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Clinical outcomes of adjuvant chemotherapy and vaginal brachytherapy with or without pelvic radiation for surgical Stage I-II uterine serous carcinoma

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Summary

Objective(s): To evaluate the benefit of adding pelvic radiation treatment (EBRT) to vaginal cuff brachytherapy (VB) for women with early stage uterine serous carcinoma (USC) treated with adjuvant chemotherapy.

Materials and Methods: After institutional review board (IRB) approval, the authors retrospectively identified 56 patients with 2009 International Federation of Gynecology and Obstetrics (FIGO) Stage I-II USC treated with hysterectomy, bilateral salphingo-oophorectomy, and radiation therapy with either VB alone (n = 33) or VB + EBRT (n = 23) between July 1998 and August 2009.

Results: Median age and follow-up were 68.5 years and 54 months respectively. Median VB alone surface dose was 37.5 Gy and median pelvic EBRT dose was 45 Gy. The prevalence of lower uterine segment involvement, >50% myometrial invasion, and Stage II disease were higher for patients receiving VB+EBRT. Overall, only one vaginal recurrence was observed. Pelvic recurrence rate was 26% for VB + EBRT compared to 12% for VB alone (p = 0.179). The five-year recurrence-free survival (RFS) was 80.5% for VB vs 67.3% for VB + EBRT (p = 0.3847), and the five-year overall survival (OS) was 65.9% for VB vs 66.7% for VB + EBRT (p = 0.7159). On univariate and multivariate analysis, radiation treatment modality was not a predictor for local control or survival.

Conclusions: In this cohort, there was no significant clinical benefit of adding pelvic EBRT to the adjuvant management of early stage uterine serous carcinoma. The higher prevalence of high-risk features in the VB + EBRT group may underestimate the value of this treatment. Further investigation is warranted to identify the optimal radiation treatment regimen for early stage USC treated with surgery and adjuvant chemotherapy.

Key words: Endometrial carcinoma; Serous; Brachytherapy; Adjuvant; Radiation treatment.

Introduction

Endometrial cancer is the fourth most common malignancy in females and the most common gynecological malignancy in the United States with an estimated number of 43,470 cases and 7,950 deaths in 2010 [1]. Although most patients with endometrial cancer will not die from their disease, some subsets of patients are at much higher risk for recurrence and death. USC is a relatively rare subset of endometrial cancer representing less than 10% of all cases, but accounts for a disproportionate 39% of the total uterine cancer deaths [2]. Its aggressive nature is manifested by its propensity for deep myometrial invasion, extensive vascular space invasion, and early metastasis to lymph nodes with approximately 60 to 70 percent of women with USC having disease spread outside of the uterus at the time of presentation [3, 4]. These features of USC lead to high recurrence rates and poor prognosis, and only a relatively small portion of patients with early stage disease (Stage I-II) [3, 4].

Due to the infrequency of early-stage disease, there is a lack of randomized evidence to support optimal adjuvant management in this setting, and most recommendations are based on small retrospective series. In this light, it is generally accepted that all patients with USC should undergo comprehensive surgical staging, which includes a total hysterectomy, bilateral salphingo-oophorectomy, pelvic washings, and pelvic and para-aortic lymphadenectomy ± omentectomy [5-7]. A high risk of distant recurrence led to the use of adjuvant platinum-based chemotherapies, which have been shown to improve recurrence rates, progression-free, and overall survival [6, 8, 9].

Several studies have shown the benefit of adjuvant radiation treatment (RT) for patients with early stage USC [5, 6, 10-12]. A recent Surveillance Epidemiology End Results (SEER) study showed a survival benefit for adjuvant radiation after surgery for patients with early stage USC, which was most pronounced in patients with more than 50% myometrial involvement [12]. However, the optimal treatment volume and technique of adjuvant RT is less defined. Various RT volumes include vaginal brachytherapy alone [5, 9, 11, 13, 14] EBRT to the pelvis [6, 10, 15] combination of VB + EBRT [6, 7, 10] and whole abdominal RT incorporating pelvic boost, with or without vaginal brachytherapy [6, 7, 15].
The purpose of this study was to compare the outcomes of patients with early stage USC treated with surgery followed by adjuvant chemotherapy and RT, with either vaginal brachytherapy alone or in combination with pelvic external beam RT, and to determine the potential benefits, if any, of adding pelvic external beam RT to the adjuvant management of these patients.

Materials and Methods

Following approval from the IRB, this prospectively-maintained database of 1,280 patients with uterine carcinoma and the database of Karmanos Cancer Center were examined to identify patients with 2009 FIGO Stage I-II uterine serous carcinoma. This group was further refined to include only patients who had hysterectomy, bilateral oophorectomy ≥ lymphadenectomy, ≥ omentectomy, adjuvant chemotherapy, and RT. Patient demographics, surgical-pathological information, clinical data, and follow-up details were obtained for the Institutions’ computerized medical record system.

Surgical staging included: total abdominal hysterectomy (TAH), bilateral salpingo-oophorectomy (BSO), selective pelvic and para-aortic lymph node sampling with dissection of suspicious nodes, ≥ omentectomy, and peritoneal cytology. All surgeries were performed by gynecologic oncologists.

On pathological evaluation, the following factors were assessed: tumor grade, depth of myometrial invasion (< 50% vs ≥ 50%), angiolymphatic space invasion (ALI), lower uterine segment involvement (LUSI), and number of lymph nodes dissected. All cases met the microscopic criteria for USC according to World Health Organization (WHO) pathology manual [16] and were reviewed and confirmed by one gynecologic pathologist (RA). No patients with mixed tumors were included in this study.

Adjuvant treatments included both chemotherapy and RT. Chemotherapy was three-six cycles of carboplatin and paclitaxel regimen given adjuvantly after hysterectomy with the number of cycles determined by the discretion of the gynecologic oncologist.

All patients received adjuvant RT in the form of VB alone or with additional pelvic external beam RT (VB + EBRT). RT was in the form of VB delivered using 192-Ir high dose rate (HDR). A median total surface dose of 37.5 Gy was delivered in five to six fractions, one to two fractions per week. The target volume treated was the upper four cm of the length of the vagina. The treatment was delivered using a single-channel cylinder that was connected to a HDR 192-Ir microSelectron (Nucletron, Veenendaal, Netherlands). The diameter of the cylinders used ranged from three to 3.5 cm (median, 3.5 cm). The median dose per fraction for VB alone was 7.5 Gy (range, 6 - 7.5 Gy). The dose was prescribed to the surface of the cylinder. Dose optimization was used in all the patients to ensure uniform dose distribution at depth and to eliminate the potential dose reduction at the vaginal apex because of source anisotropy. A Foley catheter was not used during these procedures. The bladder and rectal doses were not calculated. Pelvic external beam radiation consisted of standard 4-field box technique with a median dose of 45 Gy (range, 44-50.4), 1.8-2.0 Gy per fraction with daily treatments over five to six weeks.

After completion of adjuvant treatment, patients were regularly followed-up with clinical examination, Pap smear, and appropriate imaging studies. Survival curves were generated according to Kaplan-Meier product-limit method calculated from the date of hysterectomy. These were compared using the log-rank test to determine the rates of local control, Recurrence-Free Survival (RFS), Disease Specific Survival (DSS), and Overall Survival (OS). Cox regression analysis was used to explore relationships between various factors and outcomes using both univariate (UNA) and multivariate (MVA) models. The Fischer exact and χ² tests were used to determine significant differences (p < 0.05) in demographics, patient factors, and tumor characteristics between the VB and VB + EBRT groups.

Results

The authors identified 56 patients with pathologic Stages I-II uterine serous carcinoma treated at the Institutions that had surgery from July 1998 to August 2009. Table 1 shows the demographic, pathological, and recurrence compar-

Table 1. — Patient characteristics between treatment groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>VB alone (n = 33)</th>
<th>VB + EBRT (n = 23)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>10 (30%)</td>
<td>10 (43%)</td>
<td>0.311</td>
</tr>
<tr>
<td>African American</td>
<td>23 (70%)</td>
<td>13 (57%)</td>
<td></td>
</tr>
<tr>
<td><strong>Median follow-up (months)</strong></td>
<td>54.0 (range 18 - 151)</td>
<td>56.0 (range 19 - 134)</td>
<td>0.686</td>
</tr>
<tr>
<td><strong>Median age</strong> 64.0 (range 59 - 83)</td>
<td>69.0 (range 59 - 81)</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 60</td>
<td>13 (39%)</td>
<td>3 (13%)</td>
<td>0.043</td>
</tr>
<tr>
<td>61-70</td>
<td>7 (21%)</td>
<td>11 (48%)</td>
<td></td>
</tr>
<tr>
<td>≥ 71</td>
<td>13 (39%)</td>
<td>9 (39%)</td>
<td></td>
</tr>
<tr>
<td><strong>Cancer Stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>31 (94%)</td>
<td>14 (61%)</td>
<td>0.002</td>
</tr>
<tr>
<td>II</td>
<td>2 (6%)</td>
<td>9 (39%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 50% myometrial invasion</td>
<td>3 (9%)</td>
<td>7 (30%)</td>
<td>0.040</td>
</tr>
<tr>
<td>Lower uterine segment invasion</td>
<td>8 (24%)</td>
<td>3 (13%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Angiolymphatic invasion</td>
<td>11 (33%)</td>
<td>6 (26%)</td>
<td>0.562</td>
</tr>
<tr>
<td>Median # of lymph nodes removed</td>
<td>8 (range 0 - 21)</td>
<td>8 (range 0 - 30)</td>
<td></td>
</tr>
<tr>
<td>No lymph nodes removed</td>
<td>9 (27%)</td>
<td>5 (22%)</td>
<td>0.641</td>
</tr>
<tr>
<td>1 - 9 lymph nodes removed</td>
<td>13 (39%)</td>
<td>7 (30%)</td>
<td>0.496</td>
</tr>
<tr>
<td>≥ 10 lymph nodes removed</td>
<td>11 (33%)</td>
<td>11 (48%)</td>
<td>0.067</td>
</tr>
<tr>
<td>Median # of paraaortic lymph nodes removed</td>
<td>2 (range 0 - 6)</td>
<td>2 (range 0 - 4)</td>
<td></td>
</tr>
<tr>
<td>Omentectomy</td>
<td>22 (67%)</td>
<td>16 (70%)</td>
<td>0.234</td>
</tr>
<tr>
<td>Treatment-related toxicity (diarrhea ≥ Grade II)</td>
<td>4 (12%)</td>
<td>4 (12%)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 2. — Patterns of failure by treatment and stage.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Overall recurrence</th>
<th>Vaginal</th>
<th>Pelvic</th>
<th>Distant</th>
<th>Death from disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>VB alone (n = 31)</td>
<td>5 (16%)</td>
<td>1 (3%)</td>
<td>3 (10%)</td>
<td>5 (16%)</td>
</tr>
<tr>
<td></td>
<td>VB + EBRT (n = 14)</td>
<td>1 (7%)</td>
<td>0 (0%)</td>
<td>1 (7%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>II</td>
<td>Overall (n = 45)</td>
<td>6 (13%)</td>
<td>1 (2%)</td>
<td>4 (9%)</td>
<td>6 (13%)</td>
</tr>
<tr>
<td></td>
<td>VB alone (n = 2)</td>
<td>1 (50%)</td>
<td>0 (0%)</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td></td>
<td>VB + EBRT (n = 9)</td>
<td>6 (66%)</td>
<td>0 (0%)</td>
<td>5 (56%)</td>
<td>4 (44%)</td>
</tr>
<tr>
<td></td>
<td>Overall (n = 11)</td>
<td>7 (64%)</td>
<td>0 (0%)</td>
<td>6 (55%)</td>
<td>5 (45%)</td>
</tr>
</tbody>
</table>

VB = vaginal brachytherapy; VB + EBRT = vaginal brachytherapy with pelvic external beam radiation treatment.
isons between these groups. The median follow-up time for all patients was 54 months (range 18 - 151 months) and the median age was 68.5 years (range 48 - 91 years). African American comprised 63% of patients. The median number of examined lymph nodes was 8 (range 0 - 30).

For the entire cohort of patients, the five-year clinical outcomes were as follows: recurrence-free survival 74.0%; local-regional control 79.6%; disease-specific survival 77.4%; and overall survival 63.4%. Thirty-three (59%) of the patients received VB alone and twenty-three (41%) received VB + EBRT. Patients in the VB + EBRT group were more likely to have Stage II disease (p = 0.002), lower uterine segment involvement (LUS) (p = 0.006), ≥ 50% myometrial invasion (MI) (p = 0.04), and older age (p = 0.043).

Figure 1 shows the Kaplan-Meier plots for survival outcomes for patients who received VB alone compared to those who received VB + EBRT. The five-year local regional control was 87.1% for VB and 71.8% for VB + EBRT. The five-year RFS was 80.5% for VB versus 67.3 for VB + EBRT. In regards to DSS, the estimated five-year rate was 78.9% for VB alone compared to 78.9% for V, and the five-year OS rates was 65.9% for VB versus 66.7% for VB + EBRT. These are shown in Table 2. Statistically significant differences were not detected between treatment groups for any of the outcome parameters (local control, RFS, DSS, and OS).

As expected, patients with Stage I disease had superior outcomes compared to Stage II patients. Stage II patients had significant worse five-year recurrence-free survival rates (30% vs 85.6%, p = 0.001), five-year local-regional control rates (35.0% vs 90.6%, p = 0.001), and a trend towards worse DSS (54.5% vs 84.8%, p = 0.063) compared to Stage I patients, but differences in OS were not statistically different (45.5% vs 71.9%, p = 0.152). While the extent of lymph node dissection did not appear to impact clinical outcomes (OS, DSS, RFS) for the entire cohort, Stage II patients with limited lymph node dissection (0 - 9) lymph nodes dissection had worse five-year DSS compared to those with 10+ lymph nodes removes (40% vs 83%, p = 0.045).

There were no significant differences between patterns of failure between treatment groups, but there were clear differences between Stage I and II. As Stage increased, so did the propensity for overall recurrence, pelvic recurrence, and distant recurrence. For example the overall recurrence rate for Stage I was 13% compared to 64% for Stage II patients (Table 2).

Univariate analyses determined that ALI, ≥ 50% MI, Stage II, and LUSI were predictive for local control, RFS, and DSS, while for OS, univariate analysis identified only angiolymphatic invasion and ≥ 50% myometrial invasion as significantly predictive. After multivariable modeling, performed on an exploratory basis, only ALI remained statistically predictive for local control, RFS, DSS and OS (Table 3). In addition, MI ≥ 50% was found to be independent predictive of OS and DSS. RT type (VB compared to VB + EBRT) was not found to be significantly predictive in this patient population for any of the observed outcomes.

### Discussion

This is the first study investigating the potential benefits of adding pelvic RT to the adjuvant treatment of patients with 2009 FIGO Stage I-II uterine serous carcinoma in the setting of adjuvant chemotherapy and VB. In this study, excellent vaginal control and good local-regional control was observed for the entire cohort, but the authors were unable to detect a difference in OS, DSS, LR control or RFS between the groups treated with VB or VB + EBRT.

In previously published studies, one of the greatest difficulties determining the role and type of adjuvant RT for early stage USC is the heterogeneity of treatments and patient characteristics existing even in a single study. Often, analysis is performed combining all types of RT modalities into one treatment group “adjuvant radiation”, [6, 7, 9, 10, 17] but this approach does not aid in the discussion about which modality and type of adjuvant RT for early stage USC is the most effective. This controversy is compounded by the relative scarcity of serous histology and the retrospective nature of most studies and creates debate defining specific success rates by treatment volume for each type of RT.

The main objective of the current study was to suggest the appropriate treatment volume for adjuvant RT (VB alone or in combination with pelvic RT) in women with early stage USC who received adjuvant chemotherapy after hysterectomy. The overall results suggest that there is no difference between RT options, but this may mis-

### Table 3. — Patterns of failure by treatment and stage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Local control</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Recurrence - free survival</td>
<td>Disease - specific survival</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>HR (CI)</td>
<td>p value</td>
</tr>
<tr>
<td>Stage II</td>
<td>0.012</td>
<td>14.9 (1.8 - 124)</td>
<td>0.009</td>
</tr>
<tr>
<td>LUSI</td>
<td>0.003</td>
<td>7.1 (2.0 - 25)</td>
<td>0.002</td>
</tr>
<tr>
<td>≥ 50% MI</td>
<td>0.050</td>
<td>3.5 (0.97 - 12.3)</td>
<td>0.022</td>
</tr>
<tr>
<td>ALI</td>
<td>0.012</td>
<td>5.2 (1.4 - 18.6)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

CI = confidence interval; HR = hazard ratio; LUSI = lower uterine segment involvement; MI = myometrial invasion; ALI = angiolymphatic space invasion.
represent the data due to the retrospective nature of this study and the fact that the two treatment groups were not equally weighted. Significantly, more patients receiving VB + EBRT had Stage II disease, LUSI, higher incidence of deep MI, and a trend towards older age. These features likely contributed to the final outcomes and may mitigate any benefit of EBRT. With this in mind, and in light of previously published reports, for patients with surgical Stage I USC treated with adjuvant chemotherapy, VB alone may suffice, but adding pelvic RT may be potentially beneficial to patients with Stage II disease or Stage I patients with adverse prognostic factors (e.g., angiolymphatic invasion, and deep MI).

In this study, only one patient had isolated vaginal cuff recurrence alone. All others with recurrences, had local recurrence as a component of distant failure. The rate of local-regional control at five years was 80% for the entire group, but rates were significantly worse in Stage II patients compared to Stage I, 35% vs 90.6% at five years respectively. Stage II patients also had increased distant metastasis with crude distant failure rates of 55% vs 13% compared to Stage I patients. This study also showed differences between Stage I and Stage II patients for RFS and local control with a trend towards worse DSS for Stage II patients.

In their series of patients treated with chemotherapy and vaginal brachytherapy, Alketiar et al. also reported worse outcomes with Stage II patients compared to Stage I patients with two out of five (40%) Stage II patients recurring opposed to only two out of 20 (10%) Stage I patients [11]. This may suggest that VB alone may not be adequate to treat Stage II patients even with chemotheraphy and surgical staging.

The authors report a five-year OS rate of 63.4% for the mixed population of Stage I and II patients. The rate in this study was lower than the 88% rate that was reported by Alektiar et al. in a small group (n = 25) of Stage I-II patients treated with surgical staging, carboplatin/paclitaxel chemotherapy and intravaginal brachytherapy, but the median follow-up in this study was almost double (54 months vs 30 months) [11]. Turner et al. reported a 95% five-year overall survival and disease-free survival in 18 patients with Stage I uterine serous carcinoma who underwent surgical staging, and adjuvant vaginal HDR brachytherapy (five received chemotherapy) and a 100% five-year overall survival for those who underwent comprehensive surgical staging [5]. Two possible explanations for the slightly worse outcomes in this study compared to Turner et al. is the lack of comprehensive surgical staging for fewer patients in this study.
and the inclusion of adjuvant chemotherapy and vaginal brachytherapy with or without pelvic radiation for surgical Stage I-II etc.

Conclusion

In this series of Stage I-II patients with USC who received adjuvant chemotherapy, clinical outcomes were similar between patients receiving adjuvant RT in the form of HDR VB alone or in combination with pelvic external beam RT, but some bias may have mitigated the benefit of additional pelvic RT. Even with adequate local treatment, distant failure remains a problem. Further work defining the most appropriate adjuvant therapies should include prospective multicenter randomized trials to better determine the type of adjuvant radiation therapies for these patients.

References


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Clinical significance of Mena and Her-2 expression in breast cancer

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Summary

Objective: The aim of this study was to determine the expression patterns of Mena and Her-2 in breast cancer tissues and to explore their clinical significance and correlation with clinicopathological parameters. Methods: The expression of Mena and Her-2 was detected in 40 breast cancer tissues and 14 normal breast tissues by immunohistochemistry, and the relationship of Mena and Her-2 expression with clinicopathological parameters was analyzed. Results: Both Mena (70%) and Her-2 (40%) were more commonly expressed in breast cancer than in normal breast tissue (7.1%, 0%, respectively; \( p < 0.05 \)), further, Mena and Her-2 expression in breast cancer were positively correlated \( (r = 0.530, p < 0.05) \). In comparing expression with clinicopathological parameters of tumor samples, Mena and Her-2 were both associated with axillary lymph node metastasis and TNM stage \( (p < 0.05) \), but not with patient age or pathological type. Conclusions: Mena and Her-2 are related to the malignancy degree and metastasis of breast cancer, and thus may play a coordinating role in the occurrence and progression of breast cancer.

Key words: Breast cancer; Mena; Her-2; Immunohistochemistry; Clinicopathological parameter.

Introduction

In many countries, the incidence of breast cancer has continued to increase in recent years, and it is commonly ranked as the most frequent malignancy in women [1-3]. Indeed, although mortality rates have declined through improved screening and treatment, this cancer still claims many lives each year [2]. Breast cancer prognosis and mortality are heavily influenced by tumor invasion and metastasis [4, 5]. Thus, the development of novel clinical prognostic indicators is crucial to improving survival rate and reducing mortality of patients with breast cancer [6]. Both diagnosis and therapy can be improved by understanding the molecular mechanisms behind tumor initiation and metastasis and by identifying factors that can estimate metastasis potential and/or alter this ability of tumors to invade other sites.

One factor of great interest as a promoter of metastasis in breast cancer is Mena. This protein is a member of the Ena/VASP family of actin regulatory proteins and is rarely expressed in normal breast tissue. However, Mena expression correlates with tumor development and increases with malignancy grade. Further, overexpression of Mena can promote infiltration and metastasis of breast cancer cells [7, 8]. The association of Mena with tumor development, progression, and metastasis has made it an important molecule for clinical investigation, with potential wide applications as a diagnostic, prognostic, and therapeutic marker for breast cancer [9].

Another molecule that promotes metastasis of breast tumors [10, 11], human epidermal growth factor receptor 2 (Her-2), also known as c-erbB2, is commonly used as a marker in clinical research and practice. However, although both Mena and Her-2 are known to promote metastasis, no report has demonstrated whether they share the same mechanism or have synergistic effects on metastasis. Further research is needed to know whether both molecules may be targeted at once to block tumor cell invasion and metastasis more effectively, lengthening patient survival and improving cure rates.

To attempt to understand whether Mena and Her-2 share similar mechanisms and/or synergistic effects for promoting breast cancer metastasis, we used immunohistochemistry to detect Mena and Her-2 protein expression in 40 cases of breast cancer and 14 cases of normal breast tissue. The relationship between Mena and Her-2 expressions and their relationship with clinicopathological parameters were determined.

Materials and Methods

General information

Specimens were collected from 40 breast cancer patients who had been pathologically confirmed and received surgical resection in the Affiliated Hospital of Anhui University of Science and Technology (Huainan City, Anhui Province, China) from June 2010 to October 2011. For each case detailed clinical and pathological data were available; none had received any preoperative chemotherapy or radiotherapy. All patients were female. Patients ranged in age from 24 to 72 years (mean age 54.1 ± 9.4 years); 21 patients were < 55 years, and 19 patients were ≥ 55 years. Pathological diagnoses revealed that 36 patients had invasive ductal carcinoma, three patients had invasive lobular carcinoma, and one case had medullary carcinoma; 32 patients had axillary lymph node metastasis, and eight had no axillary lymph node metastasis. TNM stages were classified according to standards established in 2002 by Union Internationale Contre Le Cancer (UICC): 25 cases were in Stage I+II, and 15 cases were in Stage III+IV. In addition, 14 specimens were also collected from normal breast tissues (> 5 cm away from edge of cancer and confirmed by pathological diagnosis) as control.
**Immunohistochemistry**

Tissues were fixed in neutral formalin, dehydrated, and embedded in paraffin by conventional methods. Samples were sectioned (4 µm) and collected onto glass slides. Slices were then dewaxed with dimethylbenzene, rehydrated, soaked, and heated for antigen retrieval. Hydrogen peroxide solution (3%) was used to block endogenous peroxidase activity, then sections were covered with non-specific serum, placed in a wet box, and incubated at room temperature. Primary antibodies (Mena, goat anti-mouse monoclonal; Her-2/neu, mouse anti-human monoclonal; Santa Cruz Biotechnology) were added to the wet box, and the box was incubated at 4°C overnight. Slides were washed three times with PBS before addition of biotinylated secondary antibodies and incubation at room temperature. Slides were again washed three times in PBS before adding streptococcus avidin-peroxidase (Zhongshang-Golden Bridge Biotechnology, Ltd., Beijing) and incubating at 37°C for 30 minutes. Staining was developed with DAB chromogen (Zhongshang-Golden Bridge Biotechnology). Sections were counterstained with hematoxylin, dehydrated with ethanol gradient, and sealed. Known positive tissue slices were used as positive controls, and PBS was used as a negative control instead of primary antibodies.

Staining patterns indicating expression were yellowish-brown granules in cancer cell cytoplasm for Mena, and for Her-2 brownish-yellow in the membranes of cancer cells. To assess staining, ten high-power fields were selected per sample. Staining intensity was classified into four grades as follows: no staining visible was scored as 0; faint yellow was scored as 1; yellow as 2; and brownish-yellow as 3. Staining frequency was assessed by the proportion of positive cells per total tumor cells, as follows: ≤ 5% positive cells was assigned a 0; 6-25% positive, 1; 26-50% positive, 2; 51-75% positive, 3; and ≥ 76% positive was assigned a 4. Total scores for each case reflect the positive cells within the samples of staining intensity and frequency, with total scores of 0 indicated as (-), total scores of 1-2 as (+), total scores of 3-5 as (++), and total scores of 6-7 as (+++).

**Statistical methods**

SPSS17.0 statistical software was used for statistical analysis; \( \chi^2 \) test was used to compare expression of Mena and Her-2 protein among groups, and Spearman rank correlation was used to analyze correlations between Mena and Her-2 protein expression. Analyses were performed with two-sided tests; \( p < 0.05 \) was considered statistically significant.

**Results**

**Expression of Mena in breast cancer and normal breast tissue**

Breast tumor samples more commonly exhibited both Mena and Her-2 expression than did normal breast tissues. Specifically, for 40 breast cancer tumors, 70% (Table 1) and 40% (Table 2) expressed Mena and Her-2, respectively. In contrast, as few as 7.1% of normal breast samples expressed Mena, while 0% exhibited staining for Her-2. The positive rates of Mena and Her-2 expression in breast cancer were significantly higher compared to normal breast tissue (\( p < 0.05 \)). Further, Mena and Her-2 expression in breast cancer were positively correlated with one another (\( r = 0.530, p < 0.05 \); Table 3).

**Table 1. — Expression of Mena in breast cancer and normal breast tissue [n (%)] detected by.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>–</th>
<th>+</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>40</td>
<td>12</td>
<td>13</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Normal breast tissue</td>
<td>14</td>
<td>13</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>25</td>
<td>14</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

\( \chi^2 = 16.672, p = 0.001. \)

**Table 2. — Expression of Her-2 in breast cancer and normal breast tissue [n (%)] detected by.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>–</th>
<th>+</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>40</td>
<td>24</td>
<td>7</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Normal breast tissue</td>
<td>14</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>38</td>
<td>7</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

\( \chi^2 = 7.958, p = 0.047. \)

**Table 3. — Correlation between Mena and Her-2 expression in breast cancer tissue.**

<table>
<thead>
<tr>
<th>Mena</th>
<th>( n )</th>
<th>–</th>
<th>+</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>12</td>
<td>11</td>
<td>(91.7)</td>
<td>0</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>+</td>
<td>13</td>
<td>9</td>
<td>(69.2)</td>
<td>2</td>
<td>(15.4)</td>
</tr>
<tr>
<td>++</td>
<td>7</td>
<td>2</td>
<td>(28.6)</td>
<td>3</td>
<td>(42.9)</td>
</tr>
<tr>
<td>+++</td>
<td>8</td>
<td>2</td>
<td>(25.0)</td>
<td>2</td>
<td>(25.0)</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>24</td>
<td>(60.0)</td>
<td>7</td>
<td>(17.5)</td>
</tr>
</tbody>
</table>

\( r = 0.590, p = 0.001 \)

**Relationship between expression of Mena and Her-2 protein and clinical/pathological parameters**

To determine whether the increased expression of both Mena and Her-2 correlated with the severity of breast tumors, we assessed expression patterns in comparison with clinical and pathological features of the samples. Both Mena and Her-2 protein expression in breast cancer were correlated with axillary lymph node metastasis and TNM stage (\( p < 0.05 \)), but not with age or pathological type.

**Discussion**

Breast cancer is one of the most common malignancies among women, often metastasizing to the lungs, bone, brain, and other organs. With rapid advances in molecular biology and cytobiology many genes have been found to play important roles in the occurrence, development, and metastasis of breast tumors. At least a subset of these genes may offer potential as molecular markers or therapeutic targets to aid in diagnosis and treatment of the disease.

To invade and metastasize, cancer cells must first evade normal defense systems, then disseminate and infiltrate into the surrounding tissues, blood vessels, and lymphatic vessels. Mena, as a member of the Ena/VASP family of actin regulatory proteins, can enhance motor activity of cancer cells, enabling them to infiltrate lymph nodes and distant organs. Indeed, this protein is associated with the occurrence, development, invasion, and metastasis of various tumors and is overexpressed in lung, colon, and other cancers. Studies performed by DiModugno et al.
growth and development of tissues and organs; however, human embryonic development, Her-2 participates in been studied intensively. Typically expressed during biological behaviors of tumors, and may provide new avenues for tumor diagnosis and treatment.

These findings suggest that changes in Mena expression with increased disease severity. Thus, both Mena and Her-2 expression were positively correlated with each other, suggesting that Mena and Her-2 may collectively participate in proliferation of breast cancer cells and have synergistic effects on invasion and metastasis of tumor cells.

In summary, expression of both Mena and Her-2 protein in breast cancer were significantly increased and positively correlated. Further, expression was associated with increased disease severity. Thus, both Mena and Her-2 may be used as reference indicators for evaluating biological behavior and prognosis of breast cancer, and Mena may offer a new therapeutic target.

### References


Table 4. — Correlation between expression of Mena protein and clinicopathological parameters in breast cancer [n (%)].

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>n</th>
<th>–</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong> &lt; 55</td>
<td>21</td>
<td>7 (33.3)</td>
<td>9 (42.9)</td>
<td>3 (14.3)</td>
<td>2 (9.5)</td>
<td>4.310</td>
<td>0.230</td>
</tr>
<tr>
<td>≥ 55</td>
<td>19</td>
<td>5 (26.3)</td>
<td>4 (21.1)</td>
<td>4 (21.1)</td>
<td>6 (31.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pathological type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>36</td>
<td>11 (30.6)</td>
<td>10 (27.8)</td>
<td>7 (19.4)</td>
<td>8 (22.2)</td>
<td>4.708</td>
<td>0.582</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>3</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medullary carcinoma</td>
<td>1</td>
<td>0</td>
<td>1 (100.0)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Axillary lymph node metastasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32</td>
<td>11 (34.4)</td>
<td>13 (40.6)</td>
<td>5 (15.6)</td>
<td>3 (9.4)</td>
<td>13.624</td>
<td>0.003</td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>1 (12.5)</td>
<td>0</td>
<td>2 (25.0)</td>
<td>5 (62.5)</td>
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</tr>
<tr>
<td><strong>TNM Stages</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I+II</td>
<td>25</td>
<td>10 (40.0)</td>
<td>10 (40.0)</td>
<td>3 (12.0)</td>
<td>2 (8.0)</td>
<td>9.328</td>
<td>0.025</td>
</tr>
<tr>
<td>III+IV</td>
<td>15</td>
<td>2 (13.3)</td>
<td>3 (20.0)</td>
<td>4 (26.7)</td>
<td>6 (40.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. — Correlation between expression of Her-2 protein and clinicopathological parameters in breast cancer [n (%)].

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>n</th>
<th>–</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong> &lt; 55</td>
<td>21</td>
<td>14 (66.7)</td>
<td>4 (19.0)</td>
<td>1 (4.8)</td>
<td>2 (9.5)</td>
<td>2.516</td>
<td>0.472</td>
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<tr>
<td>≥ 55</td>
<td>19</td>
<td>10 (52.6)</td>
<td>3 (15.8)</td>
<td>4 (21.1)</td>
<td>2 (10.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pathological type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>36</td>
<td>22 (61.1)</td>
<td>6 (16.7)</td>
<td>4 (11.1)</td>
<td>4 (11.1)</td>
<td>8.249</td>
<td>0.220</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>3</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medullary carcinoma</td>
<td>1</td>
<td>0</td>
<td>1 (100.0)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Axillary lymph node metastasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32</td>
<td>22 (68.8)</td>
<td>7 (21.9)</td>
<td>2 (6.3)</td>
<td>1 (3.1)</td>
<td>16.354</td>
<td>0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>2 (25.0)</td>
<td>0</td>
<td>3 (37.5)</td>
<td>3 (37.5)</td>
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<td></td>
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<td><strong>TNM Stages</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I+II</td>
<td>25</td>
<td>10 (80.0)</td>
<td>5 (40.0)</td>
<td>4 (32.0)</td>
<td>1 (8.0)</td>
<td>10.484</td>
<td>0.001</td>
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<td>III+IV</td>
<td>15</td>
<td>3 (20.0)</td>
<td>5 (33.3)</td>
<td>4 (26.7)</td>
<td>3 (20.0)</td>
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</tr>
</tbody>
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Evaluation of the histopathological diagnosis of patients preoperatively diagnosed with atypical endometrial hyperplasia after hysterectomy

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Summary

Objective: To evaluate the patients diagnosed with atypical endometrial hyperplasia preoperatively, and compare preoperative and postoperative results. Materials and method: We investigated the files of 58 patients diagnosed with atypical endometrial hyperplasia who were treated surgically after clinical evaluation. We compared sociodemographic diagnosis, preoperative and postoperative diagnosis. Results: Mean-age of patients was 51.7. Obesity, diabetes mellitus, hypertension and infertility were seen, respectively, in eight cases (13.7%), 12 cases (20.6%), 19 cases (32.7%) and four cases (6.8%). While endometrial cancer was not found postoperatively in patients preoperatively diagnosed with simple atypical endometrial hyperplasia, we determined well differentiated endometrial adenocarcinoma in 44.7% of the patients. Conclusion: In the literature the probability of developing well differentiated endometrial adenocarcinoma in atypical hyperplasia is 40-50%. All patients diagnosed with complex atypical hyperplasia should be evaluated preoperatively for well differentiated adenocarcinoma and undergo an appropriate surgical technique and staging.

Key words: Atypia; Biopsy; Consensus diagnosis; Endometrial hyperplasia; Endometrioid adenocarcinoma; Hysterectomy.

Introduction

Endometrial hyperplasia (EH) is a non-physiological non-invasive proliferation of the endometrium. Histologically, the disease spectrum ranges from benign to precancerous lesions. Without considering age, EH is seen in 1.5% of all abnormal uterine hemorrhage although its exact incidence is unknown [1]. This figure is about 15% in women with postmenopausal hemorrhage [1, 2]. Although prevalence of EH increases after the ages of 45 to 55, EH has been reported in young adults with anovulatory cycles and even during the teenage period [1, 2]. The most important reason for ongoing discussions about diagnosis, treatment and classification of EH is the fact that 5-10% of the women diagnosed as having this pathology have concomitant genital cancers [1-5]. The primary risk factor for EH is unbalanced estrogen and/or hyperestrogenic milieu. This is frequently associated with such conditions as anovulatory cycles (adolescence period, polycystic ovaries, perimenopausal period), excessive endogenous estrogen release (granulosa cell tumor, thecoma, adrenocortical hyperplasia, obesity), and exogenous estrogen intake (tamoxifen). Furthermore, genetic predisposition exists in women with hereditary non-polyposis colorectal cancer. Excessive estrogen leads to hyperplastic lesions by acting via PTEN mutation or k-RAS activation, and to mutagenic and carcinogenic effects in the hyperplastic endometrial lesions depending on total dose and duration of action. The standard diagnostic method for diagnosis of endometrial hyperplasia is endometrial biopsy. Differing diagnostic success rates have been reported in studies on classical dilation and curettage, endometrial sampling with pipette, hysteroscopic biopsy or office hysteroscopic biopsies. Well differentiated adenocarcinoma is found in hysterectomy specimens in 17-62% of the patients with endometrial hyperplasia with complex atypia [2]. In the present study, we investigated the accordance between preoperative and postoperative diagnoses in 58 patients who were diagnosed as having atypical endometrial hyperplasia by means of dilation and curettage (D&C) and who underwent hysterectomy in three hospitals in Izmir. We discuss current therapeutic approaches in the patients with a diagnosis of atypical endometrial hyperplasia in accordance with the literature.

