</td>
<td>Recur</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td>TAH+BSO+PLND+ RTx</td>
<td>NED</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td>RTx</td>
<td>DOD after 5 months</td>
<td></td>
</tr>
<tr>
<td>Iida (2005)</td>
<td>1</td>
<td>I</td>
<td>RH+BSO+PLND+RTx</td>
<td>NED for 17 months</td>
<td>16</td>
</tr>
<tr>
<td>Clement (1998)</td>
<td>9</td>
<td>I</td>
<td>TAH+BSO+PLND+ RTx+CTx</td>
<td>DOD after 13 yrs (due to colon cancer)</td>
<td>5</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td>Local excision</td>
<td>F/U loss</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td>TAH+BSO</td>
<td>F/U loss</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td>TAH+BSO+PLND</td>
<td>Recur after 3 yrs (vagina)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td>TAH+BSO+PLND+RTx</td>
<td>NED for 21 months</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
<td>TAH+BSO+PLND</td>
<td>NED for 13 months</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td></td>
<td>TAH+BSO+PLND</td>
<td>NED for 16 months</td>
<td></td>
</tr>
<tr>
<td>Wright (2005)</td>
<td></td>
<td></td>
<td>Local excision+ RTx</td>
<td>NED</td>
<td></td>
</tr>
<tr>
<td>Mathoulin-Portier (1998)</td>
<td>1</td>
<td>I</td>
<td>TAH+BSO</td>
<td>Recur after 12 months (abdomen)</td>
<td>17</td>
</tr>
<tr>
<td>Farley (1997)</td>
<td>1</td>
<td>IV</td>
<td>Local excision+ RTx</td>
<td>DOC after 2 months</td>
<td>18</td>
</tr>
<tr>
<td>Manhoff (1995)</td>
<td>1</td>
<td>I</td>
<td>TAH+BSO+RTx</td>
<td>NED for 6 months</td>
<td>19</td>
</tr>
<tr>
<td>Rodriguez-Escudero (1988)</td>
<td>1</td>
<td>I</td>
<td>TAH+BSO+PLND+ RTx</td>
<td>Recur after 6 months (vagina)</td>
<td>20</td>
</tr>
<tr>
<td>Young (1988)</td>
<td>1</td>
<td>I</td>
<td>TAH+BSO+PLND+ RTx</td>
<td>F/U loss</td>
<td>21</td>
</tr>
<tr>
<td>Miyazawa (1986)</td>
<td>1</td>
<td>I</td>
<td>RH+BSO+PLND+RTx</td>
<td>NED for 30 months</td>
<td>22</td>
</tr>
<tr>
<td>Waxman (1983)</td>
<td>1</td>
<td>I</td>
<td>Local resection + Chemoradiation</td>
<td>NED for 9 months</td>
<td>23</td>
</tr>
<tr>
<td>Hall-Craggs (1981)</td>
<td>1</td>
<td>I</td>
<td>TAH+BSO</td>
<td>F/U loss</td>
<td>24</td>
</tr>
<tr>
<td>NA</td>
<td></td>
<td></td>
<td>Local excision+PLND+CTx+RTx</td>
<td>NED for 15 months</td>
<td></td>
</tr>
</tbody>
</table>

NED: no evidence of disease; DOD: dead of disease; F/U: follow-up; NA: not stated; CTx: Chemotherapy; RTx: Radiation therapy; TAH: Total abdominal hysterectomy; RH: Radical hysterectomy; BSO: Both salpingo-oophorectomy; PLND: Pelvic lymph node dissection.

* The patient had an 8 cm in size cervical mass.

Reference


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Synchronous primary endometrial and fallopian tube cancers

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Summary

Background: Synchronous primary cancers are relatively uncommon in the general population. We present a case of synchronous primary endometrial and fallopian tube cancers and review the literature. Case: The patient, a 54-year-old, gravida 2, para 2 postmenopausal Greek woman presented with a complaint of abnormal vaginal bleeding. She underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and total omentectomy. The histopathology revealed synchronous primary cancers of the endometrium and right fallopian tube. The patient underwent postoperative chemotherapy and postoperative radiotherapy. She remains well without evidence of disease, 65 months after initial surgery. Conclusion: The reason for the better median overall survival of patients with synchronous primary endometrial and fallopian tube cancers is not intuitively obvious. Perhaps it is due to the detection of patients at earlier clinical stage and lower grade disease state.