Materials and Methods

Hospital files of 58 patients who were diagnosed as having atypical endometrial hyperplasia by means of fractional curettage and who underwent surgical treatment upon clinical evaluation in three hospitals in Izmir were reviewed retrospectively. In the patient files diagnostic methods (D&C, office H/S) and accordance between preoperative and postoperative diagnoses were reviewed along with risk factors predisposing to endometrial cancer. All patients who were diagnosed as having postoperative carcinoma were evaluated for grade, depth of myometrial invasion, involvement of lymphovascular area (LVA), and tumor size. The patients for whom frozen-section (FS) investigation was performed during surgery and who were diagnosed as having endometrial cancer were staged surgically. The patients were classified according to FIGO criteria.
Results

Mean age of the 58 patients meeting study criteria was 51.7 years. Eight (13.7%) of the patients were obese, 12 (20.6%) had diabetes mellitus, 19 (32.7%) had hypertension, and four (6.8%) had infertility. Three patients (5.17%) had been operated previously for breast cancer and had adjuvant chemotherapy. The most common complaint at the time of presentation was irregular vaginal bleeding in 48 (82.7%) of the patients with 31 (53.4%) patients being in the postmenopausal period and 27 (46.6%) patients being in the premenopausal period (Table 1). Forty-nine out of 58 patients underwent fractional curettage under general anesthesia and nine patients under local anesthesia. Evaluating the results of fractional curettage, we found that 38 patients had a diagnosis of complex atypical hyperplasia (CAH), and 20 had a diagnosis of simple atypical hyperplasia (SAH). Mean length of time between biopsy procedure and operation was 4.9 ± 1.7 weeks. In 58 patients with a preoperative diagnosis of atypical endometrial hyperplasia who were operated, no cancer was observed among any of the 20 patients with a preoperative diagnosis of SAH whereas endometrial cancer was found in 17 (44.7%) of the 38 patients with a preoperative diagnosis of CAH (Table 2). Grade 1 tumor was seen in 16 of these patients and grade 3 tumor in one. Fifteen patients had myometrial invasion; depth of invasion was more than one-half in three of them and involvement of lymphovascular area in two. In 14 patients who had a diagnosis of CAH, frozen-section pathologic examination was performed during surgery. Results of frozen-section procedure were reported as being malignant in six of 14 patients (42.8%) and these patients underwent surgical staging. No lymph node metastasis was observed in any patients undergoing surgical staging. Results of paraffin block examinations were reported as being cancer in all of six patients. In eight patients whose results of frozen-section examination were reported as being benign, results of paraffin block examination were benign in six patients and endometrial cancer in two patients. No lymph node metastasis in the pelvic region was observed in any of the patients with cancer found during hysterectomy. In terms of histological diagnosis, pathological diagnosis in the first biopsy was compatible with the results of examination of paraffin blocks in 25 out of 58 patients. Operative pathologies defined lower grade lesions in six patients whereas well differentiated adenocarcinoma was found in 17 patients. The compatibility rate was 43.1%.

Discussion

Associated with irregular glandular and stromal increase in the endometrium, endometrial hyperplasias take place between normal proliferative endometrium and well differentiated adenocarcinomas, and are important because of their associations with endometrial carcinomas [6]. The fact that carcinoma develops in some of the patients with untreated hyperplasia and hyperplasia is found in many areas in hysterectomy specimens which were diagnosed as having endometrial carcinoma raises the importance of diagnosing hyperplasia [2]. It has been shown in some retrospective studies where specimens were reevaluated that atypical hyperplasia existed in up to 45% of patients with endometrial cancer, and that concomitant cancers existed in 17-62% of specimens of patients with atypical hyperplasia [3]. Myometrial invasion may be found in 7.9-51% of these cancers [3-5, 7]. In our series, results of hysterectomy were reported as endometrial cancer in 44.7% of the operations being performed for complex atypical hyperplasia. Fifteen of 17 patients in whom we found endometrial cancer after hysterectomy had myometrial invasion. Depth of invasion was more than one-half in three of them. Evaluating risk factors (e.g., anovulation, obesity, estrogen replacement therapy, tamoxifen, diabetes mellitus, hypertension, estrogen-releasing ovarian tumors, and familial cancer syndrome) and symptoms are of importance [8]. Postmenopausal bleeding, thickness of postmenopausal endometrium > 5 mm on transvaginal ultrasound, endometrial fluid accumulation, endometrial irregularities, and presence of endometrial cells in postmenopausal smears are conditions warranting further investigation. The gold standard in the diagnosis is endometrial sampling. Sampling may be done with dilation and curettage (sensitivity > 90%), biopsy at the office with pipel (sensitivity 83-97%), and hysteroscopic biopsy (sensitivity 98%). Hysteroscopy may detect structural pathologies (e.g., polyps, myomas) more precisely than D&C while the macroscopic view may be confused with benign polyps in hyperplasias and endometrial cancers [9, 10]. Biopsy under hysteroscopic guidance has the highest diagnostic value but we use classical D&C in patients with suspected malignancy. Although its prognostic role is controversial, hysteroscopy has the potential to spread
cytologically into the peritoneal cavity. Hysteroscopic biopsy at the office or low-pressure hysteroscopic biopsy for patients in whom malignancy is strongly suspected clinically (in the presence of inadequate material) is more successful in providing adequate material for diagnosis from focal lesions developing from structural pathology (polyps) [1, 3, 9, 10]. Diagnosis was made with D&C in our patients. Well differentiated adenocarcinoma was found at high rates (17-62%) in hysterectomy specimens of the patients with atypical endometrial hyperplasia. Thus, diagnostic methods with higher rates of preoperative sensitivity and specificity are required to plan optimal treatment for these patients.

Accurate defining and classifying hyperplasia is especially important in samples of endometrial biopsy and curettage because a correct diagnosis leads to clinical treatment modalities that may vary substantially depending on the type of hyperplasia. Intra- and interobserver variations in the treatment of hyperplasia cause concern about reproducibility of a classification plan [11, 12]. Reasons for high risk of unknown cancer in women with a preoperative diagnosis of complex atypical endometrial hyperplasia originate from inconsistent and different interpretations of histological criteria used in distinguishing complex atypical endometrial hyperplasia from grade 1 adenocarcinoma. In concordance with this finding, well differentiated adenocarcinoma was found in surgical specimens of 17 (44.7%) of 38 patients with CAH in the present study. In a GOG study, 306 endometrial biopsy samples with diagnoses of atypical endometrial hyperplasia were evaluated. In the evaluation consisting of three separate pathological schools, a consensus was reached in 38% of diagnoses of atypical hyperplasia whereas diagnoses at later stage were reported in 29% and diagnoses at earlier stages in 25% [13]. Thus, many authors have recommended a modified classification system of WHO in order to make more standardized diagnoses and perform more appropriate treatments. Classification of endometrial intraepithelial hyperplasia (EIN) was developed to meet this requirement. EIN is a classification system developed to detect cancer precursors better than the WHO-94 and direct the treatment of patients with hyperplasia better.

In this classification system endometrial pathologies are divided in three main categories:

1) Benign hyperplasias (benign hyperplasias due to unbalanced estrogen);
2) EIN (a monoclonal and neoplastic occurrence which is first local and later diffuse);
3) Carcinoma.

Criteria determining this category have not been widely used clinically although they seem to be associated with cancer risk, and to be objective. Use of EIN in daily practice and its cost-effectiveness analysis is unknown. Its routine use is still controversial. At the current point, distinguishing atypical endometrial hyperplasia from well differentiated adenocarcinoma should be evaluated together with biological markers, age, obesity, and other known risk factors by considering the limitations of the interventional and diagnostic methods. Current studies have shown that biopsy material obtained with D&C is more adequate in terms of diagnostic evaluation compared to endometrial biopsy with pipel, and it distinguishes atypical hyperplasia better [10, 13-15]. Furthermore, the rate of unexpected cancer significantly increases in hysterectomy materials with advanced age [1, 3, 11, 13]. In a patient series of 824 patients in which sensitivity of preoperative D&C in risk of undefined adenocarcinoma and complex atypical endometrial hyperplasia was investigated, Burgman et al. found that age was an important risk factor in the diagnosis of unexpected cancer. According to the results of that study, mean age of women with cancer was 59 after hysterectomy while mean age of those without cancer was 55. Risk of cancer rises each decade after age of 50. Cancer rate is 40% in the women younger than 50 years while it has been reported to be higher than 78% in women older than 80 years [13]. In the present study, mean age was found to be 52.4 years for those patients whose result of hysterectomy was reported to be malignant and 51.7 years for those with benign hysterectomy results to be benign.

The fact that malignancy is found at high rates in patients with CAH, difficulty in defining it intraoperatively is a serious problem which may be encountered in the therapeutic process. Cancer found in the patients operated for CAH was usually well differentiated and showed superficial infiltration. The lesion in such cases is usually not observed. Thus, sections are made in this group of patients randomly through a frozen-section (FS) procedure. This implies that the tumor can not be found at high rates in FS studies. Looking at studies on intraoperative frozen-section procedures to avoid incomplete surgery in patients with CAH, the positive predictive rate of FS ranges between 39% and 75% [16-18]. Additionally, even in patients with known endometrial cancer obvious inconsistency is seen for diagnosis with FS among pathologists and upstaging diagnoses are found in the diagnosis [19]. While studies are still being continued on optimal diagnostic and therapeutic strategies of patients with atypical hyperplasia, performing surgical staging would not be an excessive option in patients with atypical hyperplasia as a consequence of a high rate of overlapping between atypical hyperplasia and well differentiated adenocarcinoma as potential advantages of surgical staging increases in early stages of endometrial adenocarcinoma [20, 21].

Conclusion

Our aim in carrying out the present study was to draw attention to the association between atypical endometrial hyperplasia and well differentiated adenocarcinoma, to mention diagnostic difficulties in that distinction, and to emphasize performing complete surgical staging by considering patients with a preoperative diagnosis of atypical endometrial hyperplasia as well differentiated endometrial carcinoma to plan optimal treatment of such patients.
References


Clinicopathological study of 112 cases of benign, pre-invasive and invasive lesions of the vagina: a 15-year review

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Summary

Objective: Benign vaginal lesions are mainly asymptomatic and often diagnosed during routine screening gynecological examination. Additionally, vaginal intraepithelial lesions are asymptomatic and diagnosis is often confirmed after vaginal biopsy under colposcopic evaluation in cases of abnormal cytological Papanicolaou examination or synchronous cervical intraepithelial neoplasia. On the other hand, primary vaginal cancer is rare representing approximately 1 - 2% of all gynecological cancers. Metastatic invasion of the vagina is common especially in cases of advanced stage cervical cancer. The aim of this study was to examine the diagnostic approach, the management strategy, and the pathological findings in cases of benign, pre-invasive and invasive vaginal lesions that were diagnosed and treated in our Department. Materials and methods: This was a 15-year retrospective study. Cases of benign, pre-invasive, and invasive vaginal lesions diagnosed during the last fifteen years at Aretaieion Hospital of the University of Athens, were analyzed. Results: During this study period 40 cases of vaginal cysts (35.7% of all vaginal lesions) were diagnosed. Surgical excision of the lesions was decided in all cases and histology showed that the most frequent cyst type was mucus-secreting Mullerian (30%). During the study period, 23 cases of vaginal intraepithelial neoplasia (VAIN, 20.5% of all vaginal lesions) were detected. In 43.5% of the cases, histological diagnosis revealed low grade VAIN, while the remaining cases were classified as high grade VAIN. Furthermore, 11 cases of primary vaginal cancer (9.8% of all vaginal lesions) were diagnosed. The vast majority of them (91%) were squamous cell carcinomas. Additionally, histology confirmed the diagnosis of metastatic invasion of the vaginal wall in 38 cases (34% of all vaginal lesions). In the majority of these cases (55.2%), primary cancer was located in the cervix. Discussion: Benign, pre-invasive and invasive vaginal lesions are relatively uncommon and usually accompany lesions in other sites of the lower genital tract. Their diagnosis is based on gynecological or colposcopic examination. Treatment depends on the type of the lesion and the progression of the disease.

Key words: Vaginal cancer; VAIN; Vaginal cysts; Colposcopy.

Introduction

Benign vaginal cysts are in the majority of cases asymptomatic and are often incidentally discovered during gynecological examination for other purposes [1]. Several classifications have been proposed based on histologic and histochemical features of cyst epithelium [2]. According to a functional classification, vaginal cysts are divided into mucus-secreting Mullerian cysts lined mainly by endocervical and occasionally by Fallopian tube epithelium, Bartholin’s duct cysts, Gartner’s duct (mesonephric) cysts, and epidermal inclusion cysts of surface mucosa.

The histogenesis of Mullerian cysts remains uncertain. Perhaps some are derived from adenosis and their distinction is made by microscopic examination. They are usually small and located anywhere in the vagina. The pathogenesis of Bartholin’s gland cysts is also uncertain, involving occlusion of the duct and are located in the region of the ducts of Bartholin glands. Gartner’s duct cysts are usually located at the anterior-lateral wall of the vagina, resulting from secretion by small isolated epithelial remnants after incomplete regression of the mesonephric duct. Epidermal inclusion cysts of surface mucosa are reported as the most common, but usually without any clinically significant type of vaginal cysts, resulting from the entrapment of fragments of mucosa during colporrhaphy or episiotomy and most commonly occur in the distal portion of the vagina.

Additionally, vaginal intraepithelial neoplasia (VAIN) is uncommon, representing 1% of lower genital tract intraepithelial neoplasias [3]. The median age at diagnosis of VAIN is 41 years (range 16 - 87 years) [4-6]. However VAIN is now being diagnosed in younger women and this rise seems to be associated with the increased incidence of human papilloma virus (HPV) infections of the lower genital tract. Generally, most patients are asymptomatic. If present, symptoms may include postcoital spotting, vaginal bleeding, unusual vaginal discharge and odor [4, 5, 7]. The majority of lesions are located in the upper one-third of the vagina and are often multifocal [4-6]. VAIN is classified in a similar manner as cervical intraepithelial neoplasia (CIN) and HPV is the primary initiator of these lesions [7, 8]. Final diagnosis is confirmed by histological examination after vaginal biopsy under colposcopy. In the majority of the cases, an abnormal cytological Papanicolaou examination or the diagnosis of CIN leads to the colposcopic evaluation of the vagina and the vulva. Also, diagnosis of VAIN is reported after hysterec- tomy among other reasons, and histological examination of vaginal specimens.
Primary vaginal cancer is a rare malignancy accounting for about 2% of all gynecological cancers with a rate of 0.5 / 100,000 women [9]. Most of the women are more than 60 years old, with mean age of 64 years at diagnosis. Histopathologically, most common are the squamous cell carcinomas (80-90%) and adenocarcinomas (4-10%). Most common symptoms include: vaginal bleeding, unusual vaginal discharge, or dysuria. Risk factors for the development of primary vaginal cancer are considered to be HPV infection, immunosuppression, irradiation, and squamous neoplasia occurring elsewhere in the lower genital tract [10, 11].

Finally, secondary spread of malignant neoplasms to the vagina is quite common, in contrast to the primary vaginal cancer. Metastatic invasion of the vagina is often by direct extension, vascular or lymphatic embolization, or infrequent direct implication. The most frequent primary sites include: cervix, vulva, endometrium, ovaries, rectum, and colon.

Materials and Methods

The present was a 15-year retrospective study. Cases of benign, pre-invasive, and invasive (primary or metastatic) vaginal lesions diagnosed during the last 15 years at the Pathology Laboratory, Aretaieion Hospital of the University of Athens, were analyzed. Pathological findings were related to the clinical parameters, including patients’ age and symptoms.

Results

During the study period, 40 cases of benign vaginal cysts were diagnosed (40 / 112, 35.7% of all vaginal lesions) (Table 1). There were 12 cases of Mullerian cysts (30%), 11 cases of Bartholin’s duct cysts (27.5%), ten cases of epidermal inclusion cysts (25%), five cases of Gartner’s duct cysts (12.5%), one endometrioid cyst (2.5%), and one unclassified cyst (2.5%). Patient’s age ranged from 20 to 75 years with a mean age of 35 years and a peak incidence between 31 - 40 years (13 cases, 32.5%). The majority of patients were asymptomatic (31 cases, 77.5%). The cyst type which was most frequently associated with symptoms was Bartholin’s duct cyst. Most lesions were located in the left-lateral vaginal wall (13 cases, 32.5%). Mullerian cysts were lined by columnar endocervical-like or cuboidal epithelium, whereas Gartner’s duct cysts were all lined by cuboidal epithelium. Epidermal inclusion cysts were lined by stratified non-keratinizing squamous epithelium. Bartholin’s duct cysts were lined by transitional, mucin-rich columnar or squamous epithelium and were frequently accompanied by inflammation.

Thirty-three cases of VAIN (23 / 112, 20.5% of all vaginal lesions) were diagnosed during this period. Ten cases were classified as VAIN I (43.5%) aged 19 - 51 years with a mean age of 36.9 years, while six patients aged 18 - 73 years with a mean age of 46.8 years were diagnosed with VAIN II (26.1%). Additionally, histology revealed VAIN III in seven patients (30.4%) aged 50 - 75 years with a mean age of 62.1 years. Two patients diagnosed with VAIN I (20% of VAIN I and 8.7% of all VAIN’s), two patients with VAIN II (33.3% of VAIN II and 8.7% of all VAIN’s) and three patients with VAIN III (42.8% of VAIN III and 13% of all VAIN’s) had previous or coexisting squamous carcinoma of the cervix. Patients with vaginal intraepithelial neoplasia were generally asymptomatic and the changes were initially detected by cytology or colposcopy. When symptoms were present, they included: postcoital spotting, vaginal bleeding, unusual vaginal discharge, and leukorrhea. In low-grade vaginal intraepithelial neoplasia (VAIN I), hypercellularity, disorganization, and increased mitotic activity occurred especially in the deep layers, but the cells maintained orderly maturation from the parabasal to the superficial layers. In high-grade vaginal intraepithelial neoplasia (VAIN II and III), more histological changes occurred. In VAIN II squamous differentiation in the upper third of the epithelium was still maintained, but there was a greater degree of proliferation, higher mitotic activity, and loss of polarity in the lower two thirds of the epithelium when compared with VAIN I. VAIN III consisted of disorderly arranged immature cells with scant cytoplasm, hyperchromatic and irregular nuclei having a coarse chromatin pattern. Mitotict figures, including abnormal forms, were found in all layers. Squamous differentiation was absent or limited to the most superficial layers.

Eleven cases of primary vaginal cancer (22.4% of invasive vaginal lesions and 9.8% of all vaginal lesions) were histologically confirmed during this study period. Ten patients (91%), aged 50 - 86 years with a mean age of 69 years were diagnosed with squamous cell carcinoma, while one 30 year-old patient (9%) was diagnosed with primary vaginal adenocarcinoma. The majority of 60% of the squamous cell carcinomas were moderately differentiated (non-keratinized), while the remaining 40% of these cases were well differentiated (keratinized). These neoplasms were composed of typical squamous cells. Their nuclei showed low or greater pleomorphism and well-developed or less distinct intercellular bridges, depending on the degree of their differentiation. The vast majority of the patients complained of abnormal vaginal bleeding or discharge and only two patients were asymptomatic. The case of adenocarcinoma was referred to a patient with a history of prenatal exposure to diethylstilbestrol (DES). It was a low differentiated clear cell adenocarcinoma, located in the lateral wall of the upper vagina, and consisted of tubules and cysts and solid areas lined by clear cells due to the presence of glycogen and fat.

Thirty-eight cases of metastatic invasion of the vagina (77.6% of invasive vaginal lesions and 34% of all vaginal lesions) were diagnosed through histology during this ten-year study period (Table 2). Most of the cases were diagnosed during gynecological examination for clinical staging of cervical cancer. In the vast majority of the cases, primary cancer was located in the cervix (21 cases aged 43 - 88 years with a mean age of 60 years, 55.3%).
Benign vaginal cysts are rare pathological lesions. The pathogenesis of most types of vaginal cysts remains to be clarified. In this study, benign vaginal cysts represented 35.7% of all diagnosed vaginal lesions. Mullerian cysts were found to be the most common type (30%). These data are in agreement with other studies supporting that the most frequent vaginal cyst type is mucus-secreting Mullerian [12]. In contrast, other studies suggest that epithelial inclusion cysts are more common [2]. Differential diagnosis between Mullerian and Gartner’s duct cysts requires histochemical evaluation of epithelial mucin production. The vast majority of the patients diagnosed with vaginal cysts in this study were asymptomatic, as reported in the majority of the literature. Symptoms are usually represented by swelling or mass in the vagina, accompanied in some by stress urinary incontinence, dyspareunia or abnormal vaginal bleeding [12]. There was no case of vaginal cyst neither in this study nor in that of Pradhan et al. [12] diagnosed with malignant change or disclosed intraepithelial neoplasia. The treatment of choice is surgical excision, although marsupialization may be indicated with good outcome in cases of Bartholin’s duct cysts.

In contrast to the high prevalence of intraepithelial lesions of the cervix and the vulva, vaginal intraepithelial neoplasia is relatively rare. VAIN may occur as an isolated lesion or as a lesion associated with CIN (65%) or vulvar intraepithelial neoplasia (VIN) (10%) [6]. These lesions may occur at the same time (synchronous lesions) or up to several years after the initial CIN lesion (metachronous lesions) [4, 13]. Most VAIN lesions occur in the vaginal vault after radical hysterectomy for invasive cervical cancer [4, 6, 7, 14] therefore long-term cytological follow-up after surgery for early or advanced-stage cervical cancer is strongly recommended. In cases of low-grade VAIN, management is conservative with regular cytological and colposcopy follow-up. Treatment of choice in cases of high-grade VAIN is the excision of the lesion. There are several surgical procedures (local excision, laser surgery, loop electrosurgical excision procedure LEEP, cavitational ultrasonic surgical aspiration) with excellent outcome, although other non-surgical methods, such as: topical medical therapy (5% 5-fluorouracil, imiquimod), immunotherapy (interferon), or radiation therapy have been used [3, 4, 15-20]. In this study, all patients diagnosed with high-grade vaginal intraepithelial neoplasia (VAIN II or III) underwent surgical excision of the lesion with laser surgery or loop electrosurgical excision procedure.

Primary vaginal cancer is a rare gynecological malignancy accounting for about two percent of all malignant neoplasms of the female genital system. In the study of Platz et al. [21], squamous cell carcinoma represents about 80% of primary vaginal carcinomas. In the same study, the incidence of primary squamous cell carcinoma of the vagina in the United States was about 1,000 cases / per year. The incidence is 0.42 / 100,000 cases in Caucasian women and 0.93 / 100,000 cases in black women. The mean age at diagnosis of invasive squamous vaginal cancer according to the published literature is about 64 years. However, vaginal adenocarcinomas more commonly affect younger women with a documented history of DES exposure in utero. The average age in which primary vaginal adenocarcinoma is diagnosed is about 19

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**Table 1. — Classification of benign vaginal cysts according to histological type.**

<table>
<thead>
<tr>
<th>Histology of benign vaginal cysts</th>
<th>No. cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mullerian cysts</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Bartholin’s duct cysts</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>Epidermal inclusion cysts</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Gartner’s duct cysts</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>Endometrioid cysts</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Unclassified</td>
<td>1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Table 2. — Primary cancer’s location in cases of metastatic invasion of the vagina.**

<table>
<thead>
<tr>
<th>Primary cancer</th>
<th>Histological type</th>
<th>No. cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical cancer</td>
<td>Squamous cell carcinoma</td>
<td>21</td>
<td>55.3</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Serous papillary adenocA</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>AdenoCa (serous papillary or endometrioid)</td>
<td>5</td>
<td>13.2</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>AdenoCa</td>
<td>4</td>
<td>10.5</td>
</tr>
</tbody>
</table>

In the remaining cases, vaginal metastasis was diagnosed in eight patients with ovarian cancer (21%) aged 39 - 71 years with a mean age of 55.5 years, in five patients with endometrial cancer (13.2%) aged 71 - 84 years with a mean age of 74 years, and in four patients with colon adenocarcinoma (10.5%) aged 41 - 80 years with a mean age of 62 years. All cervical tumors were squamous cell carcinomas characterized by neoplastic squamous cells showing either individual cell keratinization (non-keratinized tumors, 3 / 21 of the cases) or real keratin pearls (keratinized tumors, 18 / 21 of the cases). Neoplastic cells had round to oval nuclei, coarse chromatin, and numerous mitotic figures. All ovarian cancers were low-differentiated serous papillary adenocarcinomas characterized by nuclear atypia, high mitotic activity, stratification, glandular complexity, branching papillary fronts, and stromal invasion. Two out of these five endometrial cancers were low-differentiated endometrioid adenocarcinomas consisting in malignant-appearing squamous elements and glands that displayed papillary infoldings and branches. In addition, the cytoplasm of the glandular cells was diminished, granular, and lacked mucin. The remaining three cases of endometrial cancer represented serous papillary adenocarcinomas, a highly aggressive histological type with morphological characteristics similar to ovarian serous papillary adenocarcinomas. The colon adenocarcinomas (half of them were low-differentiated and the other half were moderately differentiated) consisting in neoplastic cells that represented a combination of columnar and goblet cells, with occasional participation of endocrine cells.

**Discussion**

Benign vaginal cysts are rare pathological lesions. The pathogenesis of most types of vaginal cysts remains to be clarified. In this study, benign vaginal cysts represented 35.7% of all diagnosed vaginal lesions. Mullerian cysts were found to be the most common type (30%). These
years. The data resulting from this study seem to be in agreement with the literature. In this study, the squamous cell vaginal carcinoma (91%) was more common than the adenocarcinoma (9%). Patients diagnosed with squamous cell carcinoma aged from 50 to 86 years with a mean age of 69 years in this study, is close to what the literature reports. Similarly, the patient with vaginal adenocarcinoma was a 30-years-old young woman with a history of embryonic DES exposure.

On the other hand, secondary spread of malignant neoplasms to the vagina by direct extension or lymphatic or hematogenous metastasis is quite common. It is considered that only about 15% of vaginal cancers are primary, while the remaining 85% are metastatic. The most common primary cancer that leads to metastatic invasion of the vagina is cervical cancer by direct extension. The results of this study are in agreement with the literature as only 11 out of 49 cases (22.4%) in which histology confirmed the diagnosis of cancer were primary vaginal neoplasms. The remaining 38 cases (77.6%) were metastatic tumors originated from the cervix (55.3%), ovaries (21%), endometrium (13.2%), or the colon (10.5%).

Conclusions

Benign, pre-invasive, and primary invasive vaginal lesions are relatively uncommon and usually accompany lesions in other sites of the lower genital tract. They are usually asymptomatic and diagnosis is often incidental during a routine gynecological examination or after an abnormal cytological Papanicolaou examination that leads to colposcopic evaluation of the cervix and the vagina. Treatment of these lesions depends on the type of the lesion and the progression of the disease.

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Role of the association of high-risk HPV identified by real-time PCR in cervical preneoplastic lesions

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Summary

Objective: To evaluate the effects of infection in multiple types of high-risk human papilloma virus (HPV) in cervical preneoplastic lesions in patients undergoing colposcopy following a diagnosis of atypical squamous cells of unknown significance (ASCUS) and low-grade squamous intraepithelial (LSIL) cytology. Materials and Methods: Between 2009 and 2010, 2,500 patients were recruited with a mean age of 35 ± 5 years. Screening for cervical cancer was performed and in case of ASCUS and LSIL the patients underwent colposcopy. The tests for the detection and typing of viral DNA (HPV - DNA test) were performed on cervical swab with real-time PCR amplification. Results: The prevalence of infection was 70% (1579/2256) in the patients recruited. In relation to the degree of preneoplastic lesions some high-risk HPV viral genotypes were identified: HPV 16 (319/1466), HPV 18 (164/1466), HPV 45 (76/1466), HPV 31 (215/1466), HPV 52 (145/1466), HPV 58 (55/1466) HPV 56 (79/1466), HPV 51 (110/1466), HPV 6 (138/1466), HPV 11 (88/1466), HPV 42 (34/1466), HPV 53 (43/1466). In case of high-grade lesions of CIN (CIN2 and CIN3) a greater HPV co-infection was detected and in particular the association from 16 to 18 (70%), 16-33 (18%) and 16 to 52 (12%). Conclusions: Infection caused by the simultaneous presence of multiple HPV genotypes appears to be associated with a significantly increased risk of high-grade lesions of CIN or invasive cancer than the presence of single viral infections. The infection with multiple HPV types is a significant risk factor for high-grade lesions of CIN in women undergoing colposcopy for ASCUS cytology / LSIL. The use of real-time PCR has shown the ability not only to identify the different types of HPV, but also to monitor quantitatively the same over time, and during the study phase, to evaluate the sensitivity and specificity of the method in comparison with other techniques.

Key words: Squamous cell carcinoma; Endometrial carcinoma; Ichthyosis uteri.

Introduction

Human Papilloma Virus or HPV is a common sexually transmitted infection. About 75% of sexually active women become infected during their lifetime with an HPV of any type, more than 50% infected with a high-oncogenic risk type [1-4]. Although infection may occur without symptoms (latent or subclinical) and can be resolved (immunity cell-mediated), about 40% of cases are associated with high-grade squamous intraepithelial lesion (HSIL). Across all five continents, HPV has been reported to be the most common genotype in high-grade cervical intraepithelial neoplasia (CIN2+) with an incidence rate ranging from 33.3% in Oceania to 51.8% in Europe [5, 6]. The guidelines provide, therefore, that in case of abnormal diagnostic cytology (ASCUS and LSIL), found on screening, make a colposcopy, and/or as an alternative strategy is recommended HPV molecular biology research, while others, advising molecular research as co-colposcopic examination tests [3, 7]. This research allowed the authors to detect the presence or absence of HPV in low- or high-risk virus (16,18,31,33,35, 39,45,51,52,56,58,59,68,73,82). There are several methods of molecular biology and whichever is used, only the presence/absence of the viral genome in cells and tissues, viral genotype, or quantification of the viral load can be identified. The various molecular methodologies are officially recognized, although hibryd capture II (HCII) and polymerase chain reaction (PCR) are most often used [8-12]. The infection caused by the simultaneous presence of multiple HPV genotypes in patients with ASCUS and LSIL cytology is an important risk factor for the emergence of high-grade CIN and squamous cell cervical cancer in women undergoing colposcopy. The purpose of this observational epidemiological study was to determine, through real-time PCR, the simultaneous presence of several high-risk HPV types and their incidence in the presence of high-grade CIN in patients undergoing colposcopy after US cytology ASC and LSIL [13, 14].

Materials and Methods

The study was conducted at the “San Sebastiano and Sant’Anna” Hospital of Caserta and the San Carlo testing center of Caserta. The recruitment of patients occurred prospectively and spontaneously at the Colposcopy Clinic of the Second University of Naples based in the Caserta Hospital. Subjects of the study included 2,500 patients, recruited from a strong response to the authors observation, between the years 2009-2010, with the following inclusion criteria: aged between 18 and 70 years, under screening investigation that resulted positive for ASC-US or LSIL. Exclusion criteria included: confirmed malignant disease, HIV-seropositive patients, chemotherapy or radiation therapy for pelvic diseases even before the recruitment or at any other time during the study period, hysterectomy, inadequate research and HPV cytological reading, and refusal of informed consent. All patients were subjected to: anamnesis, gynecological visit, colposcopy, and biopsy; HPV typing through real-time PCR technique.

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Results

During the study, 2,500 patients diagnosed with ASCUS/LSIL were recruited; 96% of these (2,400) were submitted to colposcopy, the remaining 4% (100) did not accept continuation in the study. Of the patients undergoing colposcopy, a group of 144 (6%) patients were excluded because they did not fit the inclusion criteria. Of the remaining 2,256 patients, 26% (587) presented with ASCUS cytology and 74% (1,669) LSIL. The pathological diagnosis of lesions of high-level and low-grade CIN was made by biopsy, performed during the course of colposcopy only in 65% (1,466) of patients. The 790 samples were not biopsied during colposcopy because, despite having cytologic results with ASC-US and LSIL, colposcopic pictures did not suggest biopsy and histological investigation. The prevalence of histological diagnosis was negative in 34% (498) patients, positive in 43% (630) patients for CIN1 lesions (253 and 377 patients with ASCUS and LSIL, respectively), the percentage of CIN2 was 22.4% (328) of patients recruited (132 and 196 patients with ASCUS and LSIL, respectively), and finally the prevalence of CIN3 or cervical cancer was 0.6% (8) (3 and 5 patients in case of ASCUS cytology and LSIL, respectively) (Figures 1-2). The prevalence of HPV was 70% (1579/2256); 65% (1,466) of patients undergoing biopsy, depending on the degree of pre-neoplastic lesions were identified as high-risk HPV: HPV 16 (319/1466), HPV 18 (164/1466), HPV 45 (76/1466), HPV 31 (215/1466), HPV 52 (145/1466), HPV 58 (55/1466), HPV 56 (79/1466), HPV 51 (110/1466), HPV 6 (138/1466), HPV 11 (88/1466), HPV 42 (34/1466), (Figure 3) HPV 53 (43/1466). The remaining 35% of patients did not have a biopsy because they did not show suspicious lesions during colposcopy examination. Research of the simultaneous presence of multiple viral types demonstrated the role played by the association of high-risk oncogenic HPV (16, 18, 31, 33, 52 and 58) in CIN2 and CIN3. In fact, in the latter (30% of cases), more HPV co-infection was detected, which was expressed mainly with associations 16-18 (70%), 16-33 (18%) and 16-52 (12%). HPV 45 merits different attention, as is clear from recent literature, even if CIN3 are present in a low percentage of high-grade lesions, while more viral cervical adenocarcinoma are present (Figure 4).

Discussion

The results of this study suggest that the infection caused by the simultaneous presence of multiple HPV genotypes in patients undergoing colposcopic examination, as a result of ASCUS or LSIL diagnosis, seems to be associated with a significantly increased risk of high-grade CIN or invasive cancer compared to cases where there is the presence of single viral infections. Since the presence of viral DNA was detected in all cervical cancers, it is clear that the finding of papillomavirus infection, at an early stage, may have a prognostic and predictive value [7]. In fact, this event may result in cellular DNA mutations that cause the appearance of abnormal clones in cervical cells until carcinoma. These biological findings allowed the authors to understand how a high-risk HPV infection contracted at a young age may have a prognostic significance when it appears worse in older age, in the presence of persons with insufficient immunological response, and at greater risk of exposure to sexually transmitted oncogenic cofactors [15]. From this rationale, is important to seek the presence of viral DNA in the lower female genital tract. Conventional cervical screening has greatly reduced the mortality of cervical cancer, but this method is inadequate and is responsible for a significant percentage of false negatives that can not be eliminated and can reach 10%. Furthermore, cytologic shortcomings may generate higher false negatives in pre-invasive forms, where the preventive role is very important compared to the invasive form. These deficiencies are due to the fact that the Pap test identifies abnormal cellular activity produced by the virus or histological pathologies that absolutely need to be confirmed by histological diagnosis. For viral infection diagnosis, HPV testing result is instead much more sensitive. In addition, the test can be used to highlight the simultaneous presence of different viral types, as most recent literature shows [16], which are correlated with high-risk injury. In fact, women with persistent HPV infection or co-infection of high-risk types have more than 300 times higher risk of developing CIN compared to women who test negative. From these observations the proposal to integrate HPV testing with cervical screening, as this combined testing would significantly reduce the mor-
Role of the association of high-risk HPV identified by real-time PCR in cervical preneoplastic lesions

In addition, HPV-DNA testing could have a rational in clearing doubts in cases of low-grade cytology or histology in order to establish an appropriate treatment program or follow-up. The test is also used to determine the persistence of infection after treatment for CIN (conization or LEEP) or in cases where the colposcopic lesion shows a normal Pap test. However, there still remains the difficulty of choosing which method is better in terms of sensitivity and specificity for the detection and typing of HPV. There are various molecular methods officially recognized, although those most often used are HCII and PCR [8, 10, 17, 18]. The real-time PCR used measures the amplification in real time during the exponential phase of the development of the method, when the amplification efficiency is affected minimally by the variables of reaction, allowing for more accurate results compared to traditional PCR. Then the progress of the reaction can be monitored while it is still in progress, and the data obtained at the end of the cycle can be used to make a relative quantification of the amplified fragment. This is possible through the use of fluorescent markers which follow the same kinetic accumulation of the PCR reaction. The fluorescence, emitted as a result of a specific radiation from the light source of the thermal cycler, is measured in real-time by a CCD camera and is directly proportional to the amount of amplified product. This also allows the degree of viral replication to be obtained by calculating the logarithm of the concentration of HPV in 100,000 human cells, with the following clinical interpretation: < 3 clinically insignificant or little meaning; evaluated clinically by 3-5, the possible risk of dysplasia; > 5 clinically important, high-risk for dysplasia.

The above differs substantially from other methods currently used, in fact: 1) The HCII is a qualitative test and semi-quantitative test, which uses the reaction between HPV DNA, if present, and specific RNA probes, with the formation of hybrid DNA / RNA in the liquid phase, which are captured on solid phase by an Ab universal microplate and finally detected by a chemiluminescent substrate. This test can identify low-risk genotypes 5 (6,11,42,43) and 13 high-risk (16,18,31,33,35,39,45,51,52,56,58,59,68) [17, 18]. The multiplex PCR agarose gel analysis allows more targets simultaneously by using mixtures of primers or degenerate primers. It
Multiple HPV infection is associated with a noticeably increased infection duration which, in turn, could increase the risk of CIN [23]. As the duration of infection increases, its chance of detection as a prevalent case increases, causing an over-representation of severe infection in prevalent studies. Additional longitudinal investigations in larger populations will be necessary to clarify this issue.

Although the ratio is much lower for ASC-US/LSIL as reported in the literature, in the authors’ laboratory, the relationship seems to be reversed, as also reported by Spinillo et al. [24].

References

Role of the association of high-risk HPV identified by real-time PCR in cervical preneoplastic lesions


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Establishment of a visualized nude mouse model of cervical carcinoma with high potential of lymph node metastasis via total orthotopic transplantation

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Summary

Objective: To screen cervical carcinoma (CC) SiHa subgroups with high potential of lymphatic metastasis and establish a visualized nude mouse model of cervical carcinoma with a total orthotopic transplantation approach. Methods: A cervical carcinoma SiHa subgroup with high potential of lymphatic metastasis was isolated by in vitro and in vivo primary culture and continuous passage screening of cervical carcinoma SiHa cells that stably expressed enhanced green fluorescent protein (EGFP). Forty male nude mice aged 6-8 weeks were equally randomized to group A receiving unscreened SiHa/EGFP cells, and group B received in vitro and in vivo screened SiHa/EGFP cells with high potential of lymph node metastasis. Results: In the 20 animals of group A receiving orthotopic transplantation of unscreened SiHa/EGFP cells, the primary tumors were small; local lymph node metastasis was observed in five animals; local organ invasion and distal lymph node metastasis were observed in two animals; and no lung metastasis was observed. In the 20 animals of group B receiving screened SiHa/EGFP cells, local lymph node metastasis occurred in all animals; distal lymph node metastasis was observed in 18 animals; and lung metastasis was observed in seven animals. Conclusion: A cervical carcinoma SiHa subgroup with high potential of lymphatic metastasis was isolated and a visualized nude mouse model of cervical carcinoma with high potential of lymph node metastasis was established through total orthotopic transplantation successfully. This provided a good platform for the further study of cervical carcinoma-related mechanisms, especially mechanisms of lymphatic metastasis in cervical carcinoma.

Key words: Cervical carcinoma; Orthotopic transplantation; Lymphatic metastasis; Visualized animal model; Enhanced green fluorescent protein (EGFP).

Introduction

Cervical carcinoma (CC) is a common malignant tumor in gynecology, affecting about 500,000 women in the world yearly. One-third of the yearly total incidence occurs in China, and is an important cause of cancer-related deaths in women next to breast cancer. Lymphatic metastasis is the main metastatic route of CC, and the presence or absence of metastasis in pelvic lymph nodes is an independent prognostic factor in CC patients. It is therefore necessary and important to establish an ideal CC animal model to further explore the pathogenesis of CC and mechanisms of lymphatic metastasis, develop new contrast agents, and evaluate potential therapeutic methods for the treatment of CC.

Materials and Methods

Materials used in this study included enhanced green fluorescent protein (EGFP)-labeled CC SiHa cell strain prepared and stored in our laboratory; RPMI1640 solution (Genome Biomedical Technologies Co., Ltd., Hangzhou, China); fetal bovine serum (FBS) (sijiqing, Hangzhou); healthy specific pathogen-free (SPF) grade male nude mice aged 6-8 weeks and weighing 20-25 g and male nude mice aged 3-4 weeks and weighing 15-20 g (Experimental Animal Center of Southern Medical University, Guangzhou, China).

Inoculation of CC SiHa/EGFP cells via the tail root

CC SiHa/EGFP cells were cultivated routinely in 10% FBS-RPMI1640 at 37°C, 5% CO₂ and saturated humidity. Non-contaminated cells growing to 80-90% confluence (exponential phase) were harvested from the medium and washed twice with PBS, added with 0.25% trypsin containing 0.02% EDTA (just submerging the cell surface), digested for 1-2 min, and observed under an inverted microscope. When most cells became round or oval, the medium containing 10% FBS-RPMI1640 (more than two-fold volume of the trypsin) was added to terminate the digestion. Confluent cells were dispersed gently to form cell suspension, which was pipetted into a centrifuge tube and centrifuged at 600 rpm for 3 min. After removing the supernatant, cells were washed with serum-free RPMI1640 twice, added with an appropriate amount of RPMI1640 for re-suspension with the cell concentration adjusted to 1 x 10⁷ cells·mL⁻¹ to prepare single-cell suspension, which was then placed on ice for use.

Three male nude mice aged 3-4 weeks were injected with 0.2 ml single-cell suspension (2 x 10⁶ cells/each) via the tail root, and raised in the SPF environment. The growth status of the animals and tumors was observed weekly.

Screening of highly metastatic CC cells

When the above tumor-bearing mice presented with obvious signs of cachexia failure, they were anesthetized by intraperi-

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touetal (IP) injection of 20 mg/kg 1% sodium pentobarbital. The
distal metastatic lesion was removed under strict aseptic condi-
tions under the guidance of total internal reflection fluorescence
(TIRF) imaging, from which tissue that was active in the
periphery were picked out, submerged in ice PBS containing
500 IU/ml penicillin and 500 IU/ml streptomycin, and trans-
ferred as soon as possible to the laboratory superclean bench for
primary culture. The tumor tissue was removed from the super-
clean bench, and washed with PBS containing penicillin and
streptomycin three times. Red blood cells and surface contami-
nants were removed. The tissue was washed more times if it
was heavily contaminated. The washed tissue was then trans-
ferred to a clean sterile penicillin vial and sheared to 0.5-1 mm³
chips with ophthalmologic scissors, which were spread evenly
over the bottom of the flask with a dripper, to which 5 ml
PRIM1640 containing 500 IU/ml penicillin, streptomycin and
20% FBS was added. The flask was placed upside down and
cultured in a 37.5% CO₂ incubator until cells grew to conflu-
ence. One hour after culture, the flask was turned over gently to
moisten the tissue chip, and at 2 h the flask was turned to the
normal position gently. By 30 h, individual cells were seen
creeping out of the periphery of the tissue chip, and increased
in number daily. By day 4, more cells were seen creeping out of
the tissue chip, and generation amplification was performed
by day 10 when even more cells were seen creeping out of the
tissue chip. Fluorescence-labeled cells were screened out by
flow cytometry and amplified. The amplified cell suspension
was injected to the tail root of the mice. This procedure was
repeated in triplicate to screen out a CC SiHa subgroup with
high potential of metastasis.

Establishment of the visualized nude mouse model through
orthotopic transplantation

Ten healthy SPF male nude mice aged 3-4 weeks were
equally randomized to two groups: animals in group A receiv-
ing unscreened CC SiHa/EGFP cells, and animals in group B
receiving CC SiHa/EGFP cells that were screened continuously
in vitro via subcutaneous neck injection of 0.2 ml single-cell
suspension of cervix carcinoma (2 x 10^6 cells/each). The
animals were then sent back and raised in the SPF environment,
and the growth status of the mice and subcutaneous tumor was
observed closely.

To raise the adaptive ability and survival of tumor cells in the
body of the mice, they were passaged continuously in vivo. When the first passage of subcutaneous cervix carcinoma cells
grew to a 0.5 cm³ tumor, the animal was anesthetized IP with
20 mg/kg 1% sodium pentobarbital. The peripheral tissue with
active tumor cell growth was removed, washed with normal saline
(NS) containing 100 IU/ml penicillin and streptomycin
three times, placed in a clean sterile container, sheared into 1-2
mm³ tissue chips, placed on ice and moistened with NS. An
additional five male nude mice aged 3-4 weeks were anes-
thetized and tumorigenized by the subcutaneous pocket method.
This inter-mouse passage procedure was repeated three times,
and each passage consisted of at least five mice. The subcuta-
aneous tumor of the last passage was used as the tumor source
for surgical orthotopic implantation (SOI) of CC.

Forty male nude mice aged 6-8 weeks were equally random-
ized to two groups: animals in group A received passage 3 sub-
cutaneous tumor tissue from inoculation of unscreened
SiHa/EGFP cells (control group), and animals in group B
received passage 3 subcutaneous tumor tissue from continuous
inoculation of screened SiHa/EGFP in vitro (study group). The
animals were anesthetized by IP with 20 mg/kg 1% sodium
pentobarbital, fixed and draped aseptically. A median incision
was made in the lower abdomen to expose the abdominal cavity.
The omentum and intestine were pushed upward and moistened
with normal saline (NS)-soaked gauze. The urinary bladder was
exposed, behind which was seen the “Y”-shaped tubular uterus.
A small incision was made at the cervical level, through which
the fresh tumor chip was fixed in the cervical muscle with a 9/0
non-traumatic suture. The abdominal organs were then restored
in place, and the abdomen was closed with 4/0 absorbable
catgut. The skin at the suture site was sterilized with alcohol.
The animals were sent back to the SPF environment. Caution
should be taken lest the uterus-related vessels and lymph node
should be damaged during the surgical procedure.

Results
1) Subcutaneous and tail root injection of CC SiHa and
SiHa/EGFP single-cell suspension (2 x 10^6 cells/each)
and orthotopic transplantation of the tumor tissue chip
were 100% successful.

2) The overall fluorescence imaging system suggested
extensive distal metastasis at week 7 of tail root injection
of tumor cells other than the primary focus, indicating
that spontaneous metastasis was likely to occur in
animals receiving tail root injection of tumor cells.

Metastasis of orthotopic transplantation of CC tumor

The 20 mice in group A collapsed and died about two
to three months after orthotopic transplantation of the CC
tumor, when autopsy showed that the tumors grew
slowly; their size was relatively small; local lymph node
metastasis was seen in five animals; local organ inva-
sion and distal lymph node metastasis were observed
in five animals; and no lung metastasis was observed. The
20 mice in group B collapsed and died about two
months after orthotopic transplantation of the CC tumor, when autopsy showed that other than the primary lesions, local lymph node metastasis was observed in all 20 animals; distal lymph node metastasis was observed in 18 animals (Figure 1), and distal lung metastasis was observed in seven animals. The metastatic lesions were removed and HE stained (Figure 2). The degree of metastasis was converted digitally as + = 1, ++ = 2, +++ = 3 and ++++ = 4, analyzed statistically, and mapped (Figure 3). The result showed that the difference between the two groups was statistically significant (p < 0.01).

A CC Siha subgroup with high potential of lymph node metastasis was screened out successfully, and a visualized nude mouse model through orthotopic transplantation was established. The overall fluorescence imaging system suggested that local lymph node metastasis was the main form of metastasis in the early phase of CC orthotopic transplantation, followed by invasion of the lung and other distal organs, and that the size of the primary tumor was positively correlated with invasion (Table 1). The volume (V) of the tumor chip was determined by measuring three mutually vertical diameter lines with a caliper, which were marked as L (length), W (width) and H (height). Calculation was made by using the equation: V = L x W x H/2.

Pathological results
Pathological specimens obtained at two weeks after orthotopic transplantation suggested that the location of orthotopic transplantation was correct. Microscopy showed that the tumor cells had a large volume, rich cytoplasm, deep-stained nuclei, and obvious atypia. The formation of cancer nests was seen (Figure 4). The cancer tissue inside the lymph node metastasis had an extremely rich blood supply; tumor cells were poorly differentiated; the nuclei were large and deeply stained, mostly showing pathological mitosis. Lymph node CK immunohistochemistry showed brown-yellow (Figure 5).
Establishment of a visualized nude mouse model of cervical carcinoma with high potential of lymph node metastasis via orthotopic etc.

Discussion

The orthotopic transplantation strategy and tumor cell heterogeneity are two important ideas promoting the rapid development of tumor animal models in recent years. They are the important and generally accepted theoretical basis for human cancer invasion and metastasis [1]. Lymphatic metastasis is the main route of CC metastasis. Early detection of lymph node metastasis in the region of primary CC is the core problem in selecting an appropriate therapeutic program. Therefore, establishment of an ideal animal model of lymph node metastasis is an indispensable tool for such research, but there is no study reporting the establishment of a nude mouse model of CC through orthotopic transplantation.

Tumor metastasis is a complex and highly selective process, based on the theory of tumor cell heterogeneity advanced by Fidler et al. [2] in the 1970s. Fidler et al. [3] succeeded in isolating B16-F10 subgroups with high potential of lung metastasis by injecting B16 melanoma cells through the C57BL/6 mouse tail vein. Subsequently, Nicolson et al. [4] isolated subgroups with high potential of adrenal and ovarian metastasis from B16. Later, many researchers successfully isolated tumor cells with high potential of metastasis by posing selection pressure intentionally. They even isolated metastasis-associated tag genes in breast cancer [5-7], digestive tract tumors [8], and respiratory tract tumors [9]. Compared with their parent cell lines, these highly metastatic subgroups presented with high abilities of invasion and metastasis, and high tumorigenesis as well.

Based on the above findings, we postulated that lymph node metastasis in CC patients may be due to subgroups of cells with high potential of metastasis in the primary tumor. In light of previous experience of other studies, we obtained a Siha subline with high potential of lymph node metastasis by primary culture and continuous passage screening via tail root injection in nude mice, and established a visualized nude mouse model through orthotopic transplantation to analyze the status of lymph node metastasis. The result confirmed the existence of a subgroup with high potential of lymph node metastasis, thus laying a foundation for exploring the molecular mechanism of lymph node metastasis in CC.

The development of bioluminescence technique provides a new approach to the study of tumor biology. In 1997, Chishima et al. [10] first used green fluorescent protein (GFP) in tumor research in vivo, thus opening up a visual precedent for cancer research. Later, Hayashi et al. [11] used GFP-bearing tumor cells and observed how cells moved in lymphatic ducts and how they entered lymph nodes. Cells used in the present study fully exhibited the necessary characteristics of the fluorescent tumor model system, whereby micrometastatic foci could be easily detected by the fluorescent label borne by tumor cells, and actual tumor growth could be reflected more accurately, thus reducing human experimental errors.

In summary, the development and progression of CC is complex. The establishment of the nude mouse model of cervical carcinoma with high potential of lymph node metastasis through orthotopic transplantation makes it possible to reproduce the biological characteristics of CC and reflect multiple clinical aspects of the disease, especially the patterns of CC tumor invasion and metastasis. It was found that metastasis in CC follows the pattern of local lymphatic metastasis in the early phase, followed by invasion of adjacent organs, distal lymphatic metastasis, and finally metastasis to the lungs and other organs. It was also found that the size of the primary tumor is positively correlated with tumor metastasis and invasion. These findings indicate that it is feasible to use the established model to explore mechanisms underlying the development and progression of CC, develop new therapeutic methods and drugs, and study mechanisms and
therapies of lymph node metastasis. In addition, the existence of a fluorescent reporter molecule makes quantitative analysis of the corresponding experimental results more flexible and effective.

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Comparison of outcomes in patients treated with multi-agent regimens of cisplatin, adriamycin, and VP-16 versus carboplatin and paclitaxel for advanced and recurrent endometrial cancer


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Introduction

The optimal chemotherapeutic regimen for advanced or recurrent endometrial cancer remains unknown. There is currently little hope of a cure for patients with metastatic or recurrent endometrial cancer. The current standard chemotherapy combination was established following the publication of Gynecologic Oncology Group (GOG) 177, which recognized the regimen of doxorubicin, cisplatin, and paclitaxel (TAP) as the optimal front line therapy, with median overall survival of 15 months [1].

Doublet therapy with carboplatin and paclitaxel, used as the comparison arm in GOG 177, has activity advanced endometrial cancer [2-4]. Other modalities including hormonal and progesterone therapies are effective in the treatment of recurrent and metastatic carcinoma with response rates of 9-34% [5-9].

The authors report a retrospective review of patients with Stage III, IV, and recurrent endometrial cancer who received adjuvant chemotherapy at Roswell Park Cancer Institute over a period of 21 years. Two patient groups were defined based on treatment received: cisplatin, adriamycin, and VP-16 with or without megace (PAV-M), or carboplatin and paclitaxel (CT).

Material and Methods

Between 1980 and 2001, 69 patients were retrospectively identified with advanced or recurrent endometrial carcinoma who received treatment with multi-agent chemotherapy. Forty-two patients were eligible for review based on pathological diagnosis and treatment with PAV/M or CT. Treatment schedule for PAV-M consisted of cisplatin (20 mg/m2) daily for three days, etoposide (75 mg/m2) daily for three days, and adriamycin (40 mg/m2) on day one, and megace 160 mg daily every three weeks until progression of disease. CT consisted of carboplatin AUC 5 and paclitaxel 175 mg/m2 every three weeks until progression of disease.

Toxicity parameters determined dose modification, a 20% dose reduction of adriamycin and etoposide occurred if white blood count of < 1,000/mm3 white blood cells or platelets < 50,000/mm3 platelets was identified. Complete blood counts and basic metabolic panels were performed weekly. Electrocardiogram and physical examination were performed every 21 days prior to next cycle of each therapy. Baseline cardiac ejection fraction was obtained before the first course of adriamycin and prior to each additional course after five courses were administered. Cardiac toxicity was defined as a decrease in cardiac ejection fraction of > 10% or the development of congestive heart failure. Baseline renal function was established. Nephrotoxicity was defined as increased serum creatinine > 2 mg/dl.

Study outcomes included overall survival and time to progression, defined by World Health Organization (WHO) criteria. Complete response was identified as disappearance of all lesions. Partial response was > 50% reduction in index lesions, stable disease a 50% reduction compared with baseline nor 25%
increase in disease. Progressive disease was defined as >25% increase in index lesion or appearance of new lesions. The duration of overall survival was the interval between diagnosis and death. Observation time was the interval between diagnosis and last contact (death or last follow-up). Data were censored at the last follow-up for patients with no evidence of recurrence, progression, or death.

Comparison between groups was calculated using the student t-test. Kaplan Meier log rank analysis was used to estimate overall survival and disease-free progression. A p value of 0.05 was considered statistically significant.

Results

The clinical characteristics of patients included in this review are shown in Table 1. No statistical difference between patient’s age, stage, grade of tumor, histology, previous surgical intervention, or primary treatment was identified. However, patients in the CT group were older, average age 70 years compared to 62 years (p = 0.7). Patients were more likely to be treated with radiation in the PAV/PAV-M group (57% vs 17%, p = 0.01). Among the PAV-M/PAV group, 42% required dose modification compared to 11% in the CT group (p = 0.07).

The median time to follow-up was 52 months. Disease-free progression appeared longer in the PAV/PAV-M group compared to CT group, but was not statistically significant (44 months vs 16 months p = 0.03) (Figure 1). No difference in overall survival between the two groups was identified (84 months vs 34 months p = 0.9) (Figure 2).

Discussion

The standard chemotherapy regimen for advanced or recurrent endometrial cancer is cisplatin, paclitaxel, and adriamycin (TAP). This regimen was shown to be superior or in a large GOG group phase III clinical trial (GOG 177). However, this regimen has significant hematologic and non-hematologic toxicities. Hematological toxicity was noted in 3% of patients; however, grade 3 neurologic toxicity was seen in 12% of patients, grade 2 neurologic toxicity was seen in 27% of patients [1]. Due to significant toxicities with TAP regimen, CT have been increasingly adopted in the treatment of women with advanced and recurrent endometrial cancer.

CT has a favorable side-effect profile as demonstrated in epithelial ovarian carcinoma [10, 11]. This has also been studied in prior phase II studies evaluating the efficacy and toxicity of CT in endometrial cancer [3, 12, 13]. Consequently, the GOG recently concluded a phase III trial of CT vs TAP (GOG 209) and results are pending. Comparative studies evaluating CT against previously utilized regimens are lacking.

This retrospective study was conducted to assess the response and toxicity associated with PAV/PAV-M, an
Comparison of outcomes in patients treated with multi-agent regimens of cisplatin, adriamycin, and VP-16 versus carboplatin etc.

established regimen in the Institution compared to CT [14, 15]. The authors found a trend towards a longer DFS and OS with the differences did not achieve statistical significance most likely due to small sample size. Dose modification was performed in 42% of patients received PAV/PAV-M combination compared to 11% in the CT group. However, all the patients in the PAV/PAV-M group completed at least seven cycles of chemotherapy.

This study is limited by the small sample size and overall poor outcomes associated with advanced or recurrent endometrial cancer. The small sample size could account for the non-significant values found in overall survival and disease-free progression. If the awaited results of GOG 209 show CT to be equivalent or superior to TAP, triplet-based therapy will be discontinued due to increased toxicity with little benefit. However, if the regimen of CT is inferior, other triple or quadruple complications such as PAV and PAV-M need to be re-evaluated in a prospective setting.

Conclusion

In conclusion, as progress is being made in the treatment of advanced and recurrent endometrial cancer, older multi-agent chemotherapy regimens such as PAV or PAV-M need to be re-evaluated since they may be as effective and similarly tolerated as other triplet therapy. Furthermore, future efforts are necessary to identify the subset of patients that will more likely respond to PAV/PAV-M as compared to CT or newer regimens in advanced or recurrent endometrial cancer.

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Evaluation of depth of myometrial invasion by magnetic resonance imaging in patients with endometrial carcinoma

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Summary

Aims: The aim of this study was to evaluate clinical accuracy of magnetic resonance imaging (MRI) staging of myometrial invasion in patients with endometrial carcinoma. Methods: The study group consisted of 37 women with endometrial carcinoma who underwent preoperative workup, including MRI, and surgical staging at Goztepe Training Hospital, Istanbul, Turkey. We collected clinical, MRI, surgical and histopathological data of the study subjects from patients’ charts. Results: The mean patient age was 57 years (range 39-76 years). Of the subjects, 32 (86.5%) had endometrioid carcinoma. After histopathological evaluation, we found that four (10.8%) patients had no myometrial invasion, 14 (37.8%) had superficial myometrial invasion, and 19 (51.3%) had deep myometrial invasion. Overall, the accuracy of MRI staging increased in accordance with the increase of surgical stage of endometrial carcinoma. Overall, clinical success of MRI staging was higher in patients with deep myometrial invasion. Conclusion: The accuracy of MRI to depict the depth of myometrial invasion increases in accordance with surgical stage in patients with endometrial cancer. The combination of MRI and clinical findings may be helpful in determining the extent of surgery.

Key words: Endometrial cancer; Magnetic resonance imaging; Myometrial invasion.

Introduction

Endometrial cancer remains the most common female genital tract malignancy in the Western world [1], with a rising incidence in developing countries. It may develop in normal, atrophic, or hyperplastic endometrium. The management of this disease is predominantly surgical. A localized endometrial cancer (Stage I) has an excellent prognosis following surgery alone [2, 3]. The current International Federation of Gynecology and Obstetrics (FIGO) staging system [4] has identified a number of prognostic factors that predict for a high risk of disease recurrence including histology, tumor grade, depth of myometrial invasion (MI), and the presence of lymph node metastases.

Magnetic resonance imaging (MRI) is a useful diagnostic tool for the evaluation of myometrial invasion depth in patients with endometrial carcinoma [5-12]; its accuracy is higher than other imaging modalities, such as ultrasoundography (US) and computed tomography (CT) [13, 14]. MRI is helpful to assess the presence of deep myometrial invasion, the extent of cervical invasion, and in identification of enlarged pelvic and paraaortic lymph nodes. These clinical findings provide important information for the gynecologist in planning surgery, especially for less invasive surgical approaches. Dynamic MRI performed during the injection of gadolinium chelates is useful in depicting endometrial carcinoma, owing to different vascularity of the areas invaded with endometrial carcinoma and in helping to differentiate it from fluid filling the endometrial cavity [15].

Materials and Methods

Study population

This study involved 37 patients with endometrial cancer who underwent preoperative workups that included an MRI and surgical staging at Goztepe Training Hospital, Istanbul, Turkey. The clinical, imaging, surgical, and histopathological data of the study population were collected.

Endometrial cancer was diagnosed on the basis of a previous endometrial sampling. Preoperative endometrial biopsy specimens were evaluated for histological type and grade according to the 1988 FIGO classification.

MRI examinations were performed with a 2 Tesla MR system (Elscint Gyrex 2T-V, Haifa, Israel) for assessing the depth of myometrial invasion before surgery. All examinations were performed with a full-filled urinary bladder when the patient was lying on her back. The images were made from the level of aortic bifurcation up to the level of symphysis pubis with a pelvic coil. T1 weighted (W) images (repetition time (TR)/echo time (TE) 400/12 ms) in axial and coronal planes and T2W images (TR/TE 4000/126 ms) in the axial and sagittal planes were obtained with a 1-8 mm slice thickness, 26-35 cm of field of view (FOV), 260 x 350 matrix, 2-4 NEX value for all images.

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Table 1. — Preoperative endometrial histopathology.

<table>
<thead>
<tr>
<th>Type</th>
<th>Premenopausal (n = 9)</th>
<th>Menopause (n = 28)</th>
<th>Total (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid carcinoma</td>
<td>9 (24.3%)</td>
<td>23 (62.2%)</td>
<td>32 (86.5%)</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>0 (0%)</td>
<td>2 (5.4%)</td>
<td>2 (5.4%)</td>
</tr>
<tr>
<td>Serous carcinoma</td>
<td>0 (0%)</td>
<td>1 (2.7%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Malignant mixed mullerian tumor</td>
<td>0 (0%)</td>
<td>1 (2.7%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Atypical complex hyperplasia</td>
<td>0 (0%)</td>
<td>1 (2.7%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (24.3%)</td>
<td>28 (75.7%)</td>
<td>37 (100%)</td>
</tr>
</tbody>
</table>

Table 2. — Preoperative magnetic resonance imaging (MRI) stages.

<table>
<thead>
<tr>
<th>MRI stage</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>11</td>
<td>29.7%</td>
</tr>
<tr>
<td>Ib</td>
<td>9</td>
<td>24.4%</td>
</tr>
<tr>
<td>Ic</td>
<td>17</td>
<td>45.9%</td>
</tr>
</tbody>
</table>

Surgical staging and pathological evaluation

All patients underwent surgical staging after preoperative evaluation. Standard procedures for surgical staging of endometrial cancer were used including peritoneal washing cytology, exploration of the peritoneal cavity, biopsy of any suspicious lesion, simple hysterectomy and bilateral salpingo-oophorectomy with laparotomy. During histopathological examination, the uterus was opened, and the endometrial cavity was inspected. The cut surfaces were inspected. One full thickness section of the endometrial wall at the point of deepest invasion, as determined in the macroscopic exam, was submitted for microscopic examination. The slide was evaluated microscopically for the histological type of the tumor, FIGO histological grade and extent of myometrial invasion.

Statistical analysis

Data are expressed as mean (min-max) or percentage. The patients were subdivided into grades 1-3 subsets. The sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of MRI were used to determine the depth of myometrial invasion. The gold standard was the pathological report.

Results

The study population consisted of 37 patients with endometrial cancer who were diagnosed by endometrial biopsy and underwent preoperative evaluation, including MRI, between endometrial biopsy and the staging operation. The mean patient age was 57 years (range, 39-76 years). A total of 35 (94.6%) of patients had adenocarcinoma of the endometrium. The remaining cases included one clear cell carcinoma of the endometrium, and one serous tumor. Four (10.8%) had no myometrial involvement, 15 (40.5%) had superficial myometrial invasion and 18 (48.6%) had deep myometrial involvement. FIGO staging was based on the pathological reports.

Table 1 presents the histopathologic findings of preoperative endometrial sampling of the study subjects. Most of the patients had endometrioid carcinoma and were in menopause.

Table 3. — Association between surgical stages and histopathologic grades of tumors.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Grade 1 (n = 13)</th>
<th>Grade 2 (n = 16)</th>
<th>Grade 3 (n = 8)</th>
<th>Total (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>4 (10.8%)</td>
<td>0</td>
<td>0</td>
<td>4 (10.8%)</td>
</tr>
<tr>
<td>Ib</td>
<td>6 (16.2%)</td>
<td>5 (13.5%)</td>
<td>3 (8.1%)</td>
<td>14 (37.8%)</td>
</tr>
<tr>
<td>Ic</td>
<td>2 (5.4%)</td>
<td>5 (13.5%)</td>
<td>1 (2.7%)</td>
<td>8 (21.6%)</td>
</tr>
<tr>
<td>IIa</td>
<td>0 (0%)</td>
<td>1 (2.7%)</td>
<td>2 (5.4%)</td>
<td>3 (8.1%)</td>
</tr>
<tr>
<td>IIb</td>
<td>0 (0%)</td>
<td>3 (8.1%)</td>
<td>1 (2.7%)</td>
<td>4 (10.8%)</td>
</tr>
<tr>
<td>IIIa</td>
<td>1 (2.7%)</td>
<td>2 (5.4%)</td>
<td>1 (2.7%)</td>
<td>4 (10.8%)</td>
</tr>
<tr>
<td>IIIb</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>13 (35.4%)</td>
<td>16 (43.2%)</td>
<td>8 (21.6%)</td>
<td>37 (100%)</td>
</tr>
</tbody>
</table>

Table 4. — Postoperative endometrial histopathology.

<table>
<thead>
<tr>
<th>Type</th>
<th>Premenopausal (n = 9)</th>
<th>Menopause (n = 28)</th>
<th>Total (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid carcinoma</td>
<td>9 (24.3%)</td>
<td>26 (70.3%)</td>
<td>35 (94.6%)</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>0 (0%)</td>
<td>1 (2.7%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Serous carcinoma</td>
<td>0 (0%)</td>
<td>1 (2.7%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (24.3%)</td>
<td>28 (75.7%)</td>
<td>37 (100%)</td>
</tr>
</tbody>
</table>

Table 5. — Depth of myometrial invasion according to histopathology.

<table>
<thead>
<tr>
<th>Myometrial invasion depth</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4</td>
<td>10.8%</td>
</tr>
<tr>
<td>Less than 50 %</td>
<td>15</td>
<td>40.5%</td>
</tr>
<tr>
<td>More than 50%</td>
<td>18</td>
<td>48.7%</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 6. — Association between myometrial invasion depth and endometrial histopathology.

<table>
<thead>
<tr>
<th>Myometrial invasion depth</th>
<th>Histopathology</th>
<th>None (n = 4)</th>
<th>Less than 50% (n = 15)</th>
<th>More than 50% (n = 18)</th>
<th>Total (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid carcinoma</td>
<td>4 (10.8%)</td>
<td>14 (37.8%)</td>
<td>17 (45.9%)</td>
<td>35 (94.6%)</td>
<td></td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>0 (0%)</td>
<td>1 (2.7%)</td>
<td>0</td>
<td>1 (2.7%)</td>
<td></td>
</tr>
<tr>
<td>Serous carcinoma</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1</td>
<td>1 (2.7%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4 (10.8%)</td>
<td>15 (40.8%)</td>
<td>18 (48.6%)</td>
<td>37 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows the preoperative MRI stages of the study population. Most of the patients had deep myometrial invasion according to MRI staging.

Table 3 displays the association between surgical stages and histopathologic grades of tumors of the participants. The ratio of patients having early stage and grade 1 endometrial cancer was higher.

Table 4 represents the postoperative histopathology findings of the study patients. Most of them had endometrioid carcinoma and were in menopause.

Table 5 shows the depth of myometrial invasion verified by histopathology after the surgery in the study population. Nearly 50% of the patients had deep myometrial invasion.

Table 6 represents the association between myometrial invasion depth and histopathologic type of endometrial carcinoma of the patients. Most of the patients had endometrioid carcinoma 94.6% and nearly 50% of those patients had deep myometrial invasion.
Table 7 displays the association between myometrial invasion depth and histopathologic grade of endometrial carcinoma of the participants; 43% of the patients had deep myometrial invasion and 27% had histopathologic grade 2.

Table 8 shows the association between preoperative magnetic resonance imaging (MRI) stages and postoperative stage of endometrial carcinoma.

Table 9-11 present numbers of patients with MRI Stage Ia, Ib, and Ic according to the postoperative surgical stages. Overall, the accuracy of MRI staging increased in accordance with the increase of surgical stage of endometrial carcinoma.

Table 12 displays the sensitivity, specificity, PPV, NPV, and accuracy of MRI in detecting depth of myometrial invasion.

Discussion

The study included 37 patients with endometrial cancer who were diagnosed by endometrial biopsy and underwent preoperative evaluation, including MRI, between endometrial biopsy and the staging operation. Of the subjects, 32 (86.5%) had endometrioid cancer before surgery. Overall, the accuracy of MRI staging increased in accordance with the increase of surgical stage of endometrial cancer and clinical success of MRI staging was higher in patients with deep myometrial invasion.

Ryoo et al. [18] evaluated the factors that are associated with the accuracy of MRI for predicting myometrial invasion and lymph node metastasis in 128 women with endometrial carcinoma. They found that the sensitivity, specificity and accuracy for identifying any myometrial invasion (superficial or deep) were 0.81, 0.61 and 0.74, respectively; those values for deep myometrial invasion were 0.60, 0.94 and 0.86, respectively. They reported that the sensitivity, specificity and accuracy of MRI for detecting lymph node metastasis were 50.0%, 96.6% and 93.0%, respectively. According to their data, the patients who were older had more deliveries and a larger tumor size more frequently had incorrect prediction of deep myometrial invasion.

After diagnosis of endometrial cancer with endometrial sampling, US (transabdominal and transvaginal with Doppler modalities) generally is the modality of choice for the initial imaging evaluation of uterus and adnexa [19, 20]. TVS is superior to CT and approaches MRI in its ability to depict endometrial carcinoma and myometrial, cervical, and, perhaps, parametrial invasion; however, US is not successful as a whole to depict the entire pelvic or abdominal anatomic regions. CT and MRI are more accurate staging modalities than US [21]. Both techniques allow survey of the entire pelvis, abdomen, thorax, and brain. CT is available more widely and provides rapid image acquisition, and has high spatial resolution. The advantages of CT also include the availability of GI and intravenous (IV) contrast materials. The recent advent of spiral/helical and multidetector technology has improved the multiplanar capability of CT [22].
MRI depicts the endometrium as a central zone of high signal intensity on T2-weighted images, while the myometrium is depicted at its inner aspect as a zone of low signal intensity (junctional zone) and at its outer aspect as a wider zone of intermediate signal intensity. On T1-weighted images, the endometrium has intermediate signal intensity similar to the myometrium; therefore, the endometrium is not visualized distinctly as separate from the myometrium [22].

The advantages of MRI include superior spatial and tissue contrast resolution, multiplanar capabilities, lack of exposure to ionizing radiation, and availability of noniodinated, nonnephrotoxic IV contrast material [23].