Key words: Synchronous primary cancers; Endometrial cancer; Fallopian tube cancer.

Introduction

Synchronous primary cancers are relatively uncommon in the general population. The etiology and pathogenesis of this phenomenon remains unclear [1, 2]. It has been postulated that embryologically similar tissues, when simultaneously exposed to hormonal influences or to carcinogens, may develop synchronous cancers [1, 2]. The occurrence of synchronous primary endometrial and fallopian tube cancers is very rare, with only a few cases documented in the literature so far [2-4]. We present a case of synchronous primary endometrial and fallopian tube cancers and review the literature.

Case Report

The patient, a 54-year-old, gravida 2, para 2 postmenopausal Greek woman presented with a complaint of abnormal vaginal bleeding. Her past surgical history was unremarkable. Her family history revealed no evidence of cancer among the first-degree relatives.

On gynecologic examination there was a palpable pelvic mass in the right adnexa. There were no palpable inguinal lymph nodes and the rest of the pelvic examination was normal.

Preoperative computed tomography (CT) of the abdomen and pelvis, and abdominal ultrasound (US) revealed an intraabdominal mass 5 x 3.5 cm in the right adnexa. The endometrium had a width of 9 mm and a monolayer appearance. Preoperative CT of the chest, chest X-ray, intravenous pyelography (IVP), colonoscopy and urethrocystoscopy were normal. Preoperative CA-125 was elevated at 71.6 U/ml.

On exploratory laparotomy, the right fallopian tube was markedly distended, measuring 5 x 3.5 cm. Frozen section showed malignancy and the patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and total omentectomy.

Histopathology revealed synchronous primary cancers of the endometrium and right fallopian tube. The endometrial cancer was adenocarcinoma endometrioid type and invaded less than one-half of the myometrium. The fallopian tube cancer was adenocarcinoma papillary serous type and it was limited to the right fallopian tube without penetrating the serosal surface. The peritoneal washing smear was negative for malignant cells. The final diagnosis was Stage Ib endometrial adenocarcinoma endometrioid type and Stage Ia fallopian tube adenocarcinoma papillary serous type.

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

Follow-up 65 months after initial surgery with CT of the chest, abdomen and pelvis, abdominal US, chest X-ray, IVP, colonoscopy and urethrocystoscopy showed no evidence of recurrence.

Discussion

The occurrence of synchronous primary endometrial and fallopian tube cancers is very rare, with only a few cases documented in the literature so far [2-4]. Patients are usually postmenopausal, obese and nulliparous [4]. The main symptoms are abdominal pain, vaginal bleeding and palpable pelvic mass [3, 4]. In our case, the patient was postmenopausal and presented with abnormal vaginal bleeding.

The theory of the “secondary Müllerian system” was proposed to explain the observation of multiple similar cancers in the female genital tract [1, 5]. According to this theory, epithelia of the cervix, uterus, fallopian tubes, ovaries and peritoneal surfaces simultaneously respond to a carcinogenic stimulus [1, 5]. Shared hormonal receptors (estrogen receptors) may be responsible for the development of multiple primary malignancies in predisposed tissue [2, 6].
It is also possible that the synchronous presence of these cancers is an indicator of an etiologically distinct condition [7]. Perhaps patients have a more fragile genome and prior genetic damage may predispose them to synchronous cancers [7-9]. Thus, embryologic, hormonal or other phenomena may be associated with the development of malignancies arising simultaneously in genital tissues [1, 2, 5-9].

Synchronous primary cancers may have a similar appearance or may be of different histologic types [2-4]. The distinction between metastatic and synchronous primary cancers is relative easy when they have different histologic types. In our case, we had endometrial adenocarcinoma endometrioid type and fallopian tube adenocarcinoma papillary serous type.