Histopathologic features of the tumor and clinical findings at presentation significantly affect the performance of imaging modality for preoperative staging of endometrial cancer. Kinkel et al. [24] reviewed the usefulness of MRI, CT, and US in imaging patients with endometrial cancer in a meta-analysis and prepared a clinical practice guideline. They concluded that CT or MRI of the abdomen and pelvis should be performed to determine the extent of tumor spread in patients at risk for disease dissemination and lymph node involvement at presentation. According to their review, MRI could depict cervical and myometrial invasion most accurately and was approximately equivalent to CT in detecting enlarged lymph nodes.

MRI, with its preeminent soft tissue contrast and multiplanar capability, is superior to US and CT in helping assess the depth of myometrial invasion, cervical invasion, and early parametrial invasion. MRI has comparable power to detect enlarged lymph nodes [22].

Frei et al. [25] evaluated whether preoperative MRI findings contribute to treatment stratification and specialist referral in patients with deep myometrial invasion of endometrial cancer. They noted that use of contrast-enhanced MRI significantly affects the post-test probability of deep myometrial invasion in patients with all grades of endometrial cancer and could be used to select patients for specialist referral.

Utsumomiy et al. [26] investigated the clinical value of T2-weighted and contrast-enhanced dynamic T1-weighted images with histologic findings in assessing the depth of myometrial invasion by endometrial carcinoma in adenomyosis. They found that when adenomyosis coexisted with endometrial cancer at the same site on T2-weighted images, contrast-enhanced dynamic T1-weighted imaging improved the accuracy of staging.

Beddy et al. [27] studied the diagnostic performance of diffusion-weighted MRI with that of dynamic contrast material-enhanced MRI in evaluating the depth of myometrial invasion and overall stage in patients with endometrial cancer. They concluded that diffusion-weighted MRI has superior diagnostic accuracy in the assessment of myometrial invasion and significantly higher staging accuracy compared with dynamic contrast material-enhanced MRI.

Lin et al. [28] reported on the diagnostic accuracy of fused T2-weighted and high-b-value diffusion-weighted MRI at 3T for evaluation of myometrial invasion in patients with endometrial cancer. They noted that fused T2-weighted and high-b-value diffusion-weighted images at 3T can provide accurate information for preoperative evaluation of myometrial invasion.

Nasi et al. [29] studied diagnostic value of fast spin echo T2-weighted and gadolinium-enhanced FMPSGR MRI sequences in assessing the depth of myometrial invasion by endometrial cancer. They noted that gadolinium-enhanced dynamic sequences increase the accuracy of MRI in diagnosing the depth of myometrial invasion. They found that could improve the visualization of the inner myometrium, the so called subendometrial enhancing zone, whose disruption or changes were essential for diagnosing myometrial invasion. The major diagnostic advantages of the enhanced sequences were found in postmenopausal women, where visualization of the junctional zone may be difficult in the T2-weighted sequences.

Frei and Kinkel [30] reviewed the role of MRI in staging of endometrial cancer. They suggested that the value of MRI in the preoperative staging of endometrial cancer is comparable to alternative strategies. They noted that contrast-enhanced MRI performs best in the pretreatment evaluation of myometrial or cervical invasion, compared to US, CT, or nonenhanced MRI. They concluded that the overall costs and accuracy are similar to those of the current methods of staging, including intraoperative gross dissection of the uterus. They emphasized that the findings of MRI might decrease the number of unnecessary lymph node dissections.

In conclusion, during preoperative evaluation of patients with endometrial cancer, MRI can be helpful in determining surgical strategies according to the extent of tumoral invasion of myometrium. Our findings suggest that the accuracy of MRI staging increases in accordance with the increase of surgical stage and presence of deep myometrial invasion in patients with endometrial cancer.

References


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Mersin 10 (Turkey)
e-mail: info@eyupyayci.dr.tr
Late recurrence of cervical cancer: a report of 16 cases

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2Department of Pathology, INCan, Mexico City; 3Department of Gynecological Oncology, INCan, Mexico City
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5Unit of Biomedical Research on Cancer, Instituto de Investigaciones Biomédicas (IIB), Universidad Nacional Autónoma de México (UNAM)/INCan, Mexico City (Mexico)

Introduction

Deaths resulting from cancer of the cervix occur most frequently during the first years after therapy; about half of all deaths occur during the first year, 25% occur during the second year, and 15% occur during the third year, for a total of approximately 90% by the end of the third year [1]. Paunier et al., [2] indicated that 92.5% of deaths resulting from carcinoma of the cervix occur during the first five years after diagnosis. Recurrent cervical cancer carries a dismal prognosis as most recurrences are not amenable to local curative therapies; hence, most patients receive palliative chemotherapy [3].

Currently, no prospective randomized studies have been carried out to demonstrate that post-treatment surveillance of invasive cervical cancer improve the prognosis, however, it seems clear that prognosis can be improved when patients are diagnosed with an asymptomatic recurrence compared to those who are symptomatic [4, 5], suggesting that sensitive and specific methods such as positron emission tomography-computed tomography (PET-CT) can increase probability of detecting asymptomatic recurrences [5, 6] and eventually could improve survival of recurrent cervical cancer patients.

Late recurrence after a disease-free interval of five or more years after the primary therapy is uncommon in cervical cancer. According to several reports, it has been estimated that late recurrences may occur at a rate of 0.4-7.5% [7-12]. Whether tumors recurring after such a long-term have a similar clinical and biological behavior as compared to tumors recurring within the first five years posttherapy is still unknown, due to the scarcity of publications on the issue. The authors report the clinical characteristics and outcome of 16 patients with cervical cancer who developed recurrent disease five years after completing primary treatment.

Materials and Methods

The present study was a retrospective review of 16 patients with cervical cancer who were treated between 1974 and 1999 at the Institution and whose cancer recurred after a five-year disease-free interval. Their charts were revised to obtain their clinical and demographic characteristics, primary therapy, length of the disease-free interval, clinical findings of recurrence, site of recurrence, treatment for recurrence, and outcome.

Statistical analysis

All data are expressed in terms of mean and standard deviation from the mean. Survival was calculated from the date of recurrence to the date of death. Analyses were performed using the statistical software package (StatView, SAS Institute, NC).

Results

The characteristics of patients are summarized in Table 1. The age distribution was from 28 to 62 years (mean 42) at primary therapy and from 35 to 71 years (mean 55) at recurrence. The period from initial therapy to recurrence ranged from 60 to 360 months with a mean of 162.5 months. The disease-free interval was within five to ten years in eight patients (50%), between ten and 20 years in six cases (37.5%), and more than 20 years in two patients (12.5%) - 29 and 30 years after initial therapy. The staging distribution at initial diagnosis, according to the FIGO classification, was as follows: Stage IA, one...
with respiratory complaints, diagnosis was made by a chest-X-ray. In the two cases with supraclavicular disease, the diagnosis was established by a fine-needle aspiration. A patient complaining of pain and numbness of the right inferior limb, imaging studies, and gynecological exploration showed no disease, so she underwent an exploratory laparotomy to confirm diagnosis. Once diagnosed, patients were evaluated to determine the extent of disease with CT. Two patients underwent bone scanning.

Among the 16 recurrent patients, six recurred in the pelvis as the sole site of disease (37.5%), a single case had visceral disease only (lung); four (25%) had systemic lymph node disease only; one had pelvic and systemic nodal disease, two had systemic visceral and lymph node disease, and two patients had visceral and lymph-node disease (Table 3).

The treatment of recurrence is shown in Table 4. Half of patients (eight) received pelvic radiation (four concurrent with chemotherapy and four alone); six as the only form of treatment, and two cases following systemic chemotherapy. Of note, six of these eight patients had received primary radiation, and three of these six re-irradiated patients developed a vesico-vaginal fistula, and another presented a rectal stenosis. Systemic chemotherapy was delivered in seven patients (six with carboplatin and paclitaxel and one, single agent gemcitabine). One patient refused treatment. At a maximum follow-up time of 38 months, the median survival time was 30 months and the projected survival was 32% (Figure 1).

### Table 1. — Clinical characteristics of patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at initial diagnosis (years) 28 - 62 (mean 42)</td>
<td></td>
</tr>
<tr>
<td>Age at recurrence (years) 35 - 71 (mean 55)</td>
<td></td>
</tr>
<tr>
<td>Disease-free interval (years) ≥ 5 - &lt; 10</td>
<td>8 50.0</td>
</tr>
<tr>
<td>≥ 10 - &lt; 20</td>
<td>6 37.5</td>
</tr>
<tr>
<td>≥ 20</td>
<td>2 12.5</td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>1 6.25</td>
</tr>
<tr>
<td>IB</td>
<td>4 25.0</td>
</tr>
<tr>
<td>IIA</td>
<td>2 12.5</td>
</tr>
<tr>
<td>IIIB</td>
<td>1 6.25</td>
</tr>
<tr>
<td>IIIA</td>
<td>1 6.25</td>
</tr>
<tr>
<td>IIIB</td>
<td>4 25.0</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 18.7</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>15 93.7</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1 6.25</td>
</tr>
<tr>
<td>Primary therapy</td>
<td></td>
</tr>
<tr>
<td>Radiation</td>
<td>10 62.5</td>
</tr>
<tr>
<td>Surgery</td>
<td>3 18.7</td>
</tr>
<tr>
<td>Surgery + Radiation</td>
<td>2 12.5</td>
</tr>
<tr>
<td>NACT → Surgery + Radiation</td>
<td>1 6.25</td>
</tr>
<tr>
<td>NACT: Neoadjuvant chemotherapy.</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. — Clinical findings at presentation of recurrence.

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear abnormalities*</td>
<td>2 12.5</td>
</tr>
<tr>
<td>Genital bleeding</td>
<td>1 6.25</td>
</tr>
<tr>
<td>Genital bleeding, abdominal and lumbar pain</td>
<td>3 18.7</td>
</tr>
<tr>
<td>Abdominal and lumbar pain</td>
<td>4 25.0</td>
</tr>
<tr>
<td>Dyspnea and lumbar pain</td>
<td>1 6.25</td>
</tr>
<tr>
<td>Cough and weight loss</td>
<td>1 6.25</td>
</tr>
<tr>
<td>Chest pain and hemoptysis</td>
<td>1 6.25</td>
</tr>
<tr>
<td>Dysphonia and lymph node enlargement (SC)</td>
<td>1 6.25</td>
</tr>
<tr>
<td>Lymph node enlargement (SC)</td>
<td>1 6.25</td>
</tr>
<tr>
<td>Numbness and pain of inferior limb**</td>
<td>1 6.25</td>
</tr>
</tbody>
</table>

No.: number; * these two patients were asymptomatic; SC: supraclavicular; ** this patient underwent exploratory laparotomy for diagnosis.

### Table 3. — Pattern of relapse.

<table>
<thead>
<tr>
<th>Sites of recurrence</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic site only</td>
<td>6 37.5</td>
</tr>
<tr>
<td>Systemic visceral only lung</td>
<td>1 6.25</td>
</tr>
<tr>
<td>Systemic lymph nodes only</td>
<td>4 25.0</td>
</tr>
<tr>
<td>Supra-clavicular</td>
<td>1</td>
</tr>
<tr>
<td>Supra-clavicular/para-aortic</td>
<td>1</td>
</tr>
<tr>
<td>Mediastinal/para-aortic</td>
<td>1</td>
</tr>
<tr>
<td>Inguinal/para-aortic</td>
<td>1</td>
</tr>
<tr>
<td>Pelvic/systemic lymph nodes only</td>
<td>1 6.25</td>
</tr>
<tr>
<td>Pelvis/mediastinal</td>
<td>–</td>
</tr>
<tr>
<td>Pelvic/systemic visceral</td>
<td>2 12.5</td>
</tr>
<tr>
<td>Pelvis/bone</td>
<td>1</td>
</tr>
<tr>
<td>Pelvis/lung/pleura/mediastinal</td>
<td>1</td>
</tr>
<tr>
<td>Systemic visceral/lymph nodes only</td>
<td>2 12.5</td>
</tr>
<tr>
<td>Lung/pleura/mediastinal</td>
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</tr>
<tr>
<td>Lung/mediastinal/para-aortic/mediastinal</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 4. — Treatment of recurrences.

<table>
<thead>
<tr>
<th>Treatment at recurrence (No.)</th>
<th>Site of relapse</th>
<th>Re-irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic RT (2)</td>
<td>local yes (2)</td>
<td></td>
</tr>
<tr>
<td>Pelvic RT (1)</td>
<td>local no</td>
<td></td>
</tr>
<tr>
<td>Pelvic CTRT (1)</td>
<td>local no</td>
<td></td>
</tr>
<tr>
<td>Pelvic CTRT (2)</td>
<td>local yes (2)</td>
<td></td>
</tr>
<tr>
<td>Systemic CT + Pelvic RT (1)</td>
<td>local/syst yes (1)</td>
<td></td>
</tr>
<tr>
<td>Systemic CT + pelvic CTRT (1)</td>
<td>local/syst yes (1)</td>
<td></td>
</tr>
<tr>
<td>Systemic Chemotherapy (7)</td>
<td>systemic no</td>
<td></td>
</tr>
<tr>
<td>Untreated (1)</td>
<td>no</td>
<td></td>
</tr>
</tbody>
</table>

RT: radiation therapy; CTRT: chemotherapy + radiation therapy.

With respiratory complaints, diagnosis was made by a chest-X-ray. In the two cases with supraclavicular disease, the diagnosis was established by a fine-needle aspiration. A patient complaining of pain and numbness of the right inferior limb, imaging studies, and gynecological exploration showed no disease, so she underwent an exploratory laparotomy to confirm diagnosis. Once diagnosed, patients were evaluated to determine the extent of disease with CT. Two patients underwent bone scanning.

Among the 16 recurrent patients, six recurred in the pelvis as the sole site of disease (37.5%), a single case had visceral disease only (lung); four (25%) had systemic lymph node disease only; one had pelvic and systemic nodal disease, two had systemic visceral and lymph node disease, and two patients had visceral and lymph-node disease (Table 3).

The treatment of recurrence is shown in Table 4. Half of patients (eight) received pelvic radiation (four concurrent with chemotherapy and four alone); six as the only form of treatment, and two cases following systemic chemotherapy. Of note, six of these eight patients had received primary radiation, and three of these six re-irradiated patients developed a vesico-vaginal fistula, and another presented a rectal stenosis. Systemic chemotherapy was delivered in seven patients (six with carboplatin and paclitaxel and one, single agent gemcitabine). One patient refused treatment. At a maximum follow-up time of 38 months, the median survival time was 30 months and the projected survival was 32% (Figure 1).
Late recurrence of cervical cancer: a report of 16 cases

Discussion

In this review of late recurrences of cervical cancer, which the authors considered as any recurrence beyond the fifth year post-treatment, it is shown that most occurred between five and ten years, followed by those presenting after ten years, and only two after 20 years. An unanswered question is whether in particular those recurrences after 20 years truly are recurrences or second primary tumors. Nevertheless, this kind of very late recurrences is less uncommon in neoplasias such as breast cancer, hence this may only indicate a very long period of tumor dormancy [13]. Nonetheless, it has been hypothesized that some of these tumors could be late radiation-induced cancers [11, 14, 15]. However, evidence in literature speaks of second primary tumors within radiation field (bladder, rectum) and out of the field, such as lung and leukemia [16, 17]. None of these were present in the patients studied, so it is unlikely that even those highly delayed “recurrences” are second malignancies.

Takehara et al., [1] found that the probability of late recurrence in patients with Stage II or III disease was significantly higher than in those with Stage I disease which is also seen in this study. This solely can be explained on the basis of less likelihood of control due to more advanced disease. All late recurrent cases, except one patient, were found to be squamous cell carcinoma in the present study. Other authors, such as Takehara and Van Herik [1, 11], also exclusively found this histology. In this report, all but one patient had squamous histology which suggests that there was an over-representation of squamous histology in patients with late relapse. Whether this is just random due to the small number of patients, or if it is the reflection that adenocarcinoma and adenosquamous histologies tend to have a poorer prognosis and exhibit poorer responses to radiation [18-20] and consequently prone to earlier relapses, remains to be studied.

It is known that the site of metastases in breast cancer is related to the histopathological characteristics of patients. For instances, estrogen receptor (ER)-negative tumors recur more often in visceral and soft-tissue sites, while patients with ER-positive tumors are more likely to recur in bony sites [21]. The few reports on late recurrences of cervical cancer left unclear whether there is a common pattern of sites of relapse in this condition. Van Herik et al., [11] reported that the organs and tissues within the pelvis were most frequently involved in patients who had recurrence more than ten years after primary therapy. Others authors reported a predilection of bone in late relapses of cervical cancer [1, 22, 23]. The present study is remarkable because bone metastases were also observed in two patients (one as the sole site of disease), but also that four patients exclusively presented disease at lymph nodes, whereas another three had disease at lymph nodes and other organs such as lung, liver, and pleura. These data may suggests that cervical tumors destined to have late relapses are likely to have biological characteristics that preferentially drive tumor cells to lymph nodes, where they can remain dormant for longer times. This is supported by recent data in a breast cancer model of metastases where authors found that 12 out of 17 proteins over-expressed in metastases as compared to the primary tumors were found in lung metastases, as compared to only seven of these proteins in lymph node metastasis, and three in bone metastases [24].

Metastatic, recurrent, and persistent cervical cancer patients have a dismal prognosis. The most recent data of the GOG-204 study comparing four cisplatin-containing doublets indicate a median survival rate ranging from ten to 12.9 months depending on the doublet which is not statistically significant [25]. In addition, patients who failed to first-line chemotherapy have a dismal prognosis as no second- or third-line chemotherapy is considered standard with response rates below 15% [26]. In this selected population of late-relapsed patients, tailored treatment with radiation, chemoradiation, systemic chemotherapy or combination of these yielded a median survival of 30 months. At first sight this finding may suggest that late relapses may have a less aggressive course of the disease, however, this is only a suggestion. Finally, it should be stressed that despite re-irradiation can achieve a 60% of long-term control [15], the high complication rate found in the six re-irradiated patients merits a careful selection and dose planning to decrease the complication rate.

In summary, this report stresses that cervical cancer patients surviving free of disease after the fifth year post-treatment are at risk for recurrent disease. In addition, the clinical features of this population suggest that these tumors may have a different biological behavior and can be achieved better treatment results as compared to patients who relapse within the first five years post-treatment. This conclusion however, is not supported by the data here presented and should be viewed as hypothesis-driven.

References


Treatment of malignant ovarian germ cell tumors and preservation of fertility

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Summary

Objective: To explore the prognostic factors, as well as the menstrual and reproductive outcomes, of malignant ovarian germ cell tumors following a fertility-preserving treatment. Methods: A total of 145 patients with malignant ovarian germ cell tumors who had undergone fertility-preserving management in the past 20 years were analyzed retrospectively. The correlative factors for survival, recurrence, and reproductive status were evaluated. The median follow-up time was 9.5 years. Thirty-five cases suffered from dysgerminoma, 31 cases from endodermal sinus tumor, 63 cases from immature teratoma, two cases from embryonal carcinoma, 13 cases from mixed germ cell tumor, and one case had malignant struma. The overall five-year survival rate was 90.3%, whereas the ten-year survival rate was 89.2%. The five-year survival rates for the different stages were as follows: Stage I, 98.1%; Stage II, 5/5; Stage III, 73.3%; and recurrence, 64.1%. The five-year survival rates were 100% for dysgerminoma, 84.1% for endodermal sinus tumors, 92.0% for immature teratoma, one of two cases for embryonal carcinoma, 76.9% for mixed germ cell tumors, and one case of malignant struma. Results: Thirty-five babies were delivered, whereas seven induced abortions were performed during the follow-up. The important prognostic factors included the International Federation of Gynecology and Obstetrics (FIGO) stage and standard chemotherapy. No statistical significance in the five-year survival rates was determined among the different histological types, surgery types, chemotherapy courses, and chemotherapy regimen. Fertility-preserving treatment should be considered for ovarian germ cell tumors without the limitation of the FIGO stage. Conclusion: Chemotherapy does not influence the menses, pregnancy, or offspring. Recurrent cases could obtain a good prognosis after the proper treatment.

Key words: Malignant ovarian germ cell tumor; Fertility; Surgery; Chemotherapy.

Introduction

Malignant ovarian germ cell tumor (MOGCT) accounts for 5% of all ovarian malignancies. These tumors are rare gynecologic tumors usually affecting young women and children. These malignancies from primordial germ cells consist of dysgerminomatous and non-dysgerminomatous tumors, including yolk sac tumor (endodermal sinus tumors), immature teratomas, mixed germ cell tumors, pure embryonal carcinomas, and malignant struma. Hysterectomy and bilateral salpingo-oophorectomy were employed prior to the advent of effective combined chemotherapy, and under most circumstances, the patients would still die within one to two years because of high malignancy. Gershenson reported that 18 of 20 patients treated with surgery alone or postoperative radioisotopes, radiation therapy, and alkylating agents died in 1984 [1]. The survival rate of ovarian MOGCT has increased dramatically because of the application and development of combined chemotherapy. The majority of patients exhibit long-term survival with reported five-year survival rates of 100% for dysgerminomatous and 85% for non-dysgerminomatous MOGCT. Reducing toxicity, improving the quality of life, and fertility preservation are crucial in young females. We report in the current paper a large, single institutional experience of more than 20 years in the management of MOGCT. We aimed to investigate the outcome and treatment methods for the preservation of reproductive function given this rare but important disease.

Materials and Methods

Patients

A total of 286 patients with MGCTO were treated at the Cancer Institute and Hospital of the Chinese Academy of Medical Sciences between 1985 and 2004. Reproductive function was retained in 145 cases. In this study group, the tumors were staged according to the International Federation of Gynecology and Obstetrics (FIGO) classification system, and histological typing was performed according to the World Health Organization classification. After the completion of the survival period, the patients were interviewed via correspondence or telephone regarding their menstrual period and fertility. The outpatient examinations were used as a reference. The median age at the time of the first visit was 16.6 years, with an age range of 5-39 years old. Eighty-two cases were under the age of 20 (56.6%), 106 cases were unmarried (73.1%), 39 cases were married, and 19 were childless. Eighty cases were on the right-side of the ovary, 63 cases were on the left-side of the ovary, and two cases were bilateral. Stage I malignancies accounted for 106 cases (73.1%), five cases were Stage II (3.4%), 15 cases were Stage III (10.3%), and 19 were recurrences that were not treated in our hospital but merely referred (13.1%). Of the 126 cases treated in our hospital, four cases recurred, whereas four other cases were lost in the follow-up process, and one case died because of unrelated reasons (Table 1). Thirty-five cases were dysgerminoma (24.1%), 31 cases were endodermal sinus tumor (21.3%), 63 cases were immature teratoma (43.4%), two cases were embryonal carcinoma (1.4%), 13 cases were mixed germ cell tumor (9.0%), and one case was malignant struma (0.7%) (Table 2).

Surgery types

Fifty-three cases underwent unilateral salpingo-oophorectomy, omentectomy, and appendectomy. Unilateral salpingo-oophorectomy was performed in 81 cases, whereas ovarian cystectomy was performed in 11 cases. Biopsy of the contralateral ovary was performed intraoperatively in 30 cases.
Postoperative chemotherapy

The following postoperative chemotherapy was administered to 140 cases, except for five cases of immature teratoma: bleomycin sulfate, etoposide phosphate, and cisplatin (BEP) regimen in 91 cases; cisplatin, vinblastine, and bleomycin (PVB) in 25 cases; vincristine, adriamycin, and cyclophosphamide (VAC) in six cases; a single drug in 16 cases with PDD, CTX, and EADM; and other regimens for two cases. The chemotherapy courses were 1-10 courses, and median courses were 4.5. Nineteen cases were given < 3 courses, 110 cases 3-6 courses, and 11 cases > 6 courses.

Results

Survival

The follow-up work after the treatments lasted for three years to 22 years, and the median follow-up was 9.5 years. A total of 129 women are still alive, ten died, and six women were lost in the follow-up process. For the deceased cases, one Stage I patient with dysgerminoma died after seven years of postoperative and post-chemotherapeutic treatment. Only one woman with Stage I immature teratoma died after postoperative therapy and three courses of chemotherapy due to unrelated reasons. The other eight women died because of tumor within one year; one case at Stage I, two cases at Stage III, and five cases with recurrent tumor. The life-span method was used to calculate survival rate. The overall five-year survival rate was 90.3%, whereas the ten-year survival rate was 89.2%. The five-year survival rates are as follows: Stage I, 98.1%; Stage II, 100%; Stage III, 73.3%; and recurrent cases, 64.1%. Statistical significance was determined for the five-year survival rates between the different stages ($p < 0.05$).

Five-year survival rates of the different histological types were 100% for dysgerminoma, 84.1% for endodermal sinus tumors, 92.0% for immature teratoma, one of two cases for embryonal carcinoma, 76.9% for mixed germ cell tumors, and one case for malignant struma. No statistical significance was found for the five-year survival rates between the different histological types.

No statistical significance for the five-year survival rates between the different surgery types was found. Likewise, no statistical significance for the five-year survival rates between the different chemotherapy regimens and chemotherapy courses was found.

Recurrent places and treatment

Nineteen recurrent post-treatment cases in other hospitals and referred to the hospital were included in the current study, including four cases of dysgerminoma, ten cases of immature teratoma, four cases of endodermal sinus tumor, and one case of mixed germ cell tumor. Four patients relapsed post-treatment in the hospital, including one case each of immature teratoma, malignant struma, endodermal sinus tumor, and embryonal carcinoma (Table 3). The conditions of these 23 patients before recurrence were as follows: 12 cases without treatment after operation, seven cases treated with non-standard chemotherapy plans, one case treated with only one cycle of chemotherapy, and three cases used standard schedule chemotherapy for ovarian germ cell tumors.

Metastatic diseases such as in the liver, spleen, lung, and retroperitoneal lymph nodes were detected in 11 recurrent patients, and the recurrences of the other 12 cases were limited to the abdominopelvic cavity. Surgery was performed on 14 recurrent cases, whereas 20 cases were retreated with chemotherapy. However, three recurrent cases of immature teratoma did not undergo chemotherapy post-surgery. Fifteen relapsed patients have survived, six cases died, and two cases were lost to follow-up (Table 4).
Menstruation after treatments

Eighteen cases occurred before the menarche age at the start of the therapy, but presently, all these cases have had menarche at a normal age, and the menstrual period is regular. A total of 86 cases had menorrhagia during chemotherapy and recovered normally within three to six months after completion of the chemotherapy. The other patients had their regular menses during the chemotherapy.

Pregnancy and offspring

Of the 129 survival cases, 18 cases had conceived before the therapy, and 50 cases were single. Of the 61 married cases, 42 gravidities in 41 women were recorded, seven of whom had induced abortions, 35 underwent pregnancies, and one gave birth twice. All infants were in good health. The oldest is now 18 years old, and the youngest one year old. For the conceiving patients, 15 cases suffered from dysgerminoma, 12 immature teratoma, six endodermal sinus tumor, and one mixed germ cell tumor. The interval between the chemotherapy and pregnancy is within the range of six months to 18 years.

Discussion

MOGCT is a highly malignant disease. Most cases before the 1970s were treated with bilateral salpingo-oophorectomy and hysterectomy to obtain a radical cure. No effective chemotherapy was available during that period, and only treatments with adjuvant radiological therapy and alkyl agent single-drug chemotherapy were available. The majority of the patients during this period died after recurrence even during the early stages of the disease. According to reports, the survival rate of MOGCT before the 1970s was only 13% to 22%. This phenomenon indicated that the removal of the normal ovarian tissue might be treated with cystectomy.

The effective combination chemotherapy for MOGCT that was introduced in the 1980s, especially with the application of PVB and BEP regimens containing platinum, dramatically improved the survival rate [1-4]. The survival rates reached 87% to 100% for any type of tumor, despite the high malignancy of endodermal sinus tumor and embryonal carcinoma. Weinberg et al. [5] reported five-year overall survival rates of 100% for MOGCT cases. The overall five-year survival rate in the current study was 90.3%, whereas the ten-year survival rate was 89.2%. Our study showed that MOGCTs are very sensitive to chemotherapy signifying that chemotherapy is valuable in the treatment of MOGCT. No statistical significance for the five-year survival rates between the different chemotherapy regimens and chemotherapy courses was found. Single-drug and ≤3 courses of chemotherapy may be used in cases with dysgerminoma [6, 7].

Although the current management of MOGCT emphasized comprehensive staging in the initial operation, which consists of a unilateral salpingo-oophorectomy, omentectomy, appendectomy, multiple-punch biopsy of peritoneum, and ablation of enlarged lymph nodes, some of the patients might have been upstaged to Stage III secondary to omental and peritoneal micrometastasis, or retroperitoneal lymph node metastasis. However, this kind of tumor showed high sensitivity to chemotherapy and was reported to provide long-term survival for a vast majority of patients. Several patients with large residual disease could obtain persistent relief from effective chemotherapy. Chemotherapy might cure micrometastasis. Whether this treatment could diminish the extent of surgery and could be administered alone in the case of chorioepithelioma or lymphoma remains unresolved. Several articles have reported no significant difference in the prognosis of normally staged patients compared with incompletely staged patients [8-13]. In the current study, no difference in the survival rates between normal-staged patients and incompletely staged patients was found. Eleven patients in the group, including cases of immature teratoma, dysgerminoma, and mixed germ cell tumor were treated with unilateral ovarian cystectomy or bilateral malignant MOGCT was treated with unilateral salpingo-oophorectomy and contralateral ovarian cystectomy; all patients had disease-free survival. No significant influence in the prognosis was found whether an entire staging in the initial surgery following the application of standardized chemotherapy and dose was performed. The most essential operation should be unilateral salpingooophorectomy. Although Lai et al. [8] noted in their article that bilateral malignant disease contained within the normal ovarian tissue might be treated with cystectomy – our study has indicated that the 11 cases treated with cystectomy achieved a disease-free survival – a recommendation for cystectomy of unilateral tumors of MOGCT still cannot be made at present, particularly for high-grade malignant tumors, such as endodermal sinus tumor, embryonal carcinoma, and mixed germ cell tumor. Whether several low-grade MOGCTs containing immature teratoma and dysgerminoma could be treated with cystectomy remains to be further demonstrated. Beiner et al. [10] reported on cystectomy for immature teratoma of the ovary with no observed recurrence.

The combination of surgical resection and primary systemic chemotherapy cured the majority of women affected with MOGCT, and only a small number of patients relapsed. No unified recognition with the treatment of recurrent patients was established. Long-time survivors after relapse were demonstrated to be less than 5%. Lai et al. [8] reported five recurrent cases undertaking secondary cytoreduction that had completely no residual or less than 1 cm residual disease. Among these patients, four cases obtained disease-free survival, and one case was alive with tumor. The other six patients that had residual disease larger than 2 cm or even without secondary cytoreduction died from the disease. Of the 23 relapsed cases in our group the survival rate was 78.6%. As for the other nine cases that were treated without secondary surgery, the survival rate was 44.8%. A significant difference was determined between the survival rates (p < 0.05). The total effective rates have varied widely in the reports on salvage chemotherapy of recurrent MOGCT patients. Kollmannsberger et al. [14] reported a salvage
chemotherapy schedule using gemcitabine 1g/M² IV D1, 8 + oxaliplatin 130 mg/M² IV D1 to treat incurable male malignant germ cell tumors with a total effective rate of 46%. Miki and colleagues [15] reported a group of patients with incurable GCT treated with CPT-11 100 mg/M² to 150 mg/M² IV D1, 15, + DPV 20 mg/M² IV D1-5, a combination chemotherapy with an effective rate of more than 50%. (Among the 20 patients, 2 cases of CR and 7 cases of PR were identified). Giorgi et al. [16] reported another salvage chemotherapy combined with gemcitabine 800 mg/M² + Taxol 70 mg/M² + oxaliplatin 50 mg/M² IV D1, 8, 15, repeated at every 4-week interval. Of nine platinum-resistant patients with GCT who adopted the chemotherapy plan, only one case had partial response, one case remained stable disease, and seven cases had progressive disease. In the current study, the chemotherapy regimens used BEP, PVB, and IVP for the recurrent patients. The effective rates were 42.9%, 66.7%, and 75%, respectively, which are apparently relatively high- probably because some of these patients had not been treated previously with a standard chemotherapy plan.

Large-scale case series and long-term studies are expected to determine the optimal treatment with regard to recurrent MOGCT.

In the long-term VAC regimen chemotherapy study by Gershenson et al. [12], the final irregular menstruation rate was 8%, slightly higher than that of the normal females (5%), and the infertility rate was 10%, similar to that of the normal females. In the current study group, 86 cases had irregular menstrual periods or menopause during chemotherapy, but recovered after chemotherapy. A total of 18 cases occurred before the menarche age at the start of the therapy, but presently, all these cases have had menarche at a normal age, and a regular menstrual period. Except for the cases who had conceived or not reached the marrying age, 42 gravidades in 41 women, 35 pregnancies, and seven induced abortions were recorded after the end of chemotherapy. Drugs, especially alkylating agents, have been reported to induce follicle dysfunction, and the prepubertal ovary is more resistant to the adverse effects of chemotherapy. At present, the non-alkylating drugs combined with a short-term regimen have no obvious influence on ovarian function. Successful pregnancies after treatment with chemotherapy have been well documented in other types of malignancies, including choriocarcinoma and lymphoma. The chemotherapy results for MOGCT in recent years have proved not to increase the natural abortion rate and offspring abnormality rate [17-19]. In the present study, all 35 infants were in good health, whereas the other pregnancies were stopped by induced abortion. Survival is apparently not affected by conservative surgery. Fertility is excellent after a fertility-sparing surgery and chemotherapy in patients with ovarian germ cell tumors.

References


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Preoperative evaluation, clinical characteristics, and prognostic factors of nongenital metastatic ovarian tumors: review of 48 patients

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Cukurova University School of Medicine, Adana (Turkey)

Summary

Objective: To evaluate the clinicopathologic characteristics, methods for preoperative evaluation, prognostic factors, and overall survival of nongenital ovarian metastases (NGOM). Material and Methods: Forty-eight patients with NGOM followed between January 2001 and January 2009 in Cukurova University Department of Gynecologic Oncology were included in the study. Clinical characteristics including demographics, preoperative imaging methods, endoscopic evaluations, tumor markers, histopathologic findings, prognostic factors, types of surgery, modalities for adjuvant therapy and survival were analyzed. Results: The gastrointestinal tract is the most common location of the primary tumor; colonic origin was found in 41% of the patients (n = 20). All metastatic lesions were adenocarcinoma with 23% of these classified as Krukenberg and 29% as mucinous type adenocarcinoma. When the whole group was evaluated, median survival time was 15.7 months in patients and there were significant differences between the groups according to primary site. Histopathological subtypes and presence of peritoneal carcinomatosis affected the median survival. The significant prognostic factors were primary site and histopathologic subtypes of the NGOM. Conclusions: NGOM should be kept in mind to avoid inappropriate management and therapy in patients with surgically managed ovarian tumor, especially young patients with gastrointestinal complaints.

Key words: Nongenital ovarian metastases; Prognostic factors; Preoperative evaluation; Survival.

Introduction

The ovaries represent the common site for genital metastatic disease. Gastrointestinal tumors and breast cancers are the most common sources of nongenital ovarian metastases (NGOM) [1-3]. Krukenberg tumors are metastatic tumors of the ovary and histopathologically this term describes signet ring cell adenocarcinoma. Stomach and colon/rectum carcinomas are the most common primary sites of Krukenberg tumors [1]. Occasionally hematologic malignancies and other solid tumors involve the ovaries [2]. There are several ways for metastases to the ovary; in addition to direct spread, lymphatic and hematogenous spread and transcoelomic dissemination are common routes [3].

The accurate preoperative evaluation of NGOM is very important as the misinterpretation of these tumors may cause inappropriate management and unnecessary surgery. There is insufficient information on the outcome of patients with NGOM who undergo surgery and cytoreduction [4-7]. Some authors suggest the beneficial effect of surgery especially in cases with colorectal cancer but the role of cytoreduction and surgical strategy is not clear enough [4-7]. Therefore, preoperative evaluation of these tumors is important. Imaging methods such as computed tomography (CT) and magnetic resonance imaging (MRI) are commonly used in daily practice. Gynecologic oncologists must consider the Krukenberg tumor when there are gastrointestinal symptoms and bilateral ovarian tumors, and preoperative screening must be complete [8, 9].