Endometrial cancer usually produces early symptoms, and is diagnosed in over 70% of patients when it is still confined to the uterus [2]. In patients with synchronous primary endometrial and fallopian tube cancers, a clinically silent cancer of the fallopian tube was diagnosed earlier because of the symptomatic endometrial cancer. This may account for the more favorable outcome in these patients [2]. It is also possible that synchronous primary cancers may have an inherently more favorable prognosis, because they are relatively low grade and biologically less aggressive [2].

Because of its rarity, the optimal therapeutic strategy for synchronous primary endometrial and fallopian tube cancers has not been well defined. Treatment of choice of early stage synchronous primary cancers is total abdominal hysterectomy with bilateral salpingo-oophorectomy and total omentectomy [4]. In advanced stage, patients require more aggressive management with adjuvant chemotherapy or radiotherapy after surgery [4].

The reason for the better median overall survival of the patients with synchronous primary endometrial and fallopian tube cancers is not intuitively obvious. Perhaps it is due to the detection of patients at earlier clinical stage and lower grade disease state [2, 4, 10]. More extensive clinical research must be performed to have definite etiologic, diagnostic and management modalities.

References

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Isolated osseous ovarian metaplasia: Case report

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Introduction

Some ovarian metaplasias may contain bone or osteoid tissue. The most common tumors presenting these alterations are teratomas and mixed mesodermal tumors with heterologous elements. Osseous metaplasia has been described in a case of mucinous cystadenoma [1], thecoma [2], cysto-adenocarcinoma [3], endometriosis [4] and Sertoli-Leydig cell tumor [5]. In the female genital tract, osseous metaplasia has been described in tumors of the endometrium and tubes [6].

The relevant aspect of the present report is that it is the first description of ovarian osseous metaplasia not accompanied by other concomitant ovarian pathological entities.

Case Report

A black 31-year-old nulligravida patient with diabetes mellitus (DM) since the age of 15 years was followed-up in our service because of oligomenorrheic cycles due to chronic anovulation caused by ovarian hypofunction secondary to DM. During ultrasonography examination indicated because of oligomenorrheic cycles, a solid hypoechogenic ovarian image with calcifications was identified on the right, suggestive of a dermoid cyst. All serum tumor markers, including CA125, CEA, α-fetoprotein and β-HCG, were normal. The patient was submitted to surgical laparoscopy for exeresis of the mass, with salpingo-oophorectomy being performed on the right. Macroscopically, the ovary was enlarged, with a smooth surface and hardened. Histological evaluation revealed osseous metaplasia in the ovarian stroma, with the adjacent parenchyma presenting primordial follicles and a corpus albicans, with no other abnormalities (Figure 1).

Discussion

The present case appears to be the first description of isolated ovarian ossification. Five cases of ovarian osseous metaplasia have been described previously, all of them associated with other abnormalities. Morizane et al. reported ossification of a luteinized thecoma with an extensive area of calcification [2] while Mukonoweshuro and Oriowolo reported an endometrioid adenocarcinoma associated with benign stromal osseous ovarian metaplasia [3]. Badawy et al. described a supernumerary ovary with an endometrioma and osseous ovarian metaplasia [4] and Mooney et al. reported ossification of a well-differentiated Sertoli-Leydig cell tumor [5]. Finally, Mislevich and Boss described metaplastic bone in a mucinous cystadenoma of the ovary [1].

Summary

Background: Some ovarian metaplasias may contain bone or osteoid tissue. The most common tumors presenting these alterations are teratomas and mixed mesodermal tumors with heterologous elements. Case report: We report the case of a woman who, during gynecologic follow-up for chronic anovulation at the age of 31 years, presented a solid ovarian ultrasonographic image with calcifications. After laparoscopy and histological examination it was found to be an isolated ovarian osseous metaplasia. Conclusion: A rarely occurring condition, ovarian osseous metaplasia continues to be of uncertain clinical significance.

Key words: Osseous metaplasia; Ovary; Laparoscopy.
The pathogenesis of osseous metaplasia has not been clarified but, under certain circumstances, it seems to be an uncommon reaction to tissue aggression in an attempt at repair. Up to now, osseous metaplasia appeared to follow this same pattern in the ovary. However, the present finding of osseous metaplasia in one ovary without association with other abnormalities led us to question whether this is really the mechanism by which this tissue reaction is stimulated.

In the present case, tissue aggression may have been due to excessive ovarian stimulation by circulating hyperinsulinemia leading to altered androgen production. As described, the patient had a diagnosis of ovarian hypofunction secondary to DM, which is a disease characterized by autoimmune aggression of the endocrine system of the pancreas resulting in the abolition of insulin secretion.

**Conclusion**

Ovarian osseous metaplasia associated or not with other ovarian changes is rare and its histogenesis continues to be unknown. To our knowledge, this process has no pathological prognosis or significance.

**References**


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A case of ovarian endometrioid adenocarcinoma with yolk sac tumor in a 35-year-old woman

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Summary
Ovarian yolk sac tumor (YST) is a malignant ovarian neoplasm differentiated from primordial germ cells that occur in young age, while endometrioid carcinoma (ECA) is a müllerian epithelial tumor that usually occurs in older patients. The coexistence of an ovarian ECA and YST component is very rare. Only 12 cases have been reported until now according to a Medline search of the English literatures. We present a case of a simultaneous ECA and a YST component in a 35-year-old woman. Exploratory laparotomy was performed. The parts of both ovaries that showed an endometrioid-like glandular pattern were positive for cytokeratin 7 and negative for AFP, but the YST component was negative for cytokeratin 7 and positive for AFP. After completion of four courses of BEP chemotherapy, two courses of taxane and carboplatin chemotherapy were added. The patient failed to respond and succumbed to the disease after 12 months of follow-up.

Key words: Endometrioid adenocarcinoma, Yolk sac tumor; Endometriosis.

Introduction
Ovarian yolk sac tumor (YST) is a malignant ovarian neoplasm differentiated from primordial germ cells that occur in young age, while endometrioid carcinoma (ECA) is a müllerian epithelial tumor that usually occurs in older patients. These two diseases have different origins in the malignant differentiation of tumor and different onset ages. The coexistence of ovarian ECA and a YST component is very rare: only 12 cases have been reported until now according to a Medline search of the English literatures (Table 1). A case of a simultaneous ECA and YST component in a 35-year-old woman is reported together with a review of the literature.

Case Report
An 8-cm ovarian tumor was found in a 35-year-old female, gravida 4, para 2, during a health examination. She was advised to undergo surgery but refused. One year later, she visited our hospital again due to a large amount of ascites. She had severe abdominal distension on physical examination and an abdominal mass was palpated.

The patient was found to have a huge pelvic mass which was mixed with cystic and solid portions on magnetic resonance imaging (MRI). Preoperative pigtail catherization drained 4,500 ml of ascites. Cytology was positive for malignant cells which were suspicious of metastatic adenocarcinoma.

Preoperative CA 125 was 137 U/ml (normal < 35 U/ml). Alpha-fetoprotein was not evaluated.

Exploratory laparotomy was performed. A total abdominal hysterectomy, bilateral salpingo-oophorectomy, partial omentectomy, and removal of multiple intraabdominal lesions were carried out. Both ovaries were replaced by tumors, more than 8 cm in size. The ascitic fluid (2,500 ml) was of serous nature, and positive for malignant cells. The pathologic report demonstrated ECA with a YST component, and extraovarian spread (Stage IIIC) (Figure 1). After surgery, chemotherapy including bleomycin, etoposide, and cisplatin (BEP) was followed on the tenth postoperative day.

After completion of four courses of BEP chemotherapy, abdominal computed tomography (CT) showed an aggravated state of ascites and peritoneal and omental infiltration.

The chemotherapy regimen was changed to taxane and carboplatin. Two courses were performed, but there was no response. The clinical symptoms and ascites were aggravated. There was a third change of chemotherapy with ifosfamide and cisplatin. The patient failed to respond and succumbed to the disease on July 2007.

Both ovarian tumors contained two distinct histological components. Most were endometrioid carcinomas and some were yolk sac tumors. The parts of both ovaries that showed an endometrioid-like glandular pattern were positive for cytokeratin 7 (CK 7) and negative for AFP, but the YST component was negative for CK 7 and positive for AFP. After completion of four courses of BEP chemotherapy, these slides were reviewed by six pathologists from two institutes. Microscopically, the tumors that were transferred to the omentum and pelvic wall were metastatic ECA with a YST component. These slides were reviewed by six pathologists from two institutes.