The aim of this study was to evaluate and discuss the preoperative clinical findings, surgical approach and prognosis in patients with NGOM.

Material Methods

Forty-eight patients with NGOM were identified during surgery between January 2001 and January 2009 at Cukurova University, Department of Gynecologic Oncology. Patient records were reviewed regarding the following data: age, menopausal status, main complaint, primary site, chronology, preoperative tumor markers such as CA 125, carcinoembryologic antigen (CEA), and CA 19-9, preoperative gastrointestinal endoscopic evaluation, imaging methods such as CT and MRI. Operative findings: presence/absence of ascites, bilaterality, tumor size, peritoneal carcinomatosis, residual tumor status, primary sites, surgical treatment modalities and additional surgical procedures including appendectomy, bowel resection, and cholecystectomy were noted. Adjuvant therapy modalities: chemotherapy and/or radiotherapy and survival data were reviewed.

Surgical treatment modalities were categorized as 1 - Oophorectomy or ovarian biopsy, 2 - Total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH+BSO); 3 - TAH+BSO+omectomy, 4 - TAH+BSO+omectomy+ bilateral pelvic and paraaortic lymphadenectomy (BP-PALND)+cytoreduction such as peritonectomy. Optimal cytoreduction was the term used if the diameter of the largest residue tumor was less than 1 cm. Survival was determined based on the date of surgery to the date of last follow-up or death. The follow-up was censored on September 2011.

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Table 1. — Demographic characteristics and clinical findings of the patients.

<table>
<thead>
<tr>
<th>Tumor markers (median)</th>
<th>Stomach cancer (n = 10)</th>
<th>Colorectal cancer (n = 19)</th>
<th>Breast cancer (n = 5)</th>
<th>Appendiceal cancer (n = 3)</th>
<th>Gallbladder cancer (n = 2)</th>
<th>Pancreatic cancer (n = 1)</th>
<th>Unknown primary (n = 7)</th>
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<td>247</td>
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<td>19</td>
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<td>CA 19-9 U/ml</td>
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<td>75.5</td>
<td>10.3</td>
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<td>6</td>
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<td>1</td>
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<td>GI endoscopic evaluation (n)</td>
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<td>12</td>
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<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>27 (56%)</td>
<td>0.405</td>
</tr>
<tr>
<td>done</td>
<td>3</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>21 (44%)</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS version 16.0; SPSS Inc., Chicago, IL, USA). Data are shown as mean ± SD and propriety data are shown as median, min-max value. The chi-square test for cross-tables and one-way ANOVA for comparing variables between the primary site groups were used. Survival data were computed using the Kaplan-Meier method. Differences in the survival curves were calculated using the log-rank test. The Cox proportional hazard model was used to assess the significance of multiple variables.

Results

There were 456 cases surgically identified with ovarian cancer in the study period in our institute. NGOM cases accounted for 10.5%; the mean age of the patients was 50.1 ± 14.1 years. There were statistically significant differences observed between the primary site of the NGOM according to mean age (p = 0.001). Of patients 48% were premenopausal and 52% were postmenopausal and no differences were found between the groups. The most common presenting symptoms were abdominal distension (31%). Dyspeptic complaints were dominant in nine patients (18%). Primary tumor preceded metastatic ovarian lesions in 33% of the patients and all cases with breast cancer were in this group. In two-thirds of the patients NGOM were detected during the exploration of a pelvic mass. Preoperative gastrointestinal endoscopic evaluation had been performed in 43% of the patients. Preoperative radiological imaging had been done in 70% of patients. Tumor diameter was between 6 and 10 cm in 48% of the patients and larger than 10 cm in 23% of the patients. Seventy-seven percent of the patients had bilateral ovarian masses. Thirteen percent of the patients had ascites more than three liters. Peritoneal carcinomatosis was found in 40% of the patients. The demographic characteristics and clinical findings of the patients are presented in Table 1.

Minimal surgical effort including ovarian biopsy or oophorectomy had been performed in 27% of the patients and TAH+BSO+BPPALND+cytoreduction had been done in 15% of the patients. Colon resection was performed in 42% of all patients and cytoreductive surgery for colorectal cancer had been done in 17 patients. Major surgical complications occurred in 12% of the patients. Histologically all metastatic lesions were adenocarcinoma with 23% of these classified as Krukenberg and 29% as mucinous type adenocarcinoma. Adjuvant chemotherapy had been given to 83% of the patients and 14% of the patients received radiotherapy. Intraoperative findings, histopathologic characteristics, types of surgery, adjuvant treatment modalities, and survival times of the patients are presented in Table 2. The gastrointestinal tract, especially the colon, was the most common origin of NGOM (41%). Origin of the tumor was not found in 14% of the cases (n = 7).

Survival rates were compared for primary site, bilaterality, presence of peritoneal carcinomatosis, types of operation, additional surgical procedures, histopathologic subtypes and modalities used for adjuvant aims. Median survival times were different according to the primary site (p = 0.001) and median survival time was 15.7 months. The longest survival was found in cases with colorectal cancer; it was 23 months. The shortest survival was found in cases with gastric cancer (7 months). Median overall survival time was 22 months in cases with breast cancer and eight months in cases with unknown primary. Log rank analysis showed that bilaterality of NGOM, surgery types, additional surgery procedures and adjuvant therapy modalities did not affect sur-
Table 2. — Intraoperative findings, histopathologic characteristics, types of surgery, adjuvant treatment modalities, and survival times of the patients.

<table>
<thead>
<tr>
<th></th>
<th>Stomach cancer (n = 10)</th>
<th>Colorectal cancer (n = 19)</th>
<th>Breast cancer (n = 5)</th>
<th>Appendix cancer (n = 3)</th>
<th>Gallbladder cancer (n = 2)</th>
<th>Pancreas cancer (n = 1)</th>
<th>Unknown primary (n = 7)</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td><strong>Chronology</strong></td>
<td></td>
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<tr>
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<td>7</td>
<td>13</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>32 (67%)</td>
<td>0.015</td>
</tr>
<tr>
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<td>7</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>16 (33%)</td>
<td>0.498</td>
</tr>
<tr>
<td><strong>Laterality</strong></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>11 (23%)</td>
<td>0.498</td>
</tr>
<tr>
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<td>9</td>
<td>15</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>37 (77%)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5 cm</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>0</td>
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<td>1</td>
<td>14 (29%)</td>
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</tr>
<tr>
<td>6-10 cm</td>
<td>6</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>23 (48%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 10 cm</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>11 (23%)</td>
<td></td>
</tr>
<tr>
<td><strong>Ascites</strong></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
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<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>18 (37%)</td>
<td>0.273</td>
</tr>
<tr>
<td>1-3 l</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>23 (48%)</td>
<td></td>
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<tr>
<td>&gt; 3 l</td>
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<td>16</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
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<td>1</td>
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<td>0</td>
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<td>6</td>
<td>5</td>
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<tr>
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<td>9 (19%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>6</td>
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<td>2</td>
<td>19 (39%)</td>
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<td></td>
</tr>
<tr>
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<td>4</td>
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<td>5</td>
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<td>1</td>
<td>–</td>
<td>2</td>
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<td>0</td>
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<td>3 (6%)</td>
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<td>17</td>
<td>–</td>
<td>1</td>
<td>0</td>
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<td>2</td>
<td>20 (42%)</td>
<td></td>
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<td>Splenectomy</td>
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<td>–</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<td>2</td>
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<td>1</td>
<td>2 (4%)</td>
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</tr>
<tr>
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<td>–</td>
<td>0</td>
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<td>4</td>
<td>9 (19%)</td>
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<tr>
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<td></td>
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<td></td>
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<td>1</td>
<td>5</td>
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<td>0</td>
<td>2</td>
<td>19 (40%)</td>
<td></td>
</tr>
<tr>
<td><strong>Complication</strong></td>
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<td></td>
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<td></td>
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<td>10</td>
<td>17</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>42 (87%)</td>
<td>0.277</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
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<td></td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4 (10%)</td>
<td></td>
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<tr>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (10%)</td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 (5%)</td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 (5%)</td>
<td></td>
</tr>
<tr>
<td><strong>Histopathologic subgroup</strong></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Krukenberg</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>11 (23%)</td>
<td>0.003</td>
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<td>Musinous</td>
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<td>9</td>
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<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14 (29%)</td>
<td></td>
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<tr>
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<td>5</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>23 (48%)</td>
<td></td>
</tr>
<tr>
<td><strong>Chemotherapy</strong></td>
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<td></td>
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<td></td>
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<tr>
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<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8 (17%)</td>
<td>0.737</td>
</tr>
<tr>
<td>Done</td>
<td>9</td>
<td>15</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>40 (83%)</td>
<td></td>
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<tr>
<td><strong>Radiotherapy</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>10</td>
<td>16</td>
<td>2</td>
<td>3</td>
<td>2</td>
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<td>7</td>
<td>41 (86%)</td>
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</tr>
<tr>
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<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7 (14%)</td>
<td></td>
</tr>
<tr>
<td><strong>Survival (months)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Median ± SD (95% CI)</td>
<td>7 ± 1.4</td>
<td>23 ± 6.5</td>
<td>22 ± 0.0</td>
<td>6 ± 2.4</td>
<td>4.5</td>
<td>2</td>
<td>8 ± 5.8</td>
<td>15.7 ± 12</td>
<td>0.001</td>
</tr>
<tr>
<td>Lower-upper bound</td>
<td>(4.1-9.8)</td>
<td>(10.2-35.7)</td>
<td>(1.1-10.8)</td>
<td>(0.0-19.5)</td>
<td>(8.2-15.7)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

The survival (Table 3). Multivariate analysis showed that histopathological subtypes and presence of peritoneal carcinomatosis affected the median survival. However, the presence of peritoneal carcinomatosis, types of surgery, and optimal cytoreduction were not found to be prognostic factors in NGOM (Table 4).

**Discussion**

Metastatic ovarian cancers are not rare. In clinical and autopsy series, 5-20% of patients with primary ovarian cancers present with metastatic ovarian malignancies [1]. Generally, these rates include both genital and nongenital ovarian tumors. Moore et al. [3] demonstrated an 8.2%
incidence of metastases to the ovary in patients who were thought to have a primary ovarian tumor. Detection of NGOM in gynecologic surgery has been found to be a poor prognostic indicator in a large population study with a long follow-up [10]. An important finding of this study was that in more than half of the cases with NGOM, it originated from the gastrointestinal tract. de Waal et al. [11] reported that ovarian metastases mimicking primary ovarian tumor originated from the large intestine in one fourth of the cases. In another study gastrointestinal origin was found in 42% of the cases [12]. Fusiwara et al. found that gastric primaries metastasing to the ovary made up 30% of the cases and this was followed by breast (21.6%), and colon cancers (6.7%) at the time of autopsy [13]. An explanation for this discrepancy may be the global incidence of stomach cancer. In our study identified NGOM cases consisted of 10.5% of the surgically managed ovarian cancers in the study period, and colorectal malignancies were the most common site for NGOM. This is followed by malignancies of the stomach, breast, and unknown primary site. These results are consistent with the literature [3-5]. Synchronous NGOM was found in 32 patients (66%) in our study. This high percentage indicates the importance of preoperative evaluation; 16 patients had metachronous NGOM.

The radiological features of metastatic ovarian cancers show considerable variability due to primary site [8]. Unfortunately, radiologically there are no definitive criteria for the differentiation of primary and metastatic ovarian tumors. In general, metastatic ovarian tumors present with bilateral ovarian involvement and more solid appearance while primary ovarian tumors show unilaterality and cystic nature. The shape of ovarian metastasis from colon cancer tends to be cystic, thus a solid and cystic nature cannot be an absolute milestone for the diagnosis in these metastatic cancers [9]. In our study, among the preoperative imaging modalities CT and MRI were used in 60% and 10% of the patients, respectively. Colorectal, appendiceal, and upper gastrointestinal tract primary tumors have been shown to be the most common primary malignancies associated with clinical findings suggestive of a primary ovarian cancer [2, 10, 11]. In our study preoperative endoscopic evaluation of the gastrointestinal tract had been performed in approximately half the patients (44%); an important point for clinical practice in that incomplete preoperative evaluation causes unnecessary surgery. In our cases the most common presenting complaints were abdominal distention and dyspeptic symptoms (such as constipation, weight loss, nausea and vomiting). Therefore a detailed history is very important as seen in all other clinical conditions. There were no differences for preoperative tumor marker levels according to the primary site in our study. The median serum CA 125, CEA and CA 19-9 were 146.5 U/ml, 6 ng/ml and 32 U/ml, respectively. However an elevated level of CEA may suggest gastrointestinal malignancy as the primary site [12].

NGOM is commonly bilateral in 60% of the patients and...
frequently present in smaller sizes than primary ovarian cancer. In this series, bilaterality was found in 77% of the patients and only 29% of the cases had less than 5 cm tumor diameter. Approximately a quarter of the patients had larger than 10 cm tumor. These results are in conflict with the literature because NGOM are usually smaller than primary ovarian cancer. Fusiwara et al. reported 75% of metastatic ovaries had diameters less than 5 cm in their autopsy series [13]. Such conflict was explained in that this study was retrospective and consisted of surgically identified NGOM, however the literature (aforementioned study) data were based on autopsy findings. The bilaterality, size and amount of the ascites were not different for the primary site of NGOM in our study. This may be due to the limited number of cases analyzed in our study. It is accepted that NGOM has a poor prognosis. However there is a significant difference in survival according to the primary site [15]. The longest median survival time has been found in patients with colorectal cancer and this was followed by breast cancer in our study which is compatible with the literature [4-7]. In our study, overall median survival was 15.7 months which is compatible with previous studies. Surgery is frequently indicated to detect the origin of an ovarian mass, and also for relief of symptoms in most cases. There are discordant results regarding the role of surgical resection or tumor debulking in patients with NGOM. Some of these studies showed that the prognosis was better in patients undergoing complete surgical resection, especially in cases with colorectal cancers [4, 7, 16, 17]. However some researchers suggest that tumor resection should be avoided [14]. There are conflicting results about the beneficial effect of cytoreductive surgery in patients with breast cancer. Some studies showed that debulking surgery in patients with breast cancer may be beneficial, especially in patients with late recurrences (5 or more years) [18, 19]. However this beneficial effect has not been shown in other studies [4, 15]. The results of some studies support the role of debulking surgery in the management of tumors of stomach origin [20, 21]. In our study, the primary site and histopathologic subtype of the NGOM were found to be prognostic factors in multivariate analysis. Krukenberg was a poor prognostic factor in accordance with the literature. Age, peritoneal carcinomatosis, surgical procedure and cytoreduction were not found to be prognostic factors. Although this study was retrospective, nonrandomized, included a heterogenous patient population, and had a small number of cases for some primary sites of NGOM, no comment can be made for the surgical modalities. This limited retrospective review is noteworthy for the preoperative evaluation of NGOM.

Conclusion

Surgery is frequently indicated for diagnosis of an ovarian mass and also for relief of symptoms in most cases but surgical procedures and especially maximum surgical efforts in patients with NGOM are not yet clear enough. In conclusion, preoperative evaluation methods should carefully be reviewed for these patients to avoid inappropriate procedures and complications.

References


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Evaluation of frozen-section analysis of surgical margins in the treatment of breast cancer

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Summary

Objective: To evaluate surgical margins in cases of ductal carcinoma through a histopathological exam using frozen sections. Materials and Methods: Retrospective study encompassing 242 conservative surgeries, 179 of which included intraoperative frozen-section histopathology and 63 intraoperative nonfreezing techniques (macroscopy/gross examination and cytology). The results of such analyses were compared with those of the histology processing following paraffin embedding and hematoxylin and eosin (H & E) staining. A margin was deemed free when the distance between the tumor and the surgical border was equal to or greater than two millimeters. The factors given consideration for possibly affecting the results were: age, surgical aspects (skin removal and widening of surgical margins), histopathological findings (size, affected lymph nodes, and angiolymphatic invasion), and extensive intraductal and immunohistochemical components (estrogen, progesterone, Ki-67, and HER-2 receptors). In the statistical analyses, the chi-square test was used and negative predictive values were calculated. Results: The negative predictive values were 87.1% and 79.3% for frozen and nonfrozen sections, respectively. There was no significant difference between the two groups (p = 0.14). The factors under consideration had no influence on the results of the intraoperative exam of the margins. Conclusion: The present study allowed to conclude that the intraoperative exam of the surgical margins by frozen section is not superior to a macroscopy and / or cytology exam.

Key words: Breast cancer; Conservative surgery; Intraoperative analysis of margins; Freezing of margins.

Introduction

The major issue regarding conservative surgical treatment of breast cancer is local remission (LR), which requires reintervention [1] and may produce negative psychic disturbances in women [2, 3]. Recent data indicate a decrease in local remission rates and give evidence of factors associated with greater or lesser LR in oncology follow-up [4-11]. The most relevant of such factors is the absence of compromised surgical margins. Clinical essays have shown that the LR rate ranges from 3% to 13% when margins are free. In compromised cases, these rates are much higher (21% - 31%) [4-11]. Intraoperative investigation allows immediate on-the-spot evaluation of surgical margins enabling reintervention during the same surgery, thus lowering the rates of new operations [12]. There are several ways of examining resection margins intraoperatively, such as: a macroscopic study or gross exam (visual measurement of the distance between the tumor and the resection margins) [12], a microscopic study (imprint and / or cancer cytology [13], and frozen section of the margins close to the tumor [12]. All techniques yield variable degrees of false-negative results when they are compared with the anatomopathological exam in paraffin [14-16]. However, there are not enough comparative data in the literature concerning the different techniques for analyzing resection margins in the intraoperative exam.
by the seventh edition of the American Joint Committee on Cancer (AJCC) [20].

The immunohistochemical subdivision of breast cancer by analogy with molecular subtypes met the criteria proposed by Nielsen et al. in 2004 [22], and used the following classification: luminal (ER+ and / or PR+ and HER2-), HER2 (ER-, PR, and HER2+) and triple negative (ER-, PR-, and HER2-).

Statistical analysis

To compare the medians from each group, the Mann-Whitney test was applied, and to compare proportions, the chi-square and the FISH tests were applied. A 5% significance level was adopted [23, 24]. A sample size requiring a minimum of 60 patients per group for 90% power was used based on the anatomicopathological exam.

Results

Of the 242 patients in this study, 85.1% had free margins in the histopathological exam. In group A, 87.1% in the frozen-section exam, and in group B, 79.3% in the nonfreezing tests (macroscopy / gross exam and cytology). There was no significant difference between the two groups (p = 0.135).

The parameters that were evaluated and that could have influenced the results concerning free margins did not significantly differentiate the two groups (Table 1). With respect to freezing, the patients were grouped according to final stage, i.e., compromised margins or free margins. No significant differences were found among the parameters shown in Table 2.

Table 1. — Characteristics of total number of patients and of subgroups with and without intraoperative frozen-section exam.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frozen-section (Group A)</th>
<th>Non-frozen-section (Group B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>n = 179</td>
<td>n = 63</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>58.14</td>
<td>55.59</td>
</tr>
<tr>
<td>Tumor size (pT)</td>
<td>pT0 - pTis: 2.3% 2.1%</td>
<td>pT0 - pTis: 2.3% 2.1%</td>
</tr>
<tr>
<td></td>
<td>pT1: 63.4% 59.5% 2.1%</td>
<td>pT1: 63.4% 59.5% 2.1%</td>
</tr>
<tr>
<td></td>
<td>pT2: 30.9% 27.6%</td>
<td>pT2: 30.9% 27.6%</td>
</tr>
<tr>
<td></td>
<td>pT3: 0.6% 0%</td>
<td>pT3: 0.6% 0%</td>
</tr>
<tr>
<td></td>
<td>pT4: 2.9% 10.6%</td>
<td>pT4: 2.9% 10.6%</td>
</tr>
<tr>
<td>Lymph node stage (pN)</td>
<td>pN0: 69% 65.9%</td>
<td>pN0: 69% 65.9%</td>
</tr>
<tr>
<td></td>
<td>pN1: 23.4% 25%</td>
<td>pN1: 23.4% 25%</td>
</tr>
<tr>
<td></td>
<td>pN2: 5.8% 2.2%</td>
<td>pN2: 5.8% 2.2%</td>
</tr>
<tr>
<td></td>
<td>pN3: 1.8% 6.8%</td>
<td>pN3: 1.8% 6.8%</td>
</tr>
<tr>
<td>Ki-67</td>
<td>&lt; 25%: 62.5% 65.7%</td>
<td>&lt; 25%: 62.5% 65.7%</td>
</tr>
<tr>
<td></td>
<td>&gt; 25%: 37.5% 34.3%</td>
<td>&gt; 25%: 37.5% 34.3%</td>
</tr>
<tr>
<td>ALI</td>
<td>Absent: 67.7% 77.4%</td>
<td>Absent: 67.7% 77.4%</td>
</tr>
<tr>
<td></td>
<td>Present: 32.3% 22.6%</td>
<td>Present: 32.3% 22.6%</td>
</tr>
<tr>
<td>EIC</td>
<td>Absent: 40.5% 41.4%</td>
<td>Absent: 40.5% 41.4%</td>
</tr>
<tr>
<td></td>
<td>Present: 59.5% 58.6%</td>
<td>Present: 59.5% 58.6%</td>
</tr>
<tr>
<td>Surgical cavity shaving?</td>
<td>Yes: 59.8% 50%</td>
<td>Yes: 59.8% 50%</td>
</tr>
<tr>
<td></td>
<td>No: 40.2% 50%</td>
<td>No: 40.2% 50%</td>
</tr>
<tr>
<td>Estrogen and progesterone receptors</td>
<td>Positive: 88.2% 88.8%</td>
<td>Positive: 88.2% 88.8%</td>
</tr>
<tr>
<td></td>
<td>Negative: 11.8% 11.2%</td>
<td>Negative: 11.8% 11.2%</td>
</tr>
<tr>
<td>Immunohistochemical subtypes</td>
<td>Luminal: 72.2% 55.3%</td>
<td>Luminal: 72.2% 55.3%</td>
</tr>
<tr>
<td></td>
<td>EIC: 8.3% 6.3%</td>
<td>EIC: 8.3% 6.3%</td>
</tr>
</tbody>
</table>

Table 2. — Analysis of the influence of variables of surgical margins in patients from group A subjected to the frozen-section exam.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive margins (n = 59)</th>
<th>Free margins (n = 120)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>21.74% 26.97%</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>&gt; 50 years</td>
<td>78.26% 73.03%</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Immunohistochemical subtype</td>
<td>Luminal: 58.82% 73.91%</td>
<td>Her-2: 18.26% 29.41%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Her-2: 18.26% 29.41%</td>
<td>Triple negative: 11.76% 7.83%</td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td>&lt; 25%: 66.67% 61.45%</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>&gt; 25%</td>
<td>33.33% 38.55%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAL</td>
<td>Absent: 66.67% 67.63%</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>33.33% 32.37%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical cavity shaving?</td>
<td>No: 34.78% 41.29%</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>65.22% 58.71%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size</td>
<td>&lt; 1 cm: 23.81% 27.45%</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>&gt; 1 cm</td>
<td>76.19% 72.55%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor stage (pT)</td>
<td>pT0-1: 76.19% 64.71%</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pT 2-3-4: 23.81% 35.29%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive lymph nodes/</td>
<td>61.9% 69.33% 0%</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Resected lymph nodes</td>
<td>14.29% 10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 10%: 23.81% 20.67%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node staging (pN)</td>
<td>pN0: 60% 70% 0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pN+ 40% 30%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIC</td>
<td>Absent: 25% 41.98% 0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>75% 58.02%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The chi-square test was employed to compare variables.

Discussion

The intraoperative analysis of surgical margins is a crucial stage in the conservative treatment of breast cancer, for it allows the detection of compromised margins at the time of surgery itself, thus rendering further interventions unnecessary [25] and keeping costs and morbidity from rising. The present study makes a contribution as it analyzed only IDC cases (except by lobular histology) and compared the group subjected with the other study patients who were also operated, but did not undergo such a procedure.

There is a great deal of controversy in the literature concerning the width of the ideal margin [26]. The studies from major world centers consider a margin to be free when there are no tumor cells in the resection margin regardless of the distance of the tumor [3, 4]. However, for other authors, a margin is free when it is 1-10 mm away from the tumor [9, 27]. In this study, a safety (free) margin was defined as a two mm margin in accordance with Pinotti and Carvalho in 2002 [17].

The intraoperative exam with cryoablated margins is criticized by some authors for increasing the costs and the complexity of the intraoperative exam without improving the end results [12, 16, 28]. However, there are other authors who claim such a procedure allows for greater diagnostic precision. The results in the present study, though, do not bear out their allegation.

The medical literature has shown that the intraoperative investigation of the margins effectively lowers the reoperation rate [1]. However, the frozen-section exam has produced less emphatic results. Olson et al. in 2007, estimated that it raises the cost and lengthens the time of
surgery considerably. Besides, there is a hypothetical risk of loss of material during the exam [28, 29].

Unlike Weber et al. in 2008 [30], who found that was superior to the other procedures, the present study showed that the frozen-section exam did not make a significant difference. However, the number of women in this study was greater than that in the study of the aforementioned authors. Also, all of the participants were operated and evaluated by the same surgery and pathology teams. One should keep in mind, though, that this study was retrospective. Ideally, a prospective and randomized study should be conducted to reach definitive conclusions as to the value of intraoperative diagnosis.

In contrast to a study published by Riedl et al., in 2009 [15] who also analyzed variables that might influence the frozen-section exam, this study did not find it difficult to evaluate the margins of smaller tumors. However, this study did not make a preoperative distinction between tumors previously subjected to chemotherapy and those without a previous diagnosis.

The results obtained by Moore et al. in 2007, were similar to those included in that in this study did not find predictors pointing to higher failure rates in the frozen-section exam. Moreover, the cases of lobular histology were previously excluded from analysis in this study [31].

Contrary to other studies [21], the authors did not observe important differences among the intraoperative techniques that were analyzed. In fact, the results suggest that the intraoperative evaluation of cryosublational surgical margins does not improve the intraoperative diagnosis.

References

Evaluation of frozen-section analysis of surgical margins in the treatment of breast cancer


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CD4+CD25+Foxp3+ Treg and TGF-β play important roles in pathogenesis of Uygur cervical carcinoma

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2Department of Gynecology and Obstetrics, First Affiliated Hospital of Xinjiang Medical University, Urumqi
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Summary

Objective: The aim of the study was to evaluate the function of CD4+CD25+ and Foxp3+ T (Treg) cell and related cytokine in the Uygur patients with cervical carcinoma and CIN (cervical intraepithelial neoplasia). Materials and Methods: 170 Uygur women were recruited in the study from January 2007 to January 2011. The study group was comprised of normal controls, cases of primary cervical carcinoma/CIN, and cervical carcinoma/CIN treated with surgery. The following parameters were examined: clinicopathologic features of patients, percentage of CD4+CD25+Foxp3+ Treg cell in blood and Foxp3 mRNA expression in CD4+CD25 high T cell concentration of serum cytokine. Women with primary cervical carcinoma/CIN after being treated with surgery were compared to the normal controls. Where appropriate, univariate and multivariate analyses were used to identify the function of the Treg and related cytokine. Results: The percentages of CD4+CD25+ Treg were detected as well as in the blood of carcinoma patients and CIN II/III, but the number of cells was much higher compared to both control and CIN I groups (p < 0.01). Moreover, a significant correlation between the expression of Foxp3 mRNA and pathological changes was found. The secretion levels of IL-10, and TGF-β correlated positively with the process of carcinoma. Furthermore, after surgical operation, the number of Treg cells and related cytokines were decreased. Conclusions: Finally, the authors would like to highlight that CD4+CD25+ Treg, especially the CD4+CD25+Foxp3+ Treg and TGF-β play important roles in Uygur cervical carcinoma, and may have a correlation with survival. Therefore, the inhibitory function of TGF-β depletion of Treg cells in combination with other anti-tumor therapies could optimize eradication of malignancies.

Key words: Cervical carcinoma; CD4+CD25+Foxp3+ Treg; TGF-β; Uygur.

Introduction

Cervical carcinoma is a leading cause of morbidity and mortality among women worldwide, especially in the developing countries [1]. Cervical carcinoma has a positive correlation with human papilloma virus (HPV) [2, 3] and cervical carcinoma cells may have many mechanisms to escape from host immunosurveillance. At present, it was thought that the imbalance of T cell responses were associated with the progress of cervical carcinoma [4]. It has been reported that T cell responses are regulated by CD4+CD25+Foxp3+ ‘regulatory’ T (Treg) cells, Treg accounts for 10% of the total peripheral T cell and play an important role in maintenance of immunological tolerance to self-antigens and in preventing immune pathologies. Evidences from other carcinoma suggest that increased Treg activity may be associated with poor immune responses to tumor antigens and contribute to immune dysfunction [5]. A recent study on Treg cells in cervical carcinoma patients and CIN showed increased Treg frequencies in peripheral blood of these patients. It suggests that suppression of immunity by Treg will be an impediment in designing therapeutic strategies [5].

In Xinjiang, Uygur women have higher risk for the occurrence of cervical carcinoma Han compared to the population [6]. In this study, to expand the authors’ understanding of Treg interference with immune response, the effect of Treg cells and cytokines in the blood from donors was calculated. The authors first reported that the proportion of CD4+CD25+Foxp3+ T cell in Uygur cervical carcinoma was high. They also found that after surgical operation, Treg cells and related cytokines were decreased. Using this data set may help the authors to better understand the mechanism of cervical carcinoma and provide insight for the treatment.

Materials and Methods

Patients

This study recruited 170 Uygur women, including the normal control, and cervical carcinoma, CIN I, and CIN II/III. The cases of cervical carcinoma, CIN I and CIN II/III were diagnosed in the Gynecology and Obstetrics Department of the First Affiliated Hospital of Xinjiang Medical University from January 2007 to January 2011. The characteristics of the patients are shown in Table 1. Peripheral anticoagulation blood was used to measure the phenotype of the cells of each patient by flow cytometry (FCM). Informed consent was obtained from all donors and the Institutional Review Board at Xinjiang Medical University Hospital approved this study.

Preparation of cells and FCM

Cells were isolated according to an established method with slight modifications. Briefly, peripheral blood mononuclear cells (PBMC) from donors were isolated by density centrifugation on Histopaque 1077 (Sigma, St. Louis, MO, USA). The PBMC (1 × 10^6) were subjected to FCM analysis using the ASR system (Becton Dickinson, Franklin Lakes, NJ).
CD4+CD25+Foxp3+ Treg and TGF-β play important roles in pathogenesis of Uygur cervical carcinoma

Statistical analysis

Statistical analysis was performed using SPSS 14.0. Results are presented as the means ± SD. Data were processed using the chi-square test, the Kruskal-Wallis H test, and analysis of variance, depending on the number and distribution of the compared groups. A \( p \) value of < 0.05 was considered statistically significant.

Results

Demographic profiles

One hundred and seventy women with or without primary cervical carcinoma/CIN were enrolled in the study. All cervical carcinoma/CIN patients were operated on mainly due to carcinoma/CIN. Tumor size, lymph node metastasis, and depth of invasion were confirmed by biopsy during surgery (Table 1). There were no significant differences between the two groups in terms of age, parity, and body mass index (BMI). For the cervical carcinoma group, all patients underwent radical hysterectomy and pelvic lymph node dissection. Among them, pathological results showed no lymph node metastasis.

Treg cell frequencies in peripheral blood

To investigate the association between CD4+CD25+ Treg cells, and cervical carcinoma or CIN, the number of Treg cells in the blood was determined. There were
no differences in total CD3+CD4+ T cell and balance of CD4/CD8 among all groups, but in terms of Treg cells, cervical carcinoma patients had the most abundant Treg cells in the blood (Table 2, Figure 1). In contrast to CIN II/III patients, the Treg cells in cervical carcinoma patients were higher, but not statistically significant \((p > 0.05)\). The CIN I group had a higher number of Treg cells in the blood than controls but not significant; however, both groups had a statistically significant comparison to the cervical carcinoma and CIN II/III groups \((p < 0.05)\). These results showed that there was a significant association between Treg and cervical carcinoma. Among the enrolled 170 Uygur women, 140 patients with CIN II/III or cervical carcinoma underwent surgical therapy. Then the phenotype of Treg cells in the blood was evaluated five days postoperatively (Table 3). Treg cells in all groups had decreased, especially in CIN II/III and cervical carcinoma groups \((from 19.7 \pm 5.8 to 14.5 \pm 4.6 and 20.5 \pm 5.8 to 15.7 \pm 6.5, respectively)\).

**Table 2.** Expression of Foxp3 mRNA in CD4+CD25+ T cells.

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>CD4+/CD3+ (%)</th>
<th>CD4+/CD25+ (%)</th>
<th>CD8+/CD3+ (%)</th>
<th>Foxp3 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>56.69 ± 3.02</td>
<td>1.56 ± 0.32</td>
<td>38.49 ± 6.73</td>
<td>7.87 ± 3.52</td>
</tr>
<tr>
<td>CIN I</td>
<td>40</td>
<td>55.15 ± 8.18</td>
<td>1.53 ± 0.64</td>
<td>38.70 ± 6.27</td>
<td>8.76 ± 3.62</td>
</tr>
<tr>
<td>CIN II-III</td>
<td>40</td>
<td>57.53 ± 4.53</td>
<td>1.58 ± 0.41</td>
<td>37.46 ± 6.67</td>
<td>14.67 ± 4.26*</td>
</tr>
<tr>
<td>Cervical</td>
<td>60</td>
<td>56.15 ± 4.22</td>
<td>1.55 ± 0.43</td>
<td>37.83 ± 8.57</td>
<td>17.48 ± 4.58*</td>
</tr>
</tbody>
</table>

The results represent the mean SD of three independent experiments. *\(p < 0.01\) compared to controls.

The concentration of IL-10 and TGF-\(\beta\) in the serum

The authors assayed the IL-10 and TGF-\(\beta\) secretion in serum. After collecting the serum, the IL-10, and TGF-\(\beta\) concentrations in the serum were measured by ELISA. The levels of IL-10, and TGF-\(\beta\) production in the control group were very low, but in the cervical carcinoma group, there were large amounts of IL-10 and TGF-\(\beta\) \((p < 0.01)\) (Figure 3A). Considering that both IL-10 and TGF-\(\beta\) are able to act as regulators mediated by Treg cells, IL-10 and TGF-\(\beta\) are associated with Uygur cervical carcinoma \((p < 0.05)\) (Figure 3B). These results indicate that Foxp3 + Treg cells contribute more to the progress of cervical carcinoma.

**Conclusion**

In China, the incidence of cervical cancer is 14.6/100,000. Among all ethnic groups, the Uygur women have the highest incidence. A census in 2004 showed that the prevalence of cervical cancer in Uygur women is the highest among all ethnic groups. Further studies are needed to investigate the underlying mechanisms of this association.
CD4+CD25+Foxp3+ Treg and TGF-β play important roles in pathogenesis of Uygur cervical carcinoma

was 527/100,000, which was much higher than the national average. Recently, with the development of molecular immunology, cervical cancer immunotherapy has become the hotspot for researchers all over the world. More and more clinical studies and animal experimental study confirmed that immunotherapy, cytokine therapy, and adoptive cellular immunotherapy for cervical cancer treatment has an obvious curative effect. However, due to few samples and complicated immune mechanism, it is difficult to assess the exact clinical effect of a certain kind of immunotherapy. There is still a considerable distance for immunotherapy to become a routine treatment. Regulatory T cells (Treg) were found in the peripheral blood circulation of immunocompetent mice by Sakaguchi et al. in 1995 [7]. Treg is one of the subsets of the T cell, which has a special function. Treg (CD4+CD25+) cells are essential for immune incompetence of and immune suppression. Treg cells can inhibit T cell immune responses to foreign and self-antigens, thus maintaining their self-tolerance and prevent the immune response to tumor cells, leading to tumor cell immune evasion. Foxp3 is a key control molecule for the development and function of natural CD4+CD25+ Treg cells [8]. The role of Treg cells in tolerance is that natural CD4+CD25+ Treg cells specifically express the transcription factor Foxp3, which controls their development and function in a highly Treg-specific manner [9]. Therefore, Foxp3 can be used as a gold standard for confirming Treg.