Discussion
Since a case of ovarian YST arising from an ECA was first reported in 1987, 12 cases of ovarian ECA with a YST component have been reported up to now [2-9]. There were three reported cases of ovarian epithelial mucinous tumor with YST, one of which was accompanied by ECA [2, 10, 11]. A case of malignant ovarian tumor composed of endometrioid adenocarcinoma, clear cell adenocarcinoma, squamous cell carcinoma, yolk sac tumor and immature teratoma with prominent neuroectodermal and rhabdomyosarcomatous differentiation was reported [12]. The combination of an endometrioid tumor with mesothelial origin and yolk sac tumor with early...
endodermal origin is very rare, and its accurate pathogenesis is unknown.

Proposed theories include the teratoma theory, redifferentiation, collision theory, neometaplasia, and simultaneous differentiation. The most probable is the neometaplastic theory. The neoplasms that reproduce extraembryonal tissues such as trophoblasts and yolk sac tumors do not always have a teratoid or germ cell origin. Although rare, these extraembryonal tissues originate from preexisting somatic cell tumors by an unknown pathogenesis. They are mainly generated in the upper aerodigestive tract, lung, stomach, pancreas, colon, urachus, and urothelium. A secondary trophoblastic pattern and YST can develop from somatic müllerian lesions in the female genital tract as well, though it is rare [3].

There is no consensus about the effective therapy for this rare tumor. Cisplatin-based chemotherapy, which
A case of ovarian endometrioid adenocarcinoma with yolk sac tumor in a 35-year-old woman

was used as an adjuvant treatment in the earlier reports after surgery, had no effect. Kamoi et al. tried to prevent dissemination of tumors by intraperitoneal chemotherapy with carboplatin. They conducted BEP chemotherapy to control the YST component which has a much worse prognosis than pure YST. The patient was still alive after 21 months of follow-up without recurrence [5]. After that report, other studies used BEP for postoperative chemotherapy and added cisplatin-based chemotherapy or a combination of taxane and carboplatin to control endometrioid tumors [8, 9, 13]. Abe et al. conducted three courses of the combination of taxane and carboplatin after three courses of BEP chemotherapy. The patient has been clinically free of tumor for 20 months [9]. This report seemed to show that taxane and carboplatin combination therapy after BEP chemotherapy could be an effective treatment. However we did not experience any response to this protocol in the present case.

When we review the survival rate of patients according to the change of chemotherapy regimen, the survival rate increased somewhat after the use of BEP although there was not a big difference. An effective regimen or another effective adjuvant therapy to be combined with the BEP chemotherapy is needed.

Among the 13 cases including the present case, endometriosis was accompanied in eight cases. The previous reports suggested the possibility of malignant transformation of endometriosis to ovarian clear cell carcinoma and ECA. However, little is known about the

<table>
<thead>
<tr>
<th>Report</th>
<th>Age</th>
<th>Stage</th>
<th>Endometriosis</th>
<th>CTx* / Course</th>
<th>AFP / CA125</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987 (Rutgers et al.) [2]</td>
<td>50</td>
<td>Ia</td>
<td>Y</td>
<td>MOC X 3 VP X 1</td>
<td>720 (postop) / NA</td>
<td>DOD 8 mos</td>
</tr>
<tr>
<td>1996 (Nogales et al.) [3]</td>
<td>64</td>
<td>Ia</td>
<td>–</td>
<td>MOC X 3</td>
<td>&gt; 300 (postop) / NA</td>
<td>DOD 14 mos</td>
</tr>
<tr>
<td>2002 (Kamoi et al.) [5]</td>
<td>54</td>
<td>Ic</td>
<td>Y</td>
<td>Ca (ip) BEP X5</td>
<td>13,143 / 170</td>
<td>Alive 21 mos</td>
</tr>
<tr>
<td>2008 (Abe et al.) [9]</td>
<td>52</td>
<td>Ic</td>
<td>Y</td>
<td>BEP X 3 TCa X 3</td>
<td>24,518 / 8,439</td>
<td>Alive 20 mos</td>
</tr>
<tr>
<td>2009 (current)</td>
<td>35</td>
<td>IIIc</td>
<td>Y</td>
<td>BEP X 4 TCa X 2 IP X 1</td>
<td>NA / 137</td>
<td>DOD 12 mos</td>
</tr>
</tbody>
</table>

†: postop postoperative, ‡: NA none available, §: DOD: died of disease.
pathogenesis of malignant formation in endometriosis [13]. The fact that ovarian cancer and adjacent endometriotic lesions share genetic alterations such as PTEN, p53, and bcl gene mutation and that cytokine secreted causes responses from adjacent cells which are similar to a malignant mechanism suggests the possibility of the transformation of endometriosis [14].