Table 4.—Treg and cytokines in surgical patients.

<table>
<thead>
<tr>
<th>Surgical operation</th>
<th>CIN II-III Treg</th>
<th>Cervical cancer</th>
<th>IL-10 (ng/ml)</th>
<th>CIN II-III TGF-β (ng/ml)</th>
<th>Cervical cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>14.7 ± 4.3*</td>
<td>17.48 ± 4.6*</td>
<td>517.4 ± 30.6</td>
<td>728.4 ± 47.0</td>
<td>4908.2 ± 150.3</td>
</tr>
<tr>
<td>After</td>
<td>11.5 ± 2.8*</td>
<td>13.70 ± 3.5*</td>
<td>265.2 ± 28.8*</td>
<td>460.1 ± 38.7*</td>
<td>3456.2 ± 170.6*</td>
</tr>
</tbody>
</table>

The results represent the mean SD of three independent experiments. *p < 0.01 compared to controls.
The current study found that Treg and tumor occurrence are highly relevant. The key biological characteristics of malignant tumors are the disorder of proliferation and differentiation. From the immunological point of view, the tumor is a host of cells expressing a group of “normal antigens” (over-expression) and/or “abnormal antigens” (genetic modification, mutation or deletion). In vivo, autologous T cells recognize tumor antigens as a normal self-component, thereby resulting in immune neglect and immune escape of the tumor. A mouse tumor model revealed that Treg can inhibit tumor immune response [10]. Recent studies have found that in the area around the infiltrating tumor, exists not only the effective CD8+T or CD4+T cells, but also contains a large number of regulatory T cells, mainly CD4 + CD25 + Treg cells [11].

Further studies have shown that in colon cancer, ovarian cancer, lung cancer, breast cancer, pancreatic cancer, and other tumors, the CD4+CD25+ Treg cells increased in peripheral blood and local tumor [11]. This signifies that CD4+CD25+ Treg cells may have a role in the tumor immune process. Treg cells normally inhibit the generation of effective T cell-dependent anti-tumor immune responses which has been confirmed by depletion of Treg cells using CD25-specific mAbs [10, 11]. Substantial evidence confirms that in a clinical setting, the prevalence of Treg cells was found to be increased in the peripheral blood and tumour microenvironment of carcinoma patients [12]. In humans, Treg cells have been demonstrated to impair cytotoxic T lymphocyte (CTL) function in the setting of cancer [13]. The results in this study showed that cervical lesions and the CD4+ / CD8+T cell ratio showed no significant difference (p > 0.05). However, for the cervical cancer and normal control groups, CD4 + CD25 + Foxp3+ Treg accounted in the proportion of CD4+ T lymphocytes is 20.48 ± 5.78% and 11.87 ± 6.52%, respectively, and there was significant difference between them (p < 0.05). It is speculated that during the development of cervical cancer, CD4+CD25+Foxp3+ Treg cells play an important role. In patients with cervical cancer, Treg cell mediated immune tolerance and tumor growth is highly correlated and this may directly affect the process of tumor development. The number of Treg cells in blood is gradually increased with the order of normal control, CIN I, CIN II-III, and cervical cancer. Moreover, there was a significant difference between CIN II-III, cervical cancer groups and normal control and CIN I groups. This signifies that with the progression of cervical cancer, the number of CD4+CD25+Foxp3+ Treg increased gradually, which have may inhibited immune responses, promoting tumor growth and metastasis. In this study, the authors demonstrated that there are imbalances between Treg cells and effect T cells [14]. There was no significant decrease in CD8+T cells, but both CD4+CD25+Foxp3+Treg cells and II-10 or TGF-β increased in CIN and cervical carcinoma: they suppressed the anti-tumor immunity. There was a significant relation between Treg cells and Uygur cervical carcinoma. In the current study, the authors evaluated the function of Treg, dendritic cells (DCs), and CTLs around the diseased regions. As it is known the function of DCs andCTLs was impaired. The authors hypothesized that the increased Treg suppressed the DCs and CTL, and caused decreased immunosurveillance. Moreover, the results show that after surgery, Treg cells and related cytokines were decreased. Therefore, depletion of Treg cells in combination with other anti-tumor therapies could optimize eradication of malignancies.

A number of studies have found that Treg and Foxp3 expression increased in many cancers [15, 16]. Foxp3 is a key control molecule for the development and function of natural CD4+CD25+ Treg cells [17]. The role of Treg cells in tolerance is that natural CD4+CD25+ Treg cells specifically express the transcription factor Foxp3, which controls their development and function in a highly Treg-specific manner [18]. Disruption of the Foxp3 gene, which results in production of a structurally abnormal protein, blocks the development of natural Treg cells or produces dysfunctional Treg cells [19], and causes autoimmune disease. Foxp3 is currently the most specific and reliable molecular marker for natural Treg cells in humans. Yagi et al. [20] found that in patients with metastatic melanoma, the number of Treg in metastatic lymph nodes was two times higher than normal lymph nodes, and highly expressed in Foxp3. In this study, RT-PCR was used to detect expression levels of Foxp3; the results showed that the expression levels of Foxp3 in Uygur patients gradually increased from normal control, cervical CIN to cervical cancer. There was a statistically significant difference between normal controls, CIN I and CIN II-III, cervical cancer. The results showed that Foxp3 has a positive correlation with the stage of CIN and cervical carcinoma. Combining the Treg findings, this is possible because of Uygur CD4 + CD25 + Treg cell number increased, so that the Foxp3 mRNA expression level increased. The inhibition function of Treg not only maintains their constituents in tolerance, but also prevents the body from immune responses, resulting in cervical cancer cell immune evasion. The authors also studied the correlation between Treg and Foxp3, and the data confirmed that there is a positive correlation between them. The results showed that Foxp3 may be the main regulatory genes of CD4 + CD25 + Treg, and Foxp3 is an important switch for Treg cell development.

Chen et al. [21] first proved that TGF beta can stimulate activated CD4+CD25+T cells express Foxp3 in vitro. Ghiringhelli et al. [22] also found that tumor cells not only secrete TGF - beta and or IL-10, but can stimulate immature myeloid DC changes producing TGF-beta, thereby facilitating a tumor microenvironment, promoting CD4+CD25+ T cells transformation into CD4+CD25+Treg cells. IL-10 is essential for induction of antigen-specific Treg in vivo. Lundqvist et al. [23] found that mature DC can produce high levels of IL-10 and low levels of IL-2 to induce tumor-specific Treg cell generation. As a soluble mediator, IL-10 is often crucial for the maintenance of homeostasis and plays a pivotal role in Treg cell function. Treg cells from IL-10−/− mice have been shown to be significantly less potent than wild-type counterparts. In cervical carcinoma, patients...
produce higher concentrations of IL-10, which have also been shown to exert suppressive effects on DCs [24]. They can decrease anti-tumor immunity indirectly. Moreover, the role of IL-10 also implicates TGF-β, the production and action of these two cytokines being interrelated and involving positive feedback loops. IL-10 may act locally at the site of inflammation, while TGF-β seems to have a more systemic effect on immune response. Although, the requirement for TGF-β expression by Treg cells in vitro and in vivo are controversial [25], the results in this study indicated that TGF beta and IL-10 in patients with cervical cancer and cervical CIN was significantly higher than that in the normal control and CIN I group and had a high correlation with Treg cells and stage of CIN. Moreover, after surgery, TGF-β and IL-10 levels have decreased, suggesting that increased TGF-β and IL-10 promoted the increase of CD4+CD25+ Treg; this is conducive to tumor growth in vivo. It appears that regulation is dictated primarily by both Treg cells and soluble cytokine, such as IL-10 and TGF-β [26].

In conclusion, the authors provided evidence that Treg cells, especially CD4+CD25+Foxp3+ Treg cells, and soluble cytokine, IL-10 and TGF-β mediated the anti-tumor immune response. Therefore, these factors may promote researchers to investigate their therapeutic potential. Treg cells could be used as an immunotherapeutic tool by blocking their suppressive activity in anti-tumor immunity or vaccine development. Therefore, depletion of Treg cells in combination with other anti-tumor therapies could optimize eradication of malignancies, which may construct a theoretical framework for therapeutic use.

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References


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Comparing transvaginally defined endometrial thickness with hysteroscopic and histopathologic findings in asymptomatic postmenopausal women

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¹2nd Department of Obstetrics and Gynecology University of Athens, Aretaieion Hospital, Athens
²Department of Obstetrics and Gynecology, University of Crete, Herakleion, Crete (Greece)

Summary

Purpose: To assess the diagnostic value of transvaginal sonography (TVS) measurement of the endometrium compared to hysteroscopic findings and histopathologic reports in order to facilitate clinical management in asymptomatic postmenopausal women with thickened endometrium. Methods: During the period between January 2000 and December 2008, a retrospective analysis was performed including cases of women who were preoperatively diagnosed with a sonographically thickened endometrium, while asymptomatic, and therefore underwent hysteroscopic and fractionated dilatation and curettage (D & C) under general anesthesia at the Second Department of Obstetrics and Gynecology at Aretaieion Hospital in Athens, Greece. In the present study we compare US, hysteroscopic and pathologic findings. Results: The mean age of the patients ranged between 54-74 years (mean age 65.2 ± 6.8 years). In 108 cases, sonographically measured endometrial thickness ranged between 5 and 10 mm. In 59 cases, endometrial thickness ranged between 11 and 15 mm, whereas in 22 cases, between 16 and 20 mm and finally, in 13 cases endometrial thickness was more than 20 mm. Hysteroscopic examination revealed endometrial polyps in 161 cases, focal hyperplastic lesions in 28 cases, complete hyperplastic lesions in five cases while atrophy was found in five and cancer in three cases, respectively. Pathological results of the samples taken after hysteroscopy are as follows: in 169 cases (83.67%) in women with asymptomatic abnormal endometrial thickness, an endometrial polyp was present. Endometrial thickness in these cases patients was 10.9 ± 7.5 mm. In patients with focal hyperplasia (22 cases), endometrial thickness was 7.2 ± 0.5 mm but in patients with complete hyperplasia (5 cases) endometrial thickness was higher (12.3 ± 5.1 mm). Finally, in three cases with endometrial carcinoma endometrial thickness was 15.5 ± 7.8 mm. Six cases out of 28 described in our study were diagnosed as focal hyperplasia and two out of five cases as complete hyperplasia, whereas histological reports classified these cases as endometrial polyps. The other histological diagnoses confirmed hysteroscopic findings and thus provided the same results. Conclusions: We recommend hysteroscopy to follow gynecological TVS when a thickened endometrium is found in asymptomatic postmenopausal women for better diagnostic and, in a later stage, therapeutic efficacy. Key words: Asymptomatic; Postmenopausal; Endometrial thickening; Ultrasound; Hysteroscopy.

Introduction

Transvaginal sonography (TVS) is a useful method to detect endometrial pathology [1-3]. Different cut-off values for endometrial thickness in symptomatic postmenopausal women have been used in the differential diagnosis of pathologic endometrial lesions and cancer exclusion [4-8]. However, in asymptomatic women, when a thick endometrium is discovered during a routine ultrasound (US) examination, a clinical dilemma is raised as to which therapeutic strategy should be chosen because the diagnostic value of endometrial thickness alone has not been documented till now [9]. Hysteroscopy is commonly used to offer solutions to this dilemma as it is thought to be the “gold standard” for diagnosing endometrial lesions providing close color visualization [10].

The aim of the present study was to assess the diagnostic value of TVS measurement of endometrium compared to hysteroscopic findings and histopathologic reports in order to facilitate clinical management in asymptomatic postmenopausal women with thickened endometrium.

Materials and Methods

During the period between January 2000 and December 2008, a retrospective analysis was performed including cases which were preoperatively diagnosed with a sonographically thickened endometrium and therefore underwent hysteroscopic and fractionated dilatation and curettage (D&C) under general anesthesia at the Second Department of Obstetrics and Gynecology at Aretaieion Hospital in Athens, Greece. The study group consisted of 202 postmenopausal asymptomatic women with endometrial thickness of ≥ 5 mm (double layer) on TVS scanning. It should be mentioned that the US scan was performed as a complementary method during their routine gynecological check.

Diagnostic hysteroscopy was performed with a Versapoint bipolar (Johnson and Johnson, USA) or with a Karl Storz resectoscope with the patient being under general anesthesia. Uterine distention was achieved by saline infusion. Following hysteroscopy, a fractional D&C was performed and the specimens were sent for histological examination. All specimens were characterized according to the World Health Organization criteria. Overall, the “gold standard” of diagnosis was the histological report.

Medical records including age, possible hypertension, diabetes mellitus, and body mass index were recorded for all patients of our study. Operation notes and histopathological databases were also thoroughly searched for the same period.

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Data was entered into computerized database. The Student’s t-test, Fisher’s exact test and X^2 test were used to statistically compare clinical parameters. ROC curve analysis was performed to display area under the ROC curve, with standard error and 95% confidence interval (CI). When the variable under study could not distinguish between the two groups, i.e., where there is no difference between the two distributions, the area will be equal to 0.5 (the ROC curve will coincide with the diagonal). When there is a perfect separation of the values of the two groups, i.e., there is no overlapping of the distributions, the area under the ROC curve equals 1 (the ROC curve will reach the upper left corner of the plot). The 95% CI is the interval in which the true (population) area under the ROC curve lies with 95% confidence. A probability value of less than 0.05 was considered to be statistically significant. For the statistic analysis, SPSS STATISTIC 17.0 and MedCalc (version 11.4.4.0) were used.

### Results

The mean age of the patients ranged between 54-74 years (mean age 65.2 ± 6.8 years). The main characteristics of the female patients are presented in Table 1. More specifically, 5.4% of the patients were nulliparous and 19.3% multiparous, while 75% of the patients had one or two children. Over 50% of the patients were obese, whereas 55% and 16% of the patients had hypertension and diabetes mellitus, respectively.

Results of endometrial thickness and hysteroscopic diagnoses of the patients are shown in Table 2. More specifically, in 108 cases, sonographically measured endometrial thickness ranged between 5 and 10 mm. In 59 cases, endometrial thickness ranged between 11 and 15 mm, whereas in 22 cases, between 16 and 20 mm and finally, in 13 cases endometrial thickness was more than 20 mm. Hysteroscopic examination revealed endometrial polyps in 161 cases, focal hyperplastic lesions in 28 cases, complete hyperplastic lesions in five cases while atrophy was found in five and cancer in three cases, respectively.

Statistically significant findings were identified after analysis. More specifically, sensitivity, specificity, positive [PPV) and negative predictive value (NPV) were estimated. Sensitivity and specificity for polyps were 95.27% and 100%, respectively while PPV and NPV were 100% and 80.5% respectively (p < 0.0001). The respective estimates for focal hyperplasia were 100.0%, 96.67%, 78.6% and 100.0% (p < 0.0001), while those for complete hyperplasia were 100%, 100%, 60.0% and 100.0%, respectively (p < 0.0001). The respective estimates of sensitivity, specificity, PPV and NPV for atrophy were 60.0%, 100%, 100% and 99.9% (p < 0.0143), while those for cancer were 100%, 98.99%, 100% and 100%, respectively (p < 0.0001).

A subgroup analysis was performed according to endometrial thickness which ranged between 5-10, 11-15, and 16-20 mm in the different subgroups. Regarding focal hyperplasia, the estimates of sensitivity and specificity for 5-10 mm were 72.3 and 100, respectively and PPV and NPV were 100 and 50, with a p-value < 0.0001, while the estimates of sensitivity and specificity for 11-15 mm were 27.27 and 100, respectively and PPV and NPV were 100 and 27.3, with a p-value < 0.0001. The estimates of sensitivity and specificity for 16-20 mm were 18.18 and 100, respectively and PPV and NPV were 100 and 25, with a p-value < 0.0001. Regarding polyps, the findings were not statistically significant in the performed sub-analysis, however the estimate of sensitivity for 5-10 mm was 51.5 and for PPV and NPV 100 and 0. The estimate of sensitivity for 11-15 mm was 29 and for PPV and NPV 100 and 0. The estimate of sensitivity for > 20 mm was 9.09 and 100, respectively and PPV and NPV were 100.0 and 23.1, but there was not statistical significance (p < 0.1473). Regarding cancer, the findings were not statistically significant in the performed analysis. In Table 3, we present the histopathological results of the samples taken after hysteroscopy. Thus, in 169 cases (83.67%) of women with asymptomatic abnormal endometrial thickness, an endometrial polyp was present. Endometrial thickness in these patients was 10.9 ± 7.5 mm. In patients with focal hyperplasia (22 cases), endometrial thickness was 7.2 ± 0.5 mm but in patients

### Table 1. — Patient clinical characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11</td>
<td>5.4%</td>
</tr>
<tr>
<td>1-2</td>
<td>152</td>
<td>75.3%</td>
</tr>
<tr>
<td>3+</td>
<td>39</td>
<td>19.3%</td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>30</td>
<td>14.9%</td>
</tr>
<tr>
<td>25-29.9</td>
<td>62</td>
<td>30.7%</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>110</td>
<td>54.4%</td>
</tr>
<tr>
<td>Past hormone therapy</td>
<td>26</td>
<td>12.8%</td>
</tr>
<tr>
<td>Arterial hypertension (systolic blood pressure &gt; 140 mmHg or diastolic blood pressure &gt; 90 mmHg)</td>
<td>111</td>
<td>55%</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>32</td>
<td>15.84%</td>
</tr>
<tr>
<td>Past use of oral contraceptives</td>
<td>10</td>
<td>5%</td>
</tr>
</tbody>
</table>

### Table 2. — Hysteroscopic diagnosis of uterine cavity pathology.

<table>
<thead>
<tr>
<th>Endometrial thickness (mm)</th>
<th>Polyp</th>
<th>Focal hyperplasia</th>
<th>Complete hyperplasia</th>
<th>Atrophy</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-10</td>
<td>87</td>
<td>16</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>11-15</td>
<td>49</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>16-20</td>
<td>15</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>161</td>
<td>28</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 3. — Pathological findings.

<table>
<thead>
<tr>
<th>Endometrial polyps</th>
<th>169</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple hyperplasia</td>
<td>22</td>
</tr>
<tr>
<td>Complex hyperplasia</td>
<td>3</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>3</td>
</tr>
<tr>
<td>Atrophy</td>
<td>5</td>
</tr>
</tbody>
</table>

In Table 3, we present the histopathological results of the samples taken after hysteroscopy. Thus, in 169 cases (83.67%) of women with asymptomatic abnormal endometrial thickness, an endometrial polyp was present. Endometrial thickness in these patients was 10.9 ± 7.5 mm. In patients with focal hyperplasia (22 cases), endometrial thickness was 7.2 ± 0.5 mm but in patients
with complete hyperplasia (5 cases) endometrial thickness was higher (12.3 ± 5.1 mm). Finally, in three cases with endometrial carcinoma endometrial thickness was 15.5 ± 7.8 mm. Six cases out of 28 described in our study were diagnosed as focal hyperplasia and two out of five cases as complete hyperplasia whereas the histological report classified these cases as endometrial polyps. The other histological diagnoses confirmed hysteroscopic findings and thus provided the same results.

No major events complicated the hysteroscopic process. Complications included three uterine perforations resulting in ending of the operative procedures, which were repeated in a later period.

Discussion

To our knowledge, this is one of the first studies used to evaluate TVS, hysteroscopy and histopathological reports of asymptomatic postmenopausal women with a sonographically thickened endometrium. Other researchers have compared the value of diagnostic hysteroscopy with TVS in different populations [11, 12]. According to Kasraeian et al. TVS is a moderately accurate test in asymptomatic postmenopausal women with sensitivity, specificity, PPV and NPV being 62.2%, 93.9%, 68.3% and 92.2%, respectively [13].

As is already known, TVS is a useful modality to detect endometrial lesions [1-3]. Increasing use of sonohysteroscopy improves diagnostic performance of TVS [11]. However, hysteroscopy is highly accurate and so it can be used as a diagnostic tool for diagnosing endometrial lesions by evaluating the specimens of dilatation and curettage [14]. It is known that an endometrial thickness of 4-5 mm in postmenopausal symptomatic women is generally used as a limit for excluding endometrial malignancy, and if found in a range between 5 and 8 mm further investigation of the lesion is required [1-3]. This limit is not applicable to women without postmenopausal bleeding. US measurement of endometrial thickness alone is not a useful test for diagnosing focal intruterine pathologies in these women [15-18].

In asymptomatic postmenopausal women benign focal lesions such as endometrial polyps, are the most common findings [19, 20].

In our department US scans are generally performed in symptomatic or asymptomatic women as a complementary method during their standard gynecological examination. The next diagnostic step in our clinical protocols depends on US findings and if we find an abnormally thickened endometrium (that is, thickness > 5 mm) we perform D&C and hysteroscopy. In our study, we certified that endometrial polyps were the most common findings among postmenopausal women with thickened endometrium. To be more specific, 169 out of 202 women (83.66%) were found to have an endometrial polyp, which is probably the reason for the endometrial thickness. This result is in accordance with that of Schmidt et al. [21], who reported a polyp incidence of 78.5% of their patient group, consisting of women who presented with thickened endometrium in their US examination, and with that of Dreisler et al. [22] who reported a 11.8% prevalence of uterine polyps in a large Danish population between 20 and 74 years old, in the postmenopausal women of this population.

An interesting finding of our study is that we discovered eight cases of endometrial polyps [6 were hysteroscopically considered to be focal hyperplastic lesions and 2 complete hyperplastic lesions] that were missed by hysteroscopic examination and the final diagnosis was made after histological examination of the tissue specimens. This reveals that despite the high accuracy rates of hysteroscopy, some cases (4.7%) may be missed. On the other hand, it highlights the fact that endometrial polyps, although not considered as genuine precancerous lesions, have a hyperplastic potential (as shown elsewhere) [22], and thus should be removed.

The sensitivity and specificity rates of hysteroscopy are high, and are 95.26%-100% for the diagnosis of endometrial polyps, respectively, 100%-96.67% for focal hyperplasia, 100%-98.99% for complete hyperplasia, 60%-100% for atrophic lesions and 100%-100% for the diagnosis of endometrial cancer (despite the fact that there was no statistical significance for the cases of endometrial cancer due to the small number of such cases). Those findings are similar to those described by Dreisler et al. [22], who reported sensitivity and specificity rates at the levels of 56% and 88%, respectively for a cut-off level of 5 mm for TVS, as was also used in our study.

Another finding of our study is that we discovered five cases of atrophic endometrium, in which the thickness measurement was more than 5 mm (4 cases between 5 and 10 mm, and 1 case between 11 and 20 mm). In all three cases of cancer, endometrial thickness was more than 5 mm, and further investigation was performed, including a hysteroscopy and hysteroscopic-guided D&C, due to the high sensitivity and specificity rates for endometrial cancer diagnosis [23].

Conclusion

Encouraged by the results of our study, we recommend hysteroscopy to follow gynecological TVS when a thickened endometrium is found in asymptomatic postmenopausal women for better diagnostic and, in a later stage, therapeutic efficacy.

References

Comparing transvaginally defined endometrial thickness with hysteroscopic and histopathologic findings in asymptomatic etc.


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Correlation of human papilloma virus infection with cytology, colposcopy and histopathological examination of the biotic tissue in low- and high-grade intraepithelial lesions

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Higher Education School of Professional Health Studies in Belgrade (Serbia)

Summary

Introduction: It is now believed that the majority of cervical cancer is preceded by long-term infection with high-risk types of the human papilloma virus (HPV). The presence of HPV high-risk types (HR-HPV) in the cells of intraepithelial change multiplies the possibility of its progressive development to high-grade cervical precancer and invasive disease. Aim: This study examined the correlation of HPV infection with cytology, colposcopy, and histopathological examination of the biotic tissue in low- and high-grade cervical lesions. Materials and Methods: This research was conducted as a study section. Data collection was performed during a ten-year period, at the University Clinic of Gynecology and Obstetrics - Narodni Front in Belgrade (Serbia). The basic set included 1,927 patients. Colposcopy, cytology, histopathology, and HPV test verification was made in all patients. Statistical analysis was performed using the SPSS program, version 17.0. Contingency tables were used to assess the degree of correlation of variables and chi-square test was used to determine the level of statistical significance in this study. A p value < 0.05 was considered statistically significant. Results: Among 1,927 women studied, 635 (32.95 %) had abnormal cytological findings and among these, 272 (42.83%) were HR-HPV positive. There was a statistical difference between colposcopic and cytological findings in patients with HR-HPV (x² = 35.33, p = 0.000). There was also a statistically significant difference between histopathological and colposcopic findings in patients with HR-HPV (x² = 10.171, p = 0.001). Only HR-HPV types 16 and 18 showed a statistical significance compared to histopathological findings, unlike other HR-HPV. An important finding was that the authors found an abnormal colposcopy in 93.30% patients with low-grade intraepithelial neoplasia and 68.05% patients with low-grade squamous intraepithelial lesion (LSIL) had normal cytology and was 70.15 % HR-HPV negative. Conclusion: The findings imply that among high-grade intraepithelial neoplasias, the authors found a high presence of HPV type 16 and 18, and a statistical significant presence of HPV 16 in low-grade intraepithelial neoplasia, unlike other HR-HPV types in low-grade intraepithelial findings. The authors found a significant statistical correlation with abnormal cytology and presence of HPV type 16 in both groups (LSIL and high-grade squamous intraepithelial lesion (HSIL). The authors also found an abnormal colposcopy in 93.30% of patients with low-grade intraepithelial neoplasia, while 68.05% of patients with LSIL had normal cytology and were HR-HPV negative in 70.15% of the cases.

Key words: Human papilloma virus (HPV); Colposcopy; Cytology; Histopathological examination.

Introduction

Cancer of the cervix is the second most common cancer among women worldwide [1]. Human papilloma virus (HPV) infection is a necessary cause of cervical cancer. It is now believed that the majority of cervical cancer is preceded by long-term infection with high-risk types of HPV, a sexually-transmitted infection [2, 3]. Considering the prognosis of these pathological changes, it is worth mentioning that their biological behavior is unpredictable. The presence of HPV high-risk types (HR-HPV) in the cells of intraepithelial change multiplies the possibility of its progressive development to invasive disease [3].

Persistent detection of HR-HPV types is a strong predictor of development of high-grade cervical precancer and invasive cancer [4]. The principle of preventive actions should be respected, preventing already diagnosed intraepithelial change to develop into invasive cancer. Infection with HR-HPV types results in a complex of cellular abnormalities of the cervical epithelium and is an important early precursor event in carcinogenic progression to cervical cancer [5, 6]. Due to its simplicity and cost effectiveness, cytology is the most often used diagnostic method, representing the basis of the screening program in many countries worldwide. It must be pointed out that its sensitivity in discovering the intraepithelial stages of disease is limited. In histologically verified intraepithelial stages, the percentage of false-negative results of cytodiagnostics is considerable.

Other than cytodiagnostics, colposcopy is another method for detection of the cervical neoplasias, characterized by considerably higher sensitivity in detecting the lower stages of disease. For the verification and determination of pathological change extent detected by HPV testing, colposcopic and/or cytological examination, it would also be necessary to perform biopsy and histopa-
Correlation of human papilloma virus infection with cytology, colposcopy and histopathological examination of the bioptic etc.

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and cytological findings (x2 = 0.394, p = 0.530).

Abnormal colposcopic images, abnormal cytology, and HR-HPV were found in 24 patients (17.52%). There was a statistically significant difference between histopathological and colposcopy in patients with HR-HPV (x2 =

Materials and Methods

This research was conducted as a study section. Data collection was performed during the period from 2000 to 2010, in the University Clinic of Gynecology and Obstetrics - Narodni Front in Belgrade (Serbia). The basic set included 1,927 patients, aged 16-72 years (average age 33, SD = 9.60). Colposcopy, cytology, histopathology, and HPV test verification were made in all patients. Statistical analysis was performed using the SPSS program, version 17.0. Contingency tables were used to assess the degree of correlation of variables and chi-square test was used to determine the level of statistical significance in this study. A p value less than 0.05 (p < 0.05) was considered statistically significant.

Results

Altogether, 1,927 women were all examined with an adequate HPV test, cytology, colposcopy, and histopathological examination of the biopsy tissue. Of the subjects studied, 399 were < 24 years, 738 were 25-34 years, 790 were > 35 years. Altogether, 612 women (31.76%) were positive for one or more HR-HPV types. HPV 16 and HPV 33 were the most prevalent types found in 11.36% and 6.43%, respectively, with HPV 31 found in 5.24%; HPV 18 in 5.03%; and HPV 51 in 3.68%. Among 1,927 women, 635 (32.95%) had abnormal cytological findings and 272 (42.83%) among these were HR-HPV positive.

Concern regarding the low sensitivity of conventional cytology prompted research for new methods to either supplement or replace it. Testing for HPV has higher sensitivity but lower specificity than pap smear test [9, 10].

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Table 1. — Correlation colposcopic findings with the results of HPV, cytological, and histopathological results.

<table>
<thead>
<tr>
<th></th>
<th>Normal n = 1,046</th>
<th>Abnormal n = 881</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a (%)</td>
<td>a (%)</td>
</tr>
<tr>
<td>Cytology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>732 69.98</td>
<td>105 10.04</td>
</tr>
<tr>
<td>Abnormal</td>
<td>158 15.10</td>
<td>22 2.10</td>
</tr>
<tr>
<td>Histopathological verification</td>
<td>883 84.41</td>
<td>125 11.95</td>
</tr>
<tr>
<td>LSIL</td>
<td>7 0.67</td>
<td>2 0.19</td>
</tr>
<tr>
<td>HSIL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. — Correlation between HPV and cytological findings (normal and abnormal).

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Normal cytology (n = 1292)</th>
<th>Abnormal cytology (n = 635)</th>
<th>Total infections (n = 1927)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># (%)</td>
<td># (%)</td>
<td># (%)</td>
</tr>
<tr>
<td>HPV-</td>
<td>815 63.08</td>
<td>363 57.16</td>
<td>1178 61.13</td>
</tr>
<tr>
<td>HPV+</td>
<td>477 36.92</td>
<td>272 42.84</td>
<td>749 38.87</td>
</tr>
<tr>
<td>HR-HPV+</td>
<td>369 28.56</td>
<td>243 38.26</td>
<td>612 31.76</td>
</tr>
<tr>
<td>LR-HPV+</td>
<td>108 8.35</td>
<td>45 7.33</td>
<td>153 7.93</td>
</tr>
</tbody>
</table>

Table 3. — Correlation of HPV in women with histopathological findings (LSIL and HSIL).

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Histopathological LSIL (n = 1859)</th>
<th>Histopathological HSIL (n = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># (%)</td>
<td># (%)</td>
</tr>
<tr>
<td>HPV-</td>
<td>193 9.94</td>
<td>0.000</td>
</tr>
<tr>
<td>HPV+</td>
<td>84 4.51</td>
<td>0.000</td>
</tr>
<tr>
<td>HR-HPV+</td>
<td>99 5.32</td>
<td>0.386</td>
</tr>
<tr>
<td>LR-HPV+</td>
<td>121 6.50</td>
<td>0.489</td>
</tr>
</tbody>
</table>

Table 4. — The correlation between HPV in women with LSIL and HSIL and normal cytology (PA II).

<table>
<thead>
<tr>
<th>HPV type</th>
<th>HP LSIL PA II (n = 1265)</th>
<th>HP H-SIL PA II (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># (%)</td>
<td># (%)</td>
</tr>
<tr>
<td>HPV-</td>
<td>100 7.90</td>
<td>13 48.14</td>
</tr>
<tr>
<td>HPV+</td>
<td>50 3.95</td>
<td>7 25.92</td>
</tr>
<tr>
<td>HR-HPV+</td>
<td>68 5.37</td>
<td>0 0.000</td>
</tr>
<tr>
<td>LR-HPV+</td>
<td>82 6.48</td>
<td>1 3.70</td>
</tr>
</tbody>
</table>

Table 5. — Correlation of HPV in women with histopathological findings (LSIL and HSIL).

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Histopathological LSIL (n = 1859)</th>
<th>Histopathological HSIL (n = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># (%)</td>
<td># (%)</td>
</tr>
<tr>
<td>HPV-</td>
<td>193 9.94</td>
<td>0.000</td>
</tr>
<tr>
<td>HPV+</td>
<td>84 4.51</td>
<td>0.000</td>
</tr>
<tr>
<td>HR-HPV+</td>
<td>99 5.32</td>
<td>0.386</td>
</tr>
<tr>
<td>LR-HPV+</td>
<td>121 6.50</td>
<td>0.489</td>
</tr>
</tbody>
</table>

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In Table 1 the authors found 612 patients with HR-HPV (16, 18, 31, 33, and 51) and carried out the correlation between the findings of colposcopy and cytology, as well as colposcopy correlation between the findings and histopathological examination of the biopsy tissue. There was a statistical difference between the findings of colposcopy and cytology in patients with HR-HPV (x2 = 35.33, p = 0.000). Twenty-two normal subjects had colposcopic images and abnormal cytology (3.59%). Low-risk (LR) HPV was found in 137 patients and there was no statistically significant difference between colposcopic
Based on the results of cytology and biopsy as shown in Tables 4 and 5, the authors found 1,265 (65%) patients with histopathological LSIL and normal cytology and 27 (1.40%) patients with histopathological HSIL and normal cytology. In these patients, there was a statistically significant higher incidence of HR-HPV 16 and 18. Other HR-HPV viruses did not have a statistical significance compared to the histopathological exam and cytology. In the group of patients with abnormal cytology, the authors found after biopsy, 595 patients (30.83%) with LSIL and 41 patients (2.13%) with histopathological HSIL. The patients with HPV were divided according to three age groups.

Patients with HPV-positive results were divided into three age groups (Table 6). Of all LR-HPV, LR-HPV 6 occurred in 30.65% of the 25-34 year age group, while HR-HPV 16 was most prevalently found in the ≥ 35 year age group (p < 0.05). The second most common HPV virus was HR-HPV 33, which was significantly more common in the 25-34 year age group (43.55%) compared to other age groups (p < 0.05).

An examination of the colposcopic findings (Table 7) in relation to histopathological results, the authors found a statistical significance in both groups (LSIL and HSIL, p < 0.05). Abnormal colposcopy and HSIL were found in 59 patients (3.06%), which were significantly higher than in the nine patients (0.47%) with normal colposcopic results and histopathological HSIL findings.

### Discussion

HPV is common sexually transmitted virus and a subset of high-risk types is integral to the development of cervical cancer and its cytologic precursors. HPV16 is the most common HPV type found in the cervix and cervical cancer is detected in over 50% of patients and has been reported to be the most common genotype in high-grade cervical intraepithelial neoplasia [10, 11]. Other HPV types commonly detected in cervical cancer include types 18, 31, and 45. The HPV genotypes 16, 18, 33, and 45 have been detected more frequently in invasive cervical carcinomas compared to premalignant lesions [12-14]. In this study, among high-grade intraepithelial neoplasia, the authors found in 52.94% the presence of HPV 16 and only 9.84% with HPV 16 in low-grade intraepithelial findings. Among HSIL, the authors found in 19.12% the presence of HPV 18, but other HR-HPV is present between 2.94% and 4.41%. These results are comparable with those of other studies and show that presence of HR-HPV (except for HPV 16) is low, between 3.65% and 6.50% in LSIL [11, 15-17].

In the natural history of HPV, most infections are transient, especially among younger women, and only a small fraction of infections that persist may progress to cervical cancer. In a prospective cohort of 1,075 women, Woodman demonstrated that, compared to HPV-negative women, women infected with HPV 16 and 18 had relative hazard ratios of 8.5% and 3.3%, respectively, for the development of cervical intraepithelial neoplasia 2 (CIN 2) or 3 (CIN3) [17-20].

### Table 5. — The correlation between HPV in women with LSIL and HSIL and pathological cytology (PA III).

<table>
<thead>
<tr>
<th>HPV type</th>
<th>n</th>
<th>#</th>
<th>%</th>
<th>p</th>
<th>#</th>
<th>%</th>
<th>p</th>
<th>Total</th>
<th>#</th>
<th>%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>127</td>
<td>20.75</td>
<td>239</td>
<td>39.05</td>
<td>246</td>
<td>40.2</td>
<td>612</td>
<td>31.76</td>
<td>0.899</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>239</td>
<td>43.55</td>
<td>362</td>
<td>69.33</td>
<td>313</td>
<td>56.0</td>
<td>614</td>
<td>31.76</td>
<td>0.133</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>35</td>
<td>6.56</td>
<td>63</td>
<td>12.50</td>
<td>39</td>
<td>6.56</td>
<td>137</td>
<td>7.11</td>
<td>0.080</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>31</td>
<td>5.22</td>
<td>57</td>
<td>11.39</td>
<td>24</td>
<td>4%</td>
<td>108</td>
<td>5.58</td>
<td>0.159</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>51</td>
<td>14.0</td>
<td>72</td>
<td>32.90</td>
<td>103</td>
<td>47.0</td>
<td>219</td>
<td>11.36</td>
<td>0.133</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 6. — Distribution of HPV infected patients classified by age group, Belgrade, Serbia, 2000-2010.

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Age (years)</th>
<th>n</th>
<th>#</th>
<th>%</th>
<th>p</th>
<th>#</th>
<th>%</th>
<th>p</th>
<th>Total</th>
<th>#</th>
<th>%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>594</td>
<td>21</td>
<td>239</td>
<td>46.65</td>
<td>25</td>
<td>29.5</td>
<td>88</td>
<td>45.7</td>
<td>0.045</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>700</td>
<td>22</td>
<td>41</td>
<td>50.71</td>
<td>18</td>
<td>25.6</td>
<td>48</td>
<td>26.7</td>
<td>0.159</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1,927</td>
<td>44</td>
<td>72</td>
<td>37.20</td>
<td>39</td>
<td>20.5</td>
<td>119</td>
<td>6.19</td>
<td>0.133</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>594</td>
<td>23</td>
<td>44</td>
<td>38.15</td>
<td>38</td>
<td>12.7</td>
<td>76</td>
<td>4.03</td>
<td>0.030</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>390</td>
<td>11</td>
<td>15.5</td>
<td>36.50</td>
<td>24</td>
<td>33.8</td>
<td>71</td>
<td>3.68</td>
<td>0.084</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>n = 399</td>
<td>32</td>
<td>25.8</td>
<td>34.70</td>
<td>37</td>
<td>93.30</td>
<td>124</td>
<td>6.43</td>
<td>0.030</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>n = 738</td>
<td>31</td>
<td>22.8</td>
<td>34.70</td>
<td>44</td>
<td>12.7</td>
<td>98</td>
<td>5.22</td>
<td>0.030</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 7. — The correlation between colposcopic findings and histopathological verification.

<table>
<thead>
<tr>
<th>Colposcopy</th>
<th>Normal</th>
<th>Abnormal</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSIL</td>
<td>1037</td>
<td>99.14</td>
<td>822</td>
</tr>
<tr>
<td>HSIL</td>
<td>9</td>
<td>0.86</td>
<td>59</td>
</tr>
</tbody>
</table>

10,171, p = 0.001); 438 patients had abnormal colposcopic images and histopathological LSIL (71.57%), and only two patients had normal colposcopic images and histopathological HSIL. There was no statistically significant difference between histopathological and colposcopic findings in patients with LR-HPV (x2 = 1.718, p = 0.190).

The results in Table 2 show that in 1,292 (67.04%) patients with normal cytology, 477 had HPV. The authors compared patients with HPV positive findings and cytology. There was a statistically significant difference between normal and abnormal cytology in both groups, with HR-HPV and LR-HPV. Unlike other viruses, the presence of the HPV 16 as HR-HPV and LR-HPV 11 indicated a statistical significance in patients with normal and abnormal cytology.

In Table 3 the authors found that in 1,927 patients, 1,859 had LSIL and 68 had HSIL, as confirmed by histopathological verification of the biopic tissue. Only HR-HPV 16 and 18 showed a statistical significance compared to histopathological findings, unlike other HR-HPV. HPV testing found that HPV 16 were the most common viruses in HSIL as well as in LSIL histopathological findings.
Due to its simplicity and cost-effectiveness, cytology and HPV tests are the most frequently used diagnostic methods, representing the basis of screening program in many countries worldwide [21-23].

As was expected, the authors found 48.14% HPV 16 with normal cytology and high-grade histopathological findings, while only 7.90% had HPV 16, LSIL, and normal cytology. Abnormal cytology showed that there was a higher statistically significant presence of HPV 16 in LSIL (14.0%) and HSIL (56.10%). These results confirmed that HPV infection is mostly a transient phenomenon resulting in no cervical lesions or mostly low-grade lesions that often regress spontaneously. The persistence of an HPV infection appears to be a prerequisite for the development of CIN 3 and cervical cancer. Viral, host, and environmental factors may influence the course of HPV infection [21, 24].

Other than cytodiagnostics, colposcopy is another basic method for detection of cervical neoplasias, characterized by a considerably higher sensitivity of detecting the lowest stages of disease. The baseline of colposcopy is the recognition of pathological changes of the cervical epithelium that are based on significant protein increase in dysplastic cells and a considerable loss of glycogen, as well as on the changes of the stromal vascular net regarding the number, appearance, and capillary arrangement. SIL undergoing a colposcopic examination are manifested as different pathological picture of acetowhite epithelium, mosaic, punctuation, leukoplakias or atypical blood vessels. They are a sign of the pathological events in the epithelium and need to be histologically explained [7, 22-24].

The study of Kulasingam et al. showed that all women with abnormal cytology resulted in colposcopy. The strategy of requiring two positive PCR tests for HPV was both more sensitive and specific than the referral of women with abnormal cytology results for colposcopy [25, 26]. The authors found normal colposcopy in only two patients (0.19%) with HR-HPV presence and HSIL and normal colposcopy in 11.95% patients with HR-HPV positive test and LSIL. Important findings include that 93.30% of the patients had LSIL and normal colposcopy, and only 6.70% of patients had HSIL and abnormal colposcopy. These results showed that colposcopy is effective for detecting the lowest stage of disease.

Conclusion

The findings in this study imply that among high-grade intraepithelial neoplasias, a high presence of HPV 16 and 18 was found and a statistically significant presence of HPV 16 was found in low-grade intraepithelial neoplasia, unlike other HR-HPV types in low-grade intraepithelial findings. The authors also found a significant statistical correlation between abnormal cytology and the presence of HPV 16 in both groups (LSIL and HSIL). The most prevalent HR-HPV 16 was found in the age group ≥ 35 years, while HPV 33 was more common in the age group of 25-34 years. Important findings include that abnormal colposcopy was found in 93.30% of patients with low-grade intraepithelial neoplasia and 68.05% patients with LSIL that had normal cytology and that was 70.15% HR-HPV negative.

Acknowledgments

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References


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Diagnostic value of thrombocytosis and high CA 125 level in women with adnexal masses

T. Atacag

Department of Obstetrics and Gynecology, Near East University Faculty of Medicine, Lefkosa, TRNC, Mersin (Turkey)

Summary

Aim: The aim of this study was to determine the diagnostic value of thrombocytosis and high CA 125 levels in women with benign and malign adnexal masses. Thrombocytosis (platelet counts > 400 × 10^9/l) has been identified as a poor prognostic factor in many cancers including certain gynecologic malignant tumors such as endometrial, cervical, and ovarian cancers. Methods: Medical charts of 180 patients with adnexal masses were retrospectively reviewed and analyzed for the association of preoperative thrombocytosis and high CA 125 level with other clinical prognostic factors. Results: Of the 180 participants, 68 (68% of malignant adnexal masses) had thrombocytosis and 74 patients (74% of malignant adnexal masses) had elevated CA 125 levels. The patients with preoperative thrombocytosis were found to have greater elevations of CA 125 levels, more advanced stage disease, and higher grade tumors. Conclusion: Presence of thrombocytosis and high CA 125 alone and in combination may be used as a prognostic factor in the management of women with adnexal masses since they are already used as clinical tests for several purposes.

Key words: Thrombocytosis; CA125; Adnexal masses.

Introduction

In gynecology, the adnexa refer to the region adjoining the uterus and contain the ovary and Fallopian tube, as well as associated vessels, ligaments, and supporting connective tissue. Some of adnexal masses can regress spontaneously while others require a surgical procedure for histopathologic diagnosis and treatment. A mass in the adnexa may be symptomatic or discovered incidentally [1, 2]. Pathology in the adnexal area may arise from the uterus, bowel, retroperitoneum, or metastatic disease from another site, such as the breast or stomach [1]. Adnexal masses often include simple ovarian cysts, tubal pregnancies, and benign or malignant (cancerous) tumors. As tubal neoplasms are rare, adnexal masses generally remind us of ovarian tumors. The masses related to the tubes generally originate from inflammation [2].

Thrombocytosis is considered as platelet count greater than 400 × 10^9/l, consistent with published criteria [3-11]. Malignant cells produce cytokines such as IL-6 and other growth factors capable of inducing platelet production, and platelets in turn may contribute to tumor growth and metastases. Platelets are a rich source of both platelet-derived growth factor (PDGF) and thrombospondin. PDGF has been shown to function as a potent mitogen for a variety of cell types. Thrombospondin is an adhesive glycoprotein that supports the adhesion of tumor cells to the endothelium, and may promote metastasis through urokinase-type plasminogen activator mediation of cell invasion. Preoperative thrombocytosis may favor the diagnosis of malignancy in women undergoing surgical evaluation of pelvic masses [12, 13].

The original CA 125 as a tumor marker test is a homologous double-determinant (OC 125 monoclonal antibody based) assay for the quantification of tumor associated mucin-like CA 125 molecules present in the serum. It is found in small amounts like below 35 U/ml in the vast majority of cases. Therefore, its cutoff level for “normal” has been arbitrarily set at 35. CA 125 is a protein produced in response to mainly irritation of the peritoneal cavity. Anything that irritates the peritoneal cavity, the pleural cavity, even the pericardial sac, has a potential to cause an elevation of the CA 125. Thus, many benign disease processes may cause an increase in the level of CA 125. Some of these conditions are gynecological such as endometriosis, fibroids, and even menstruation. Other cancers such as colon cancer metastatic to the peritoneal cavity, metastatic stomach cancer, and pancreatic cancer besides ovarian can increase CA 125 levels [14, 15].

There is limited data evaluating usefulness of thrombocytosis and high CA 125 level in gynecologic practice to differentiate the types of adnexal masses. The aim of this study was to evaluate the diagnostic value of thrombocytosis and high CA 125 levels in patients with a benign or malign adnexal mass.

Materials and Methods

This retrospective study included 180 women undergoing gynecologic surgery for an adnexal mass at the Dr. Zekai Tahir Burak Women’s Hospital after approval of the local ethics committee. Patients that were not subjected to preoperative examination of platelet and CA 125 level, patients with an internal disease that could cause thrombocytosis, those with asplenism, those who take medicine that could cause thrombocytosis, and...
patients in postpartum and postoperative status were not included in the study. Patients with thrombocyte number > 400,000/mm³ were diagnosed as having thrombocytosis. Serum CA level > 125 UI/ml was considered high.

We collected demographic, clinical, and histopathologic data and FIGO stage of ovarian cancer of the study subjects. The study subjects were divided into benign and malign adnexal mass groups according to the histopathologic diagnosis.

Statistical analyses

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of thrombocytosis and high CA 125 levels were calculated. After categorization of patients according to age ≤ 48 or ≥ 48 years, these calculations were repeated. In patients with a benign or malign adnexal mass, the association of thrombocytosis and high CA 125 levels were evaluated with Pearson’s correlation analysis; p < 0.05 was considered significant.

Results

We included clinical data of 180 patients with adnexal masses in the study. Mean age of patients with a benign or malign adnexal mass was 44.2 (16-68 years) and 49.7 (16-81 years), respectively (Table 1).

Table 1. — Histopathologic types of adnexal masses in the study population.

<table>
<thead>
<tr>
<th></th>
<th>Benign (n = 80) n (%)</th>
<th>Malign (n = 100) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple cyst</td>
<td>27 (33.5%)</td>
<td>Serous cystadenocarcinoma 46 (46%)</td>
</tr>
<tr>
<td>Serous cystadenoma</td>
<td>12 (15%)</td>
<td>Mucinou 23 (23%)</td>
</tr>
<tr>
<td>Mucinous cystadenoma</td>
<td>8 (10%)</td>
<td>Endometrioid carcinoma 1 (1%)</td>
</tr>
<tr>
<td>Mature teratoma</td>
<td>8 (10%)</td>
<td>Clear cell carcinoma 3 (3%)</td>
</tr>
<tr>
<td>Thecoma of the ovary</td>
<td>6 (7.5%)</td>
<td>Undifferentiated carcinoma 5 (5%)</td>
</tr>
<tr>
<td>Endometrioma</td>
<td>6 (7.5%)</td>
<td>Borderline ovarian tumor 3 (3%)</td>
</tr>
<tr>
<td>Intraglandular myoma</td>
<td>4 (5%)</td>
<td>Dysgerminoma 3 (3%)</td>
</tr>
<tr>
<td>Tuboovarian abscess</td>
<td>3 (3.75%)</td>
<td>Endodermal sinus tumor 2 (2%)</td>
</tr>
<tr>
<td>Fibroma</td>
<td>3 (3.75%)</td>
<td>Immature teratoma 1 (1%)</td>
</tr>
<tr>
<td>Dermoid cyst</td>
<td>2 (2.5%)</td>
<td>Malign lymphoma 2 (2%)</td>
</tr>
<tr>
<td>Chronic ecptic pregnancy</td>
<td>1 (1.25%)</td>
<td>Granulosa cell tumor 6 (6%)</td>
</tr>
<tr>
<td>Chronic ectopic pregnancy</td>
<td>1 (1.25%)</td>
<td>Epidermoid carcinoma 1 (1%)</td>
</tr>
<tr>
<td>Chronic ectopic pregnancy</td>
<td>1 (1.25%)</td>
<td>Malign mixed Mullerian tumor 3 (3%)</td>
</tr>
<tr>
<td>Chronic ectopic pregnancy</td>
<td>1 (1.25%)</td>
<td>Metastatic tumors 1 (1%)</td>
</tr>
</tbody>
</table>

Table 2. — Number and percentage of benign and malign adnexal masses according to age groups of the study population.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Benign (n = 80) n (%)</th>
<th>Malign (n = 100) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>12.5% (10)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>21-30</td>
<td>16.25% (13)</td>
<td>13 (13)</td>
</tr>
<tr>
<td>31-40</td>
<td>17.50% (14)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>41-50</td>
<td>21.25% (17)</td>
<td>28 (28)</td>
</tr>
<tr>
<td>51-60</td>
<td>13.75% (11)</td>
<td>17 (17)</td>
</tr>
<tr>
<td>61-70</td>
<td>18.75% (15)</td>
<td>22 (22)</td>
</tr>
<tr>
<td>71-80</td>
<td>0 (0)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>≥ 81</td>
<td>0 (0)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

Table 3. — Number and percentage of patients according to thrombocytosis and high CA 125 level alone and in combination in the study population.

<table>
<thead>
<tr>
<th></th>
<th>Benign (n = 80) n (%)</th>
<th>Malign (n = 100) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytosis</td>
<td>11 (13.8%)</td>
<td>68 (68%)</td>
</tr>
<tr>
<td>High CA 125 level</td>
<td>42 (52.5%)</td>
<td>74 (74%)</td>
</tr>
<tr>
<td>Thrombocytosis plus high CA 125 level</td>
<td>10 (12.5%)</td>
<td>65 (65%)</td>
</tr>
</tbody>
</table>

Table 4. — Sensitivity, specificity, PPV and NPV of thrombocytosis, high CA 125 level, and thrombocytosis plus high CA 125 level in patients aged less than 48 or higher.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Thrombocytosis</th>
<th>High CA 125 level</th>
<th>Thrombocytosis plus high CA 125 level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>&lt; 48 71%</td>
<td>73%</td>
<td>64%</td>
</tr>
<tr>
<td>≥ 48</td>
<td>65%</td>
<td>75%</td>
<td>65%</td>
</tr>
<tr>
<td>Specificity</td>
<td>&lt; 48 80%</td>
<td>41%</td>
<td>89%</td>
</tr>
<tr>
<td>≥ 48</td>
<td>94%</td>
<td>59%</td>
<td>86%</td>
</tr>
<tr>
<td>PPV</td>
<td>&lt; 48 78%</td>
<td>56%</td>
<td>85%</td>
</tr>
<tr>
<td>≥ 48</td>
<td>95%</td>
<td>72%</td>
<td>88%</td>
</tr>
<tr>
<td>NPV</td>
<td>&lt; 48 73%</td>
<td>60%</td>
<td>71%</td>
</tr>
<tr>
<td>≥ 48</td>
<td>64%</td>
<td>59%</td>
<td>62%</td>
</tr>
</tbody>
</table>

In patients with a benign adnexal mass, the most common histopathologic types were simple ovarian cyst and serous cystadenoma. In patients with a malign adnexal mass, the most common types of tumor were serous and mucinous cystadenocarcinoma (Table 2).

The highest benign and malign adnexal mass ratios were found in women aged 41-50 years. In women aged more than 40 years, the malignity of the adnexal mass was higher compared to that found in women aged less than 40 years (Table 3).

In patients with malign adnexal masses, the ratios of thrombocytosis, high CA 125 level, or thrombocytosis plus high CA 125 level were significantly higher than those in patients with benign adnexal masses (p < 0.05) (Table 4).

Overall, in women aged < 48 or ≥ 48 years, the presence of thrombocytosis or high CA 125 alone and in combination provided comparable sensitivity and specificity (Table 5).

Overall, the ratio of thrombocytosis and higher CA 125 levels were increased according to the stage of ovarian cancer (Table 6).

In patients with adnexal masses, the sensitivity of high CA 125 level was the highest compared to thrombocytosis and thrombocytosis plus high CA 125 level. The specificity of thrombocytosis plus high CA 125 level was the highest compared to thrombocytosis and high CA 125 level. Overall, sensitivity, specificity, PPV and NPV of thrombocytosis and high CA 125 level in patients aged 48 years or higher were similar.

Discussion

In this retrospective study, we evaluated the clinical data of 180 women undergoing surgery for an adnexal
mass. We assessed the diagnostic value of presence thrombocytosis, high CA 125 level, and thrombocytosis plus high CA 125 level to rule out malign tumors. Simple ovarian cysts and serous cystadenoma were the most common benign masses. The most common malign types of tumor were serous and mucinous cystadenocarcinoma.

The highest benign and malign adnexal mass ratios were between age 41 and 50 years. The malignity of adnexal masses was higher at age 40 years or higher. For malign adnexal mass cases, the ratios of thrombocytosis and high CA 125 level alone or in combination were higher compared to those found in benign adnexal mass. Overall, in women aged less than 48 years or higher, the presence of thrombocytosis, high CA 125 level, and thrombocytosis plus high CA 125 level was higher compared to those found in benign adnexal mass cases. We assessed the diagnostic value of presence thrombocytosis, high CA 125 level, and thrombocytosis plus high CA 125 level in patients with malign adnexal mass.

Table 5. — Ratios of thrombocytosis and high CA 125 level in patients with malign adnexal mass according to stage of ovarian cancer.

<table>
<thead>
<tr>
<th>Thrombocyte number</th>
<th>CA 125 level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage n = 100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤ 400,000/mm³</td>
</tr>
<tr>
<td>I (n = 31)</td>
<td>19 (61.3%)</td>
</tr>
<tr>
<td>II (n = 12)</td>
<td>2 (16.7%)</td>
</tr>
<tr>
<td>III (n = 41)</td>
<td>7 (17.1%)</td>
</tr>
<tr>
<td>IV (n = 16)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td></td>
<td>13 (41.9%)</td>
</tr>
<tr>
<td></td>
<td>10 (83.3%)</td>
</tr>
<tr>
<td></td>
<td>5 (12.2%)</td>
</tr>
<tr>
<td></td>
<td>4 (25%)</td>
</tr>
</tbody>
</table>

Table 6. — Sensitivity, specificity, PPV and NPV of thrombocytosis, high CA 125 level, and thrombocytosis plus high CA 125 level in patients with malign adnexal mass.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytosis</td>
<td>68%</td>
<td>86%</td>
<td>86%</td>
<td>68%</td>
</tr>
<tr>
<td>High CA 125 level</td>
<td>74%</td>
<td>48%</td>
<td>59%</td>
<td>59%</td>
</tr>
<tr>
<td>Thrombocytosis plus high CA 125 level</td>
<td>65%</td>
<td>88%</td>
<td>87%</td>
<td>67%</td>
</tr>
</tbody>
</table>

that those findings were comparable for premenopausal and postmenopausal patients. They concluded that the clinical value of CA 125 was limited for preoperative discrimination between benign and malignant ovarian pathology, and that CA 125 may be used in a different way according to other clinical findings to increase its usefulness in patients with adnexal masses.

Liu et al. [17] reviewed the management of adnexal masses that were commonly encountered in gynecologic practice which were causing both diagnostic and management challenges. They reported that this was partly because of the fact that the majority of adnexal masses identified represented benign entities not requiring active intervention; however, a small subset had malignant potential. They concluded that for the best diagnostic and management strategies, effectively triage risk for malignancy was required by having a thorough understanding of the entities in the differential diagnosis, and carefully considering the clinical context for each individual patient. They suggested that optimal selection and interpretation of diagnostic tests were enhanced by both an accurate clinical risk assessment and an understanding of the inherent accuracy of diagnostic tests according to evidence-based management algorithms to optimize outcomes for women with adnexal masses.

Nolen and Lokshin [18] suggested that the goal of effective population-based screening for ovarian cancer remains elusive despite intense efforts aimed at improving biomarker and imaging modalities. While dozens of potential serum biomarkers for ovarian cancer have been identified in recent years, none have yet overcome the limitations that have hindered the clinical use of CA 125.

Avenues of opportunity in biomarker development are emerging as investigators are beginning to appreciate the significance of remote, as well as local or regional, sources of biomarkers in the construction of diagnostic panels, as well as the importance of evaluating biomarkers in prediagnostic settings. As the list of candidate biomarkers of ovarian cancer continues to grow, refinements in the methods through which specific proteins are selected for further development as components of diagnostic panels are desperately sought. Such refinements must take into account both the bioinformatic and biological significance of each candidate. Approaches incorporating these considerations may potentially overcome the challenges to early detection posed by the histological heterogeneity of ovarian cancer.

Verheijen et al. [19] studied the use of cancer antigen 125 (CA 125) in the follow-up of patients with epithelial ovarian cancer after complete response to primary treatment. As it has been suggested to refrain from CA 125 altogether, the European Society of Gynaecological Oncology report has also reviewed possible disadvantages, even possible harm, and potentially missed opportunities when such policy would be implemented. They concluded that indeed routine use of CA 125 does not provide patient benefit in survival or quality of life. They noted that the lack of benefit of CA 125 monitoring has only been proven for a specific subset of ovarian cancer.

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patients with serous histology and frequent follow-up visits including imaging and in a clinical environment where, particularly, surgery for recurrent disease and clinical studies on new second-line agents will not be considered. They warned not to stop tumor marker follow-up in other than epithelial ovarian cancers and in follow-up of patients who have not been treated with chemotherapy.

Mury et al. [20] studied the role of pre- and postoperative CA 125 levels in patients with ovarian cancer. Despite radical surgery and chemotherapy, most patients with ovarian cancer will develop recurrence and die due to progressive disease. To stratify patients for optimal therapy, prognostic and predictive factors are needed. Although CA 125 serum levels differed significantly before and after surgery in early and advanced stage ovarian cancer and preoperative CA 125 values correlated with surgical outcome in advanced stage disease, they advised not to stop tumor marker follow-up in other than epithelial ovarian cancers and in follow-up of patients who have not been treated with chemotherapy.

In a study by Li et al. [21], the prognostic value of thrombocytosis in epithelial ovarian cancer was evaluated in 183 women. They found that thrombocytosis was an independent negative prognostic factor for overall survival in patients with advanced stage ovarian epithelial cancer. In another study by that group of researchers [22], incidence of thrombocytosis in patients with uterine papillary serous carcinomas was evaluated. They concluded that thrombocytosis might identify patients at greater risk for recurrence. Our findings, in accordance with those data, supported diagnostic value of thrombocytosis alone and in combination with high CA 125 levels since thrombocyte counting is a routine procedure for women evaluated for several gynecologic diseases. Careful interpretation and assessment of simple clinical data provide important clues to diagnose many difficult cases.

In conclusion, presence of thrombocytosis and high CA 125 alone and in combination may be used as a prognostic factor in the management of women with adnexal masses since they are an already used clinical test for several purposes. Further investigation is needed to elucidate the clinical value of the presence of these tests in a large group of women with adnexal masses grouped according to histopathologic diagnoses.

References

Seasonal variation in breast cancer incidence. Circumstantial or default event?

C. Markopoulos, D. Mantas, E. Kouskos, Z. Antonopoulou

Breast Unit, 2nd Propedeutic Surgical Department, Faculty of Medicine, University of Athens, Laiko General Hospital, Athens (Greece)

Summary

It has been previously suggested that seasonality in the detection of breast cancer is mostly seen in countries with distinct climatic variations. Patient characteristics and delays have been implicated in the etiology of peak presentation. Seasonality has been more marked in premenopausal women, while delays have been attributed to both patients and health care systems. Patients: A total of 1,411 women who presented with breast cancer to our department were analyzed according to their age, menopausal status, site, stage, grade, ER and PR status, c-erb-2 and Ki-67 (412) during the year. Results: The seasonal variation was statistically significant, but no statistically significant differences were established between corresponding subgroups. Conclusion: The seasonal variation most probably reflects temporal, psychosocial and behavioral patterns in the Greek female population. Since we do not have the ability to recognize the actual onset of any cancer and then correlate it with various different independent factors we can not correlate its influence on survival or biological marker manifestations.

Key words: Seasonal variation; Breast cancer.

Introduction

There are few reports of seasonal variation worldwide in the diagnosis of malignant neoplasms as well as other potentially lethal medical conditions. There are reports on seasonal variation in the diagnosis and reporting of malignant melanomas, lymphomas, leukemias, breast and childhood cancers. Such variations were first noted in the 1960s and some show intriguing temporal patterns, reflecting on either biological phenomena or administrative differences in the likelihood of tumor detection and registration [1]. Especially for breast cancer there are several retrospective reports that relate, positively or not, to its prognosis with the season of occurrence. It is not remarkable that there are reports which either confirm a positive relationship between seasonal distribution and biological factors as well as prognosis and survival as independent phenomena or totally disagree with evidence based on epidemiological data. When we noticed that this variation might occur in our patients we decided to retrospectively analyze our breast cancer registry to determine whether any seasonal variations exist and to correlate the seasonal incidence of breast cancer with the most known independent factors related to its prognosis.

Material and Methods

The study is based on all female breast cancer cases diagnosed and treated in our Breast Unit over a 22-year period between 1988 through 2009. Cases with an unknown month of diagnosis were excluded from the analysis. In total, 1,411 cases were diagnosed with invasive or in situ breast carcinoma. Depending on the data, we separated the patients according to their age (1,374), menopausal status (1,336), site (1,368), stage (1,128), grade (1,062), ER and PR status (1,374), c-erb-2 (729) and Ki-67 (412) during the year. We noted that during August the cases were eliminated, so it was decided to exclude this particular month to avoid bias of the results. The time of diagnosis was the day of the first presentation of the patient to the Breast Unit and the diagnosis was clinically or histologically confirmed.

Results

Data from each calendar year were standardized to 12 months of equal duration and the month of peak diagnosis was identified thereafter. Percentages were also calculated to achieve comparable monthly results. For statistical analysis the χ² test for heterogeneity was used. Statistical significance was determined at the 95% confidence interval (CI).

The distribution of month of cancer detection by patients is shown in Figure 1. There was a significant annual cyclic variation in month of diagnosis with the months of highest detection being early summer and autumn and the month of lowest detection being August following by January and February. As shown in Table 1, the seasonal variation was statistically significant (p < 0.0001) at the 95% CI with August not included in the measurement. The χ² test was used to detect any deviation from a uniform monthly distribution according to the patient’s age, menstrual status, pTNM stage and grade of the tumor, ER and PR status, tumor localization (right or left breast) and the presence of c-erb-2 and ki-67 expression (p = 0.3963, 0.3397, 0.4274, 0.1955, 0.1764, 0.1455, 0.8777 and 0.3013, respectively). No statistically significant differences were established between corre-
sponding subgroups. Pre- and postmenopausal women showed a seasonal variation in the presentation of breast malignancy, with both groups peaking in July and the premenopausal group, also peaking in October and December. The observed differences were not however statistically significant. The case was similar for the dual peaks seen in the variation of the hormonal status of tumors. A statistically significant trend was observed in cases with positive lymph nodes. Again, bimodal peaks occurred in July and December. They were considered however to be of low magnitude.

Discussion

The major problem of all published reports concerning seasonal variation of breast cancer incidence is the fact that we are unable to evaluate the exact time of cancer development; therefore it is difficult to extract safe results or correlate independent factors with cancer occurrence. It has been previously suggested that seasonality in the detection of breast cancer is mostly seen in countries with distinct climatic variations, with Israel peaking in spring [2], the USA in spring and late fall [3] and Southern England in June [4]. In the southern hemisphere peaks were detected in late spring [5] and early summer [6] in New Zealand, while there are reports of dual peaks occurring in spring and late autumn in the USA and Bulgaria, for self-detected tumors requiring surgery [3, 7]. Galea and Blamey reported no significant variations in the monthly frequencies of breast tumor detection in Nottingham, England [8].

The time between transition from a clinical, non-detectable state to a detectable state may be too long for a reliable association with a specific month. Cohen et al. found a clear seasonal pattern in symptomatology especially for premenopausal disease presenting as a self-detected tumor assuming that the detection of symptoms indicates a change in tumor growth rather than actual onset of the disease [2]. On the other hand, Mason et al. showed that the season of tumor detection may influence known factors that predict survival of patients such as ER or PR status and positive axillary lymph nodes [6].

Hormonal variations and tumor-hormone receptor status have, in many cases, been related to the season of detection of breast neoplasms [3]. Melatonin seasonal variation and its effect on ovarian steroidogenesis may play a role in breast tumor pathology and time of presentation. Melatonin inhibits proliferation of estrogen-responsive breast cancer cell lines and its levels are higher in the winter. Vizula et al. reported an increased frequency of both positive estrogen and progesterone receptors in the autumn months in premenopausal women and a decrease in the spring months [9]. On the other hand, in postmenopausal women positive receptor status peaked in the summer months and was negative in the late winter months. Despite these results the cause of the relationship between season of detection and survival remains uncertain. Although some of the hypotheses are intriguing, our results do not establish a statistically significant relationship between oestrogen and progesterone receptor status and seasonality. However, it might be worth looking into the relationship between survival rates and time of tumor detection, as previous studies suggest that the time of the first detection relates significantly to the later behavior of the tumor, and may reflect seasonal changes in hormone dependent growth [6].

Comparable results concerning cancer survival and the season of diagnosis were found by Lim et al. who tried to correlate both season of diagnosis and sunlight exposure with cancer survival with limited influence of the latter [10], and by Roychoudhuri et al. who found that diagnosis in the summer is associated with substantially decreased mortality among women with breast cancer without it being able to indicate a specific factor that may influence these results [11]. On the contrary, Galea and Blamey, reported no relationship either for frequency of tumor detection and seasonal pattern, or season of detection and survival [8]. As shown in our results from a statistically strong number of cases in our country, even though we have a totally different climate compared with Sweden, we are closest to Lambe et al.’s results – not in

Table 1. — Statistically significant results in seasonal variation for 1,411 patients.

<table>
<thead>
<tr>
<th>Location</th>
<th>Variability</th>
<th>Chi-Square Test for Equal Proportions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>6.73</td>
<td>Std deviation 3.36 Chi-square 45.7365</td>
</tr>
<tr>
<td>Median</td>
<td>7.00</td>
<td>Variance 11.28 DF 10</td>
</tr>
<tr>
<td>Mode</td>
<td>7.00</td>
<td>Range 11.00 p value &lt; .0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interquartile range 6.00</td>
</tr>
</tbody>
</table>

*August not included.
terms of equivalent month distribution, but in the assumption of social factors that may influence the breast cancer seasonal variation rather than the biological matters. Previous reports on the seasonal variation in breast cancer diagnosis in Greece demonstrate dual peaks in spring and autumn [12]. In contrast to this study, we have shown the highest frequency distributions of breast cancer diagnosis in early summer and the beginning of winter.

The seasonal variation most probably reflects temporal, psychosocial and behavioral patterns in the Greek female population. During the summer months women change into lighter clothing and tend to be more observant about any changes in their body. Most women likely perform breast self-examination (BSE) and visit their physician prior to their holidays. As the majority of Greeks take their holiday in August, the low frequency distribution in this month, together with the peak in July could reflect behavioral characteristics in self-referral, rather than an etiological determinant in breast malignancy presentation. In addition, the reduced diagnostic capacity and evaluation of patients with suspected breast cancers in August could also contribute to the low frequency numbers observed. Similarly, the early winter peak could be attributed to the upcoming Christmas holiday and could represent another female internal reminder to perform a BSE, as has been previously pointed out by Ross et al. [3].

In conclusion, this study contributes to the overall understanding of breast cancer epidemiology in Greece and adds to the existing knowledge on seasonal variation in malignant breast tumor detection. It would be of great interest if we had the possibility in the future to substantially recognize the actual onset of any cancer and then correlate it with various different independent factors that may influence survival or biological marker manifestations.

References

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Calcaneal metastasis in uterine cervical cancer: a case report and a review of the literature

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Summary
A 64-year-old woman with FIGO Stage IB2 cervical cancer was treated with radical surgery. Six months after her initial surgery, the patient developed calcaneal metastasis. Significant relief in bone pain was achieved with palliative radiotherapy followed by platinum-based combination chemotherapy, and the patient is currently alive with disease at eight months after the development of recurrence. Bone metastasis from uterine cervical cancer is uncommon, especially in the distal appendicular skeleton. Currently, and to the best of the authors’ knowledge, calcaneal metastasis derived from cervical cancer has never been reported in English literature. As the prognosis of patients with bone metastasis is dismal and most patients die within a year, treatment should be directed towards improving the patient’s quality of life and palliating their symptoms.

Key words: Calcaneus; Bone metastasis; Cervical cancer; Treatments.

Introduction
Bone metastasis from uterine cervical cancer is uncommon, especially in the distal appendicular skeleton. The authors describe a case of calcaneal metastasis derived from uterine cervical cancer.

Case Report
A 64-year-old woman presented with postmenopausal vaginal bleeding. Her past surgical and medical history was unremarkable. On evaluation, she was found to have a 4.5 cm friable cervical mass, which was limited to the uterine cervix. Biopsies from the lesion demonstrated a non-keratinizing type squamous cell carcinoma. A pretreatment work-up revealed no evidence of adenopathy or metastatic disease. A diagnosis of FIGO Stage IB2 cervical cancer was confirmed, and radical hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy were performed. Despite the presence of pathological risk factors including deep stromal invasion and lymph vascular space involvement, the patient refused adjuvant radiotherapy. At six months follow-up, she presented with claudication. A four cm painful mass was observed in her left heel, which was fixed to the underlying structures. An X-ray of her foot showed an osteolytic lesion in her calcaneus, and a magnetic resonance imaging (MRI) showed a four cm mass located on her calcaneus (Figure 1). Biopsy samples from the calcaneal mass revealed metastatic poorly differentiated squamous cell carcinoma. A 2-deoxy-2-18F fluro-D-glucose position emission tomography/computed tomography (FDG PET/CT) revealed increased FDG uptake in the calcaneus, pelvic sidewall, and paraaortic lymph nodes, which were consistent with metastases. Significant relief of bone pain was achieved with palliative radiotherapy followed by platinum-based combination chemotherapy, and the patient is currently alive with disease at eight months after the development of recurrence.