The likelihood of accompaniment with ovarian cancer increases if endometriosis is large or discovered in the menopausal period [15]. Even though the malignant transformation of endometriosis into clear cell and ECA is accepted as true, there is as yet no report on the relationships between endometriosis and yolk sac tumor.

In conclusion, even though the number of reports is small, we will have to find an effective protocol by revealing the histological and biological features of the past cases of ECA with a YST component. Additionally, more studies are needed on the relationships of endometriosis with ovarian cancer and YST.

References


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Vaginal hysterectomy aided with surgery by the abdominal approach as a method of hysterectomy with salpingo-oophorectomy due to endometrial carcinoma in a woman with morbid obesity. Case report

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Introduction

Endometrial carcinoma is the fourth most frequent malignant neoplasm among women in Poland. Most often this refers to women in postmenopausal age in whom the most important risk factors are fat consumption, obesity (BMI ≥ 25 kg/m²) and use of unbalanced estrogen therapy. Other factors include lack of physical activity, a high-calorie diet, arterial blood pressure above 140/90 mmHg and high concentrations of glucose in the blood. The basic treatment in cases of endometrial carcinoma is surgery including hysterectomy with salpingo-oophorectomy and complete interoperative assessment of the development degree of the disease. Basic operational treatment is difficult as far as obese women are concerned (BMI ≥ 50 kg/m²). This is linked with poor access to operated tissues and limited visibility, mainly in the area of the bottom of the pelvis minor. Our 69-year-old patient was admitted to and operated on at the Gynecological Department due to endometrial carcinoma. Because of her giant obesity, BMI – 51.30 kg/m², surgery by the abdominal approach was very difficult to perform, so vaginal hysterectomy with salpingo-oophorectomy was carried out.

Key words: Endometrial carcinoma; Vaginal hysterectomy; Giant obesity.

Case Report

The patient, aged 69, was admitted to the Gynecological Department on November 7, 2007 because of the earlier diagnosed endometrial carcinoma.

In October 2007 due to the occurrence of bleeding after menopause and thickened (18.6 mm) endometrium at the transvaginal ultrasonographic examination (TVS), hysteroscopy was conducted. During the endoscopic examination a significantly thickened, uneven and hyperemic endometrium and hyperemic endocervix were found. The result of the histopathological examination of the collected material from the cervical canal and uterine cavity was as follows: endometrial adenocarcinoma. When interviewed, the patient did not report any ailments or systemic diseases. She has been operated on ten years before for cholecystectomy due to cholecystolithiasis.

The first menstruation was at the age of 14 with regular cycles every 28 days, lasting three to four days. The patient had given birth twice (the second delivery was with a forceps). The last menstruation was at the age of 55. There was no remarkable family history.

Physical examination showed morbid obesity, BMI – 51.30 kg/m² (height 141 cm, weight 102 kg, circumference of the abdomen 130 cm, thickness of fatty tissue 21 cm). Efficient circulation and respiration were noted.

The results of the diagnostic examinations were within normal limits.

TVS examination showed the body of the uterus in anteflexion measuring 44.5 x 39 mm. The central part of the body of the uterus was filled with a heteroechoic change of uneven contour measuring 19.7 x 14.4 mm. The muscular layer on the back wall of the uterus was 5.5 mm thick, while the muscular layer on the front wall was 10 mm thick. The echostructure of both appendages appeared normal but the examination was difficult to carry out due to morbid obesity.
The patient did not agree to be moved to a higher level health care facility. Due to life indications a decision was made to begin surgical treatment and the patient was qualified for hysterectomy with salpingo-oophorectomy by the abdominal approach.