Discussion
Bone is the third most common site of hematogenous spread from uterine cervical cancer after the lungs and liver [1]. According to recent reports, the incidence of clinical bone metastasis derived from uterine cervical cancer varies from 1.1% to 8.2% [2-5]. A large autopsy study reported an incidence of 8.6% [6]. The vertebrae, followed by the pelvic bone and long bones, are the most common sites of bone metastasis, and the distal appendicular skeleton is rarely involved [3-5]. In the lower extremities, the femur [3], fibula [7], and tibia [8] have been reported to be involved during the progression of uterine cervical cancer. However, to the best of the authors’ knowledge, calcaneal metastasis derived from cervical cancer has never been reported in English literature.

Skeletal scintigraphy or FDG PET/CT is useful for the detection of bone metastases [5, 9], however, the diagnostic value of these methods has never been directly compared in patients with cervical cancer. X-rays, CT scans, and MRI are also useful for excluding the possibility of the false-positive accumulation of radioisotopes or FDG in osteoporotic or inflammatory lesions [9]. As more than 50% of patients have multiple metastatic lesions at the time of the diagnosis of bone metastasis [2-5], a systemic metastatic work-up is also necessary.

There are no guidelines regarding the therapeutic options for this condition. Palliative radiotherapy may be beneficial for pain relief and to decrease the risk of fractures [3-5]. Matsuyama et al. reported that 67% of patients experienced pain relief after being treated with 30 Gy of external beam radiotherapy in ten fractions [3]. For systemic disease, chemotherapy following palliative radiotherapy may also provide adequate symptom control [3, 5]. In a case involving a solitary bone lesion, the surgical excision of metastatic bone has also been reported.

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Despite these treatments, survival after the diagnosis of bone metastasis was dismal and was not associated with the number of bone metastasis or the treatment modality [5]. As most patients die within 12 months from the discovery of bone metastatic lesions [2-5], treatment should be directed towards improving the patient’s quality of life and palliating their symptoms.

Conclusion

This case highlights the fact that the calcaneus can be affected during the progression of uterine cervical cancer.

References


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A case of a borderline mucinous tumor of the ovary with sarcoma-like mural nodules producing granulocyte colony-stimulating factor

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Summary

Borderline ovarian tumor with sarcoma-like mural nodule is rare. Malignant mural nodules usually occur in the wall of an atypical proliferative mucinous tumor or a mucinous carcinoma. The authors report one case of unfavorably progressive borderline tumor of the ovary with sarcoma-like mural nodule that produced granulocyte colony-stimulating factor (G-CSF).

Key words: Borderline ovarian tumor; Mural nodule; Granulocyte colony stimulating factor.

Introduction

Mucinous ovarian tumors with sarcoma-like mural nodules are rare [1]. Among diverse histologic features of mural nodules, sarcoma-like (benign) and malignant nodules are generally categorized. It has been reported that borderline tumors with sarcoma-like mural nodules have more favorable outcomes than malignant nodules [1]. Leukemoid reactions (white blood cell [WBC] count > 50 x 10⁹/l) and production of granulocyte colony-stimulating factor (G-CSF) are observed in many types of tumors, but seldom reported in gynecologic tumors.

The authors report a rare case of a progressive borderline mucinous tumor of the ovary with sarcoma-like mural nodules producing G-CSF.

Case Report

A 33-year-old woman presented to the Clinic for evaluation of abdominal fullness. A 22 cm by 10 cm multi-loculated cystic mass was noted on pelvic computed tomography (CT), suggesting a mucinous cystic adenoma or adenocarcinoma (Figure 1A). The levels of CA-125 and CA 19-9 were 1,516 and 27.21 U/ml, respectively. On exploratory laparatomy, a 18 cm by 13 cm ruptured cystic mass was noted on the left ovary. The cyst contained multiple chambers, solid portions, and areas of papillary growth (Figure 1B). The final diagnosis was a borderline mucinous tumor of the ovary with sarcoma-like mural nodules.

Along with nodularities on liver surface, cancerous masses had spread to the omentum and to small and large bowel mesentery. Staging surgery, including a total hysterectomy, bilateral salpingo-oophorectomy, total omentectomy, partial peritoneectomy, and pelvic and para-aortic lymphadenectomies, were performed. Poorly-differentiated malignant tumor involved the ascites, omentum, bowel mesentery, and 22 of 86 lymph nodes. Immunohistochemistry staining was strongly positive for vimentin, actin, CK7, and CK20, and negative for desmin, S-100, and CEA. Severe leukocytosis (WBC count, 53.6 x 10⁹/l) was noted and expression of G-CSF was strongly positive on the borderline tumor and the sarcoma-like nodules based on immunohistochemistry (Figure 1C).

Two small nodules were noted in the right middle and lower lobe fissures of the lungs, suggesting metastasis, and rapidly progressing new masses were demonstrated in the peritoneal cavity, suggestive of carcinomatosis peritonei on a post-operative 12-day CT scan (Figure 1D). An incisional wound hernia developed, for which she underwent a primary wound closure on the 13th post-operative day. She passed away without recovery from disease in hospital on day 22.

In this report, the intense neutrophilic infiltration of the tumor and the extremely high G-CSF concentration suggested that such a phenomenon might be involved in dramatic tumor growth.

Discussion

Prat and Cully first reported sarcoma-like mural nodules in mucinous ovarian tumors in 1979 [1]. In their report, none of the cases had spread to the ovary and the type of mural nodules had no effect on prognosis. Pratt and Scully suggested dividing mural nodules into either benign or malignant types [2]. Compared to malignant nodules, which are liable to recur and be fatal in 50% of cases, sarcoma-like nodules are associated with a favorable outcome [3].

A leukemoid reaction is diagnosed when a WBC count > 50 x 10⁹/l without bone marrow involvement is shown and is observed in association with the production of G-CSF in a wide variety of tumors, including carcinomas of the lung, stomach, and pancreas. In proportion to the degree of leukocytosis, G-CSF expression has been implicated in the progression of malignant behavior in non-hematopoietic cancers. Thus a high mortality rate has been reported among patients with leukemoid reactions, but only one report of ovarian cancer associated with a leukemoid reaction exists in the English literature [4].

This is the first case of a borderline mucinous tumor with sarcoma-like nodules associated with an intense paraneoplastic neutrophilic leukemoid reaction related to the production of G-CSF. Recurrences are rare and slow...
A case of a borderline mucinous tumor of the ovary with sarcoma-like mural nodules producing granulocyte colony-stimulating factor


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Primary mucinous borderline tumor of the vermiform appendix mimicking ovarian carcinoma; case report

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Summary

Introduction: Primary adenocarcinoma of the vermiform appendix is a rare entity. Borderline mucinous tumor of the appendix is a much more rare disease. Case: We present a 71-year-old woman with primary mucinous borderline tumor of the vermiform appendix. She was misdiagnosed as an ovarian carcinoma patient and operated on by a gynecologic oncology team. Her frozen section analysis of an appendical mass resulted as borderline tumor of appendix vermis, and right hemicolectomy, as is advised in invasive colon tumors, was not carried out. Conclusion: Borderline mucinous tumor of the appendix vermis should be kept in mind in patients who have pseudomyxoma peritonei during surgery. Tumor resection may be the definitive therapy in these patients.

Key words: Pseudomyxoma peritonei; Borderline; Tumor of appendix vermis.

Introduction

Primary adenocarcinoma of the vermiform appendix is a rare entity and usually discovered by the pathologist in appendectomy specimens. Neoplasms of the appendix are found in 1% of all appendectomy specimens [1]. Primary appendical carcinoma varies from 0.01 to 0.2 per 100,000 persons per year [2, 3]. Primary borderline tumor of the vermiform appendix has been reported in very few case reports and is a new entity. Treatment choices and prognostic factors are still debated for this type of neoplasm.

Case Report

A 71-year-old woman (G3/P3) was admitted to our hospital with the complaint of increasing abdominal girth. Ultrasonographic evaluation revealed a right pelvic complicated mass located in the ovarian region. Pelvic examination also revealed a right ovarian conglomorlated mass; the uterus and left ovary were free and the Douglas pouch was filled with ascites. CA125, 19-9 and 15-3 were all in normal ranges preoperatively.

The patient was diagnosed as having ovarian carcinoma and staging surgery was planned. Our clinic’s gynecologic oncology team prepared the patient for surgery and all of her preoperative tests were within normal ranges.

A laparotomy was performed and the pelvic cavity was found to be full of mucin, with a right pelvic mass consisting of the appendix vermis and right ovary. All abdominal organs were covered with mucin and debris. The mucin was cleared with repeated washings. Staging surgery consisting of total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and bilateral pelvic and paraaortic lymphadenectomy was carried out. General surgeons were invited to the operation room to evaluate the appendix vermis and an appendectomy was carried out. The lesion was a cystic mass, with a mucinous part of 2 cm in diameter on the outer portion (Figure 1). Intraoperative pathologic evaluation of appendectomy material with frozen section was reported as “mucinous appendiceal tumor of unknown behavior” and the operation ended after this pathologic diagnosis. Pathologic evaluation of paraffin-embedded specimens (the entire lesion was submitted to microscopic evaluation) revealed a mucinous tumor with low-moderate degree atypia, and no stromal invasion or epithelial cells in the mucin pools outside the appendix (Figure 2); the case was diagnosed as “mucinous tumor of uncertain malignant potential (borderline mucinous tumor) of appendix vermis”. There was no tumor or metastasis in the other ovary, uterus or lymph nodes. The right ovary was free of tumor but there were some tumoral adhesions on it arising from the appendix mucinous cyst. There was no tumor on the colon side (base) of the appendix vermis. A decision for follow-up without adjuvant therapy was decided by the tumor board, and the patient is planned to have a 3-month period of follow-up visits.

Discussion

Primary adenocarcinoma of the appendix is a rare neoplasm diagnosed in less then 0.5% of all gastrointestinal system malignancies [1]. Borderline mucinous tumor of the appendix is a much more rare and interesting entity. Treatment regimens and the type of surgery needed are still debated [4]. The extent of surgery may differ from simple appendectomy to right hemicolectomy with regional lymph node dissection. Prognosis and survival figures for this type of borderline tumor and the effect of surgery are still controversial [5, 6]. Gynecologic oncologists and also colon surgeons may misdiagnose these patients because of interfering symptoms and findings. Borderline mucinous tumor of the appendix vermis should be kept in mind in patients who have pseudomyx-
oma peritonei during surgery, as well as during intraoperative pathologic evaluation. The pathological diagnosis must be made with nearly all the specimen for microscopic evaluation. Tumoral resection may be the definitive therapy in these patients.

References


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Systematic lymphadenectomy in patients with clinical Stage II endometrial carcinoma: a case report and review of the literature

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Summary
Objective: The aim of this case report and review of the literature was to evaluate the effect of adding pelvic and/or para-aortic lymphadenectomy to hysterectomy and bilateral salpingo-oophorectomy (BSO) on the five year recurrence-free survival in patients with clinical Stage II endometrial carcinoma. Materials and Methods: A Pubmed, Embase, and Cochrane library search was performed to identify relevant articles. After screening, using predetermined exclusion and inclusion criteria, and critical appraisal, a final of four articles remained. Results: This search only revealed studies with a retrospective design. Two articles showed a significant disease-specific survival benefit in patients undergoing systematic lymphadenectomy for Stage II endometrial carcinoma. In multivariate analyses, conducted in both studies, this improvement in survival was also evident (HR 0.75, 95% CI 0.69 - 0.81, p < 0.001 and HR 0.74, 95% CI 0.58 - 0.93, p = 0.0096). The remaining studies revealed a non-significant ten-year recurrence-free survival (77% vs 65%) and five-year overall survival (72% vs 70%) in favour of patients undergoing systematic lymphadenectomy. Conclusion: The practise of performing a systematic lymphadenectomy in patients with clinical Stage II endometrial carcinoma as advocated in guidelines, is not based on evidence from randomised clinical trials. However, lymph node dissection seems to improve the five-year disease-specific survival in retrospective studies.

Key words: Endometrial cancer; Lymphadenectomy; Stage II.

Introduction
Endometrial cancer is the most common female reproductive tract malignancy in the Netherlands with an incidence of 1,900 patients and a mortality rate of 400 each year. More specific, the five-year survival for patients with Stage II disease is 76% [1].

By the International Federation of Gynecology and Obstetrics (FIGO), Stage II endometrial carcinoma is defined as carcinoma involving both the uterus and cervix, while Stage I disease is limited to the corpus [2]. The differentiation between both stages is challenging and in one report from this region, a clinical Stage II disease was downgraded in 50% to Stage I post-surgery [3].

Over the years there has been a controversy in the surgical management of endometrial cancer regarding the therapeutic benefit of lymphadenectomy [4, 5]. It is associated with adverse events in 18% [5]. Moreover, Watanabe et al. concluded that parametral involvement cannot be predicted reliably by cervical involvement, suggesting that radical hysterectomy in Stage II disease to prevent parametral spread may be overtreatment [6].

The current Dutch guideline on endometrial cancer states that lymph node dissection is not recommended in Stage I disease [7]. This is based on results of two randomised controlled trials. These studies, however, only included patients with clinical Stage I disease [8, 9]. The Dutch guideline recommends to perform a radical hysterectomy with bilateral salpingo-oophorectomy (BSO) and pelvic and para-aortic lymphadenectomy in Stage II disease. Pelvic lymph nodes are more often tumor-positive in clinical Stage II disease (36%) compared to Stage I (10%) [10]. However, no evidence is provided that this recommendation results in a better patient outcome [7].

This case report and review of the literature was conducted to determine if the addition of systematic lymphadenectomy to radical hysterectomy and BSO improves the five-year recurrence-free survival in patients with clinical Stage II endometrial carcinoma.

Materials and Methods
Case presentation
A 72-year-old woman was diagnosed with endometrial cancer with uncertainty regarding the clinical Stage (I or II). She was referred to a tertiary oncology department. After re-evaluation of a preoperative magnetic resonance imaging (MRI) the patient was referred back for surgical treatment because of clinical Stage I disease. She underwent a laparoscopic hysterectomy and BSO. No lymphadenectomy was performed. Post-operative pathologic examination revealed a Stage II endometrial carcino-
Systematic lymphadenectomy in patients with clinical Stage II endometrial carcinoma: a case report and review of the literature

Results

Methodological results

The search retrieved 2,859 articles. After removing duplicates and applying exclusion and inclusion criteria, a final of six articles were qualified for critical appraisal (Table 1). Four articles were considered eligible for analysis.

Clinical results

An overview of the results is shown in Table 2. All four articles describe retrospective studies. The study of Leminen et al. [11] analysed the effect of abdominal simple hysterectomy and BSO versus Wertheim's radical hysterectomy including pelvic lymphadenectomy. The outcome measurement was ten-year recurrence-free survival in patients with clinical Stage II endometrial carcinoma. Although not significant, the ten-year disease-free survival was 65% and 77%, respectively. Even in multivariate analyses, no statistical significant difference was found.

Chan et al. [12] performed a study in 39,396 women with all stages of endometrial cancer to assess the effect of this case illustrates the difficulty to differentiate between clinical Stage I or II. It made the authors questioning 1) whether this differentiation can be clinically performed in a reliable manner, and 2) if there is evidence that a systemic lymphadenectomy in clinical Stage II endometrial carcinoma offers a benefit in terms of recurrence-free and disease-specific survival. In this report the authors limit themselves to answer the second question by performing a systematic search of the literature.

Search strategy and selection

A systematic search of Pubmed, Embase, and Cochrane library was performed. No filter or limits were used. The search syntax (appendix) was based on synonyms of the domain and determinant of the clinical question. The search strategy is shown in Figure 1.

Critical appraisal

Standardized criteria were defined for critical appraisal of the relevance and validity of the selected articles (Table 1). Appraisal was performed by two authors. Studies scoring less than fifteen points were excluded from analysis based on a pre-defined scoring system by the authors.

Figure 1. — (N): number of articles excluded based on the criterion. Selection was based on consensus of two authors.
of surgical staging on the five-year disease-specific survival. In women with surgical Stage II disease, lymphadenectomy was associated with an improvement in survival from 82.2% to 90.4% (p < 0.001). In multivariate analysis, lymphadenectomy appears to be an independent prognostic factor for improved survival.

Selvaggi et al. [13] studied the effect of performing pelvic lymphadenectomy in 410 clinical staged patients with endometrial carcinoma. In the patients with Stage II, lymph node dissection resulted in a five-year survival of 72% compared to 70% when no lymph node dissection was performed (p = 0.894).

Smith et al. [14] included 42,184 patients with endometrial cancer to assess the effect of pelvic lymphadenectomy on the five-year uterine specific survival. In patients with Stage II disease (3364) 58.9% underwent lymph node dissection. Multivariate analyses showed a hazard ratio of 0.74 if lymph node dissection was performed (95% CI 0.58 - 0.93, p = 0.0096). Improved uterine specific survival was most pronounced if more than eleven lymph nodes were removed.

Discussion

There are several limitations to this report. First, the search did not yield a randomised controlled trial and resulted only in retrospective studies. A retrospective design is prone to selection bias since the surgeon decides whether lymphadenectomy should be performed based on patient characteristics. This holds especially true for patients with endometrial cancer. Patients in whom lymph node sampling or lymphadenectomy may not be feasible due to comorbid factors, blood loss, or body habitus may have a much poorer prognosis quod vitam, compared to patients in whom lymphadenectomy is a realistic option.

Second, in all four studies, the adjuvant therapy follow-
ing surgical treatment with or without lymphadenectomy, was unequally distributed or unknown between patients. Third, three studies defined the five-year overall survival as an outcome, instead of the five-year recurrence free survival. Therefore, this has been used as a second best outcome.

Fourth, FIGO staging changed in 2009. Stage IIA, with superficial involvement of the cervix, was downstaged to Stage I. Stage II now requires a tumour that invades cervical stroma and thus more advanced disease. The studies represented in this report have used the former FIGO staging system and therefore may also have included patients with a more favourable prognosis related to the superficial cervical involvement of Stage IIA.

Considering the individual studies, Chan et al. [12] only included surgically staged patients. To answer the question, studies that analysed patients with clinical Stage II endometrial carcinoma were more useful, as the decision to perform lymphadenectomy is made preoperatively and based on a clinically-defined stage. After surgical staging, not all patients will have true Stage II disease. This might result in stage migration and thereby influence the outcome. Also notable, the survival rates of this particular study are substantially higher than one could expect on the bases of other studies and even demographic data. A plausible explanation for this discrepancy could not be found.

Smith et al. [14] concluded that besides performing lymphadenectomy, the amount of lymph nodes removed is also of relevance for survival. This can be explained by the fact that with an increasing number of nodes removed, there is a higher statistical probability of obtaining sufficient nodes to adequately stage patients. Also, by removing micrometastatic disease within the node, a patient’s survival may improve. This benefit associated with the extent of lymph node dissection is reported by more studies [4, 5, 17, 18].

Both Selvaggi et al. and Chan et al. included only patients with a histologic subtype of endometrioid endometrial carcinoma [12, 13]. Thereby, it is questionable if the results of these studies are applicable to patients with other histologic subtypes.

Moreover, the studies of Selvaggi et al. and Leminen et al. are also less to answer the question because they included small numbers of patients [11, 13]. Therefore, the answer to the question can only be based on the studies of Chan et al. and Smith et al. [12, 14].

Conclusion

This study shows that there still is uncertainty in regards to the benefit of performing a systematic lymphadenectomy in clinical Stage II endometrial carcinoma. Systematic lymphadenectomy seems to improve the five-year disease-specific survival in patients with clinical Stage II disease. However, the limitations of retrospective study designs prevent a strong recommendation. The individual situation of each patient should be taken in consideration when planning therapy modalities.

References


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Synchronous of breast and vulvar Paget’s disease: a case report

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Summary

Background: Synchronous Paget’s disease of breast and vulva is extremely rare and has only been reported in the literature in one other case. Case: A 58-year-old postmenopausal woman was found to have crusting, bleeding, and discharge from left nipple, as well as vulvar pruritis at the same time. Biopsy of breast lesion demonstrated Paget’s disease with an underlying foci of ductal carcinoma in-situ that required total mastectomy of left breast with sentinel node biopsy and breast reconstruction. For vulvar symptoms, the patient was initially diagnosed with dermatitis and topical ointment was prescribed. However, her symptoms persisted for the next several months, and she underwent vulvar biopsy that demonstrated Paget’s disease. She underwent partial vulvectomy. Multiple episodes of recurrent vulvar Paget’s disease were noted in the postoperative course that medical therapy with Imiquimod and a second partial vulvectomy was performed. Conclusion: Synchronous of breast and vulvar Paget’s disease is presented. There was a delay in diagnosing vulvar Paget’s disease in this experienced case. While coincidence of breast and vulvar Paget’s disease is likely, ectopic mammary tissue in vulvar as well as secondary metastasis from a focal lesion of breast Paget’s disease needs to be carefully evaluated whenever the patient complains of vulvar symptoms in the setting of breast Paget’s disease.

Key words: Paget disease; Vulvar; Breast; Mammary; Synchronous.

Introduction

Mammary Paget’s disease (MPD) is an adenocarcinoma localized within the epidermis of the nipple and/or areola and is typically associated with an in-situ or invasive carcinoma of the underlying breast tissue [1, 2]. Extra-mammary Paget’s disease (EMPD) could be found in vulvar, perianal, or axillary regions, but is most commonly found in the vulvar region of postmenopausal Caucasians with ages around 65 to 70 years [1, 2]. EMPD shares histologic similarities with MPD, in that they both may be found within the epithelial sheaths of hair follicles, sweat gland excretory ducts, or secretory coils and they are characterized by large cells with clear cytoplasm, prominent nucleoli, and potential for invasion into the dermis [1, 3]. Although MPD has been found to be in association with underlying carcinoma in 67 to 100% of cases [4], EMPD is less often reported to be associated with underlying neoplasm [1, 3,11]. Co-incidence of MPD and EMPD are extremely rare clinical entity. To date, there have been only five reported cases of Paget’s disease present in the breast and vulva of the same individual [3, 5]. Among these five cases, there is only one report demonstrating synchronous presence of breast and vulvar Paget’s disease [5], highlighting possible lack of awareness of the two conditions that may possibly co-exist. Here, the authors report a case of synchronous MPD and EMPD where the diagnosis of vulvar Paget’s disease was delayed and developed multiple recurrences.

Case Report

A postmenopausal 58-year-old Caucasian female, gradiva 3 para 3, presented for gynecologic care with the chief complaint of occasional external vulvar pruritis for two to four months, as well as crusting, discharge, and bleeding from left nipple which did not resolve with treatment for presumptive eczema by primary care physician. She stated that these symptoms in vulvar and breast developed simultaneously. Past medical history was significant for prolonged QT syndrome, hypercholesterolemia, migraine headaches, and arthritis. Past surgical history included tubal sterilization, partial thyroidectomy, uterine curettage, and cold knife conization for cervical dysplasia. Family history was significant for breast cancer in her paternal grandmother. She did not smoke cigarettes, was on no special diet, and reported occasional alcohol use. Her last mammogram was one year prior to the visit and results were within normal limits. On initial physical examination, she had mild vulvo-vaginal atrophy and no external vaginal lesions were visualized. At time of initial presentation, she was being evaluated for possible MPD and underwent wedge biopsy of the left nipple.

Pathology evaluation of the breast biopsy revealed Paget’s disease with an underlying foci of ductal carcinoma in-situ (DCIS) involving underlying ducts with grade 2 nuclear atypia and associated ulceration with granulation tissue. Immunohistochemistry was marked for negative staining for both estrogen and progesterone receptors. She was taken to operating room, and total mastectomy of left breast with sentinel node biopsy and breast reconstruction was performed without complication. Pathological evaluation of breast tissue confirmed Paget’s disease of the nipple with no invasive tumor and negative lymph nodes. During her postoperative course, she kept complaining of continued right labial irritation that had not improved. The patient sought the second opinion for the evaluation of vulvar symptoms. On physical examination, right proximal labia majora was irritated, ulcerated, and erythematosus with a white
patchy lesion measuring approximately three cm. Biopsy of right labia showed EMPD with positive immunohistochemistry staining for mucicarmine, PAS-F, CEA, and CK7.

The patient was taken to the operative room and partial vulvectomy was performed and entire lesion was removed with a one cm margin. Pathology evaluation showed presence of Paget’s disease in all surgical margins. No evidence of stromal invasion was seen at that time. She was placed on Imiquimod topical cream for five months for considering positive margin results. One year after vulvectomy, the patient was found to have a recurrence of EMPD proven by biopsy, with new erythematous lesions on the right periclitoral area and the left proximal labial near the labia minora. She underwent a second partial vulvectomy. Following two years after the second vulvectomy, she developed multiple recurrences in the vulvar and underwent two additional partial vulvectomy, as well as intermittent Imiquimod treatment. There was no sign of Paget’s disease in contralateral breast.

Discussion

In 1998, Popiolek et al. reported the first case of synchronous Paget’s disease of the breast and vulva diagnosed within a period of seven months of each other [5]. In this experienced case, a 58-year-old female presented with vulvar pruritis, as well as crusting, bleeding, and discharge from the left nipple, which were both found to be Paget’s disease. To the knowledge of the authors, this is the second case of synchronous Paget’s disease of the mammary and extra-mammary tissues that is unique in that the diseases in the two areas developed simultaneously. The coexistence of Paget’s disease in breast and vulva is extremely rare, and thus, entirely incidental existence of the two conditions may be a possibility. While the concurrent nature of these two lesions is most likely unrelated, the authors propose two additional hypotheses: the first hypothesis is that ectopic mammary tissue in vulva may exist; and the second is that secondary metastasis occurred from a focal lesion.

EMPD is a slow-growing tumor and therefore may result in a delayed initial diagnosis. In addition, it has relatively non-specific characteristics such as a scaling, crusty, and rash-like appearance that may lead to delayed treatment due to diagnosis as an inflammatory or infective skin condition [2]. In the experienced case, symptoms of EMPD were presented at the initial time of diagnosis of MPD and the authors estimated that they arose within the same chronicologic time period due to the existence of external vulvar pruritis for several months prior to the initial diagnosis of MPD. This case of synchronous Paget’s disease is very unusual because it is difficult to pinpoint which neoplasm arose first. The MPD was diagnosed prior to the vulvar EMPD, however, the patient complained of symptoms of vaginal pruritis several months prior to being diagnosed with MPD. Therefore, the authors believe that simultaneous formation of Paget’s disease in the two distinct areas of the body has occurred as well as coexistence of these two entities during the same time period. It is extremely rare for synchronous Paget’s disease to occur and the authors speculate that this experienced case may be the first to be reported in which Paget’s disease arose in two separate areas of the body at the same time.

To explain the simultaneous presentation of these lesions, the authors explored the possibility that either of the two may have been due to secondary metastasis. In general, vulvar Paget’s disease is considered to be a localized and non-invasive lesion. In a retrospective review of tumor registries for Paget’s disease of the vulva, only 8% were associated with an underlying adenocarcinoma and 10% were associated with invasive disease [8]. The presented patient did not have underlying adenocarcinoma and did not exhibit inguinal lymphadenopathy at any time from initial presentation and throughout all follow up exams. In addition, initial biopsy of the vulvar lesion showed no invasion of underlying tissue. When MPD is confined to the nipple, the survival for patients is typically promising. Patients with MPD who only have nipple changes have been shown to have a five-year survival rate of 90 to 100%, while those with palpable breast tumors are at increased risk for invasive disease and have a five-year survival rate of 20 to 60% [9]. In the presented patient, the MPD pathology report showed evidence of associated DCIS, with no invasion of underlying tissues and negative lymph nodes on sentinel node biopsy. The pathology reports from either lesion do not support the invasive nature necessary for metastasis. Due to the non-invasive nature of these mammary and vulvar lesions, it is highly unlikely that either of these are secondary metastatic lesions and the authors believe they arose independently.

Several cases of primary breast cancer of the vulva have been reported in which the vulvar lesions simulate breast cancer histologically [6]. Ohira et al. reported a case of vulvar EMPD with underlying adenocarcinoma simulating breast cancer in which they proposed that ectopic breast tissue may exist in the vulva and be responsible for EMPD [6]. Primary breast cancer of the vulva is an infrequent occurrence and may be identified by three criteria: 1) a morphologic pattern consistent with breast carcinoma; 2) presence of estrogen and progesterone receptors; and 3) positivity of common biomarkers of breast cancer such as CEA, EMA, and glandular keratin [6]. Although the authors found histological similarities in the Pagetoid nature of the cells of both vulvar and breast tissues, they did not look for features of mammary tissue in the vulvar EMPD. The authors did find that the breast tissue was negative for both estrogen and progesterone receptors, however, they did not test the vulvar tissue for these receptors and cannot extrapolate that the vulvar tissue was negative for these two receptors as well. The authors did however find that the vulvar tissue was positive for biomarkers of breast cancer such as CEA and cytokeratin [7]. Due to the presence of two of the three criteria sufficient to describe primary breast cancer of the vulva, as well as the similarity in the Pagetoid nature of the two types of cells, it is possible that this case of EMPD may have derived from ectopic breast tissue present in the vulva, which appears to be uniquely susceptible to dysplastic and malignant change [7].
The existence of synchronous MPD and EMPD is still considered extremely rare, with only one other case reported by Popiolek et al. [5]. The authors believe that this is the first case reporting the coexistence of the two simultaneously. In addition, it is highly unlikely in this case that either breast or vulvar Paget’s was due to metastasis from a primary lesion. Due to the synchronous occurrence of these two lesions, the authors cannot rule out the possibility that ectopic mammary tissue existed in the vulvar area in this patient. The incidental occurrence in this case points out that future investigation of EMPD should involve pathological specimen examination for signs of ectopic mammary tissue. Though the authors did not rule out that the vulvar Paget’s disease arose from ectopic mammary tissue, it is an interesting theory that may prove to be true in future cases of synchronous Paget’s disease.

In summary, synchronous of breast and vulvar Paget’s disease is reported. In this experienced case, there was a delay in diagnosing vulvar Paget’s disease most likely due to the lack of awareness of the possibility of coexistence. While coincidence of breast and vulvar Paget’s disease is likely, ectopic mammary tissue in vulvar as well as secondary metastasis from a focal lesion of breast Paget’s disease needs to be carefully evaluated whenever the patient complains of vulvar symptoms in the setting of breast Paget’s disease.

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Dermatofibrosarcoma protuberans of the mons pubis

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Summary

Dermatofibrosarcoma protuberans (DFSP) is a rare fibrous tumor with intermediate malignant potential and in rare cases the vulva is involved. The most common clinical presentation is a firm plaque with surrounding red to blue discoloration, or less often with multiple small subcutaneous nodules. The authors present a case of a 66-year-old woman who came to the hospital complaining of longstanding painless nodules in the area of the mons pubis. On physical examination, a diffuse area of erythematous induration involving the mons pubis was recognized and within this area there were smaller nodules. The histological diagnosis was dermatofibrosarcoma protuberans. Microscopically DFSP has a storiform pattern of uniform cytologically bland spindle cells, with a characteristic honeycomb pattern of infiltration into the subcutaneous fat. Immunohistochemical staining demonstrates strong positivity for vimentin and CD34. The treatment has been through a wide local excision (WLE), although microscopic tumor projections beyond the central tumor nodule explain the tumors propensity for local recurrence.

Key words: Vulva; Dermatofibrosarcoma protuberans; Immunohistochemistry; Treatment.

Introduction

Dermatofibrosarcoma protuberans (DFSP) is a rare cutaneous tumor of intermediate grade malignancy, with a tendency for local recurrence [1, 2]. It rarely occurs in the vulva and in a review of the literature, less than 35 cases of DFSP in this area have been reported [3, 4]. Preferred treatment for DFSP is wide surgical excision with pathologically negative margins [5]. Despite the local invasiveness it rarely metastasizes [6].

The authors present another case of DFSP arising from the mons pubis and discuss its clinical presentations, histological characteristics, treatment modalities, and outcomes.

Case Report

A 66-year-old woman was admitted to the Hospital complaining of a long-standing painless nodule in the area of mons pubis. She had a history of breast cancer 15 years prior treated with surgery, but neither chemotherapy nor radiotherapy (RT) was given. On physical examination at the time of presentation, there was a diffuse area of erythematous induration involving the mons pubis measuring 8 x 5 cm. Within this area, there were smaller nodules. The rest of the gynecologic examination was normal. There was no lymphadenopathy or other skin abnormalities. The patient was offered vertical excision with the reconstruction of the area. The specimen was an ulcer ellipse measuring 8 x 5 cm. On gross examination, subcutaneously three nodules of various sizes (1, 2, and 2.5 cm) were found. The nodules were hard in consistency with a light brown color. Two more nodules were found in the adipose tissue. They were also hard in consistency, of pale white color and multilobulated. Microscopically, in both the dermis and subcutaneous fat a monotonous storiform pattern of uniform, bland spindle cells is recognized (Figures 1 and 2). There is little nuclear pleomorphism. The number of mitoses is less than five/ten high power fields (hpf). In some areas the myxoid change of the tumor is prominent. Immunohistochemically the tumor cells were diffusely and strongly positive for vimentin (Figure 3), and CD34 (Figure 4) and negative for smooth muscle actin, CD117, S100P, progesteron, and oestrogen.

On the basis of histological and immunohistochemical findings, the diagnosis of DFSP of mons pubis was established. The patient underwent further diagnostic procedure consisting of ultrasound scan of the thorax without evidence of the disease in any other organ or lymph node tissue.

Discussion

DFSP, first described in 1924 by Darrier and Ferrand [7], is a nodular cutaneous tumor characterized by a prominent storiform pattern. These tumors occur at any site, but they are seen most frequently in the trunk and proximal extremities. DFSP of the female genital area, such as the vulva, is extremely rare. In a review of the literature, less than 35 cases of vulvar DFSP have been reported [3, 4]. DFSP of the vulva accounts for 0.1% of all malignancies [8]. Patient age ranged from 23 to 76 years (mean age 46). Vulvar DFSP may present as a firm plaque of the skin with subcutaneous nodules with surrounding red to blue discoloration or as multiple small subcutaneous nodules, such as in the presented case. Histologically DFSP has a more distinct storiform pattern, little nuclear pleomorphism, and low to moderate mitotic activity. Necroses are absent [8, 9]. Immunohistochemically, DFSP is characterized by the presence of CD34 [10, 11]. Ultrastructurally earlier studies showed that the cells resemble fibroblasts, but the expression of CD34 (human progenitor cell antigen) supports the view that this neoplasm is a variant of nerve sheath tumor. Also, it is characterized by a chromosomal translocation between distinct regions of chromosomes 17 and 22, t(17; 22)
leading to fusion of the collagen type 1 alpha1 gene (COL1A1) to the platelet-derived growth factor B (PDGFB) gene [12]. Overproduction of PDGFB by DFSP results in autocrine stimulation. In the differential diagnosis of DFSP, other soft tissue tumors must be affected such as dermatofibrosarcoma with its variants, neurofibroma, schwannoma, malignant peripheral nerve sheath tumors (MPNST), leiomyoma, leiomyosarcoma, myxoid liposarcoma, desmoplastic melanoma, and solitary fibrous tumor. The final diagnosis is made with the help of positivity for CD34.

The treatment of DFSP has been with a wide local excision (WLE). The local recurrence rate after WLE ranges from 20-50%, because there are microscopic tumor projections beyond the central tumor nodule resulting in incomplete excision [5]. Mohs micrographic surgery (MMS) is an effective treatment of DFPS. MMS allows mapping the extension of DFSP with microscopic examination of its deep and lateral margins and enables to examine 100% of the tumor margin [5, 13]. MMS used to treat DFSP of the trunk and extremities is well-documented [13]. The local recurrence rate after the MMS treatment for patients with DFSP has been reported to be 1.6% vs 20% after wide excision and 43% after conservative excision [14].

In the vulva, the treatment of DFSP ranges from simple excision to radical vulvectomy and RT [15]. Until recently treating vulvar DFSP with MMS was referred in

Figure 1. — Dermal tumor composed of spindle cells that extend to the subcutis (H&E x 100).
Figure 2. — DFSP whorled pattern