**Description of the surgery:** the abdomen was opened by a longitudinal cut parallel to the medial body line. Due to morbid obesity and very difficult operational conditions, the surgical team retracted from removing the lymph nodes. Hysterectomy with salpingo-oophorectomy led to the stage of ligation and amputation of infundibular-pelvic ligaments in a typical way. Due to the lack of possibility to continue the surgery via the abdominal approach, a decision was made to finish the operation via the vaginal approach. After preparing the surgical area, the following was found during the specimen examination: the vagina was long and regressive, while the cervix was small, high-located, and unattainable. Vaginal hysterectomy with a suture laid around the vagina was performed. After that, peritomization was carried out from the side of the abdominal cavity. A redon drain was inserted in the recto-uterine fold. Layer suturing of the abdominal integuments was done and a drain was placed in subcutaneous tissue. Urine in the catheter was clear. The patient’s general condition after the surgery was good.

The postoperative course with difficult healing of wounds was related to obesity. The patient was discharged from hospital on the 11th day after surgery in good general condition with a recommendation of periodic controls in the Gynecological Department.

Two weeks later the final result of the histopathological test was obtained: adenocarcinoma of the endometrium, partially tubular and partially papillary. Predominating tissue with differentiation (G1), but locally fields of differentiation (G2) were found. Malignant infiltration included over half of the thickness of the myometrium and passed to the cervical canal, (pT2b). The parametrium and appendages were free from malignant infiltration.

The patient was consulted in the Oncological Clinic about continuing treatment. Currently, she is in good condition and is subject to periodic oncological controls.

**Discussion**

Surgical treatment of endometrial carcinoma includes, apart from hysterectomy with salpingo-oophorectomy and removal of the vaginal vault, removal of the pelvic and parietal pelvic lymph nodes as a method of surgical assessment of the progression of endometrial carcinoma. Lymphadenectomy has a therapeutic meaning, which is proven by the results of retrospective tests [14-16], but also a diagnostic meaning that enables the assessment of the necessity and scope of the postoperative treatment. Such a proceeding is recommended in the majority of patients suffering from endometrial carcinoma but it has not been commonly accepted [17, 18]. Many researchers claim that as far as women with a low level of progression of carcinoma are concerned, an operation without lymphadenectomy should be carried out because these women are unnecessarily exposed to possible complications and death after radical surgery. Factors that qualify patients to a low-risk group include limitation of the neoplasm to the body of the uterus, histological malignancy degree 1 or 2, endometrial subtype of the neoplasm and infiltration of the myometrium not exceeding 50% [12, 19, 20]. The assessment of these factors should be carried out interoperationally and, according to Mariani et al. [21], in women from the low-risk group, infiltration of lymph nodes or relapse of the disease are not found. In cases of more advanced disease further treatment is required depending on the degree of progression, e.g., radiotherapy or chemotherapy. Currently there are recommendations to base the postoperative treatment on the characteristics of the tumor, defining possibilities of spread of the neoplasm and not on a traditional method [22].

The situation looks different in women who cannot be operated on due to other medical contraindications. These women are recommended to have full radiotherapy, which enables a satisfactory local control of the tumor and 5-year survival rate.

Basic operational treatment of endometrial carcinoma is difficult as far as obese women are concerned (BMI ≥ 50 kg/m²). This is linked to bad access to operated tissues and limited visibility, mainly in the area of the bottom of the pelvis minor. Hysterectomy with salpingo-oophorectomy and removal of the vaginal vault, and all the more removal of the pelvic lymph nodes, were very difficult in the case of our patient and required the operation to be finished via the vaginal approach. Macroscopic and palpable assessment of the organs and lymph nodes, lack of possibility of a precise interoperationally assessment of the progression of the neoplasm as well as exceptionally difficult conditions caused by obesity made the operational team retract from the removal of the lymph nodes. Due to the fact that the final result of the histopathological examination indicated Stage IIb of progression according to FIGO and the lymph nodes were not removed, the patient was treated as a patient from the non-operational group and full radiotherapy treatment was recommended.

**References**


Vaginal hysterectomy aided with surgery by the abdominal approach as a method of hysterectomy with salpingo-oophorectomy etc.


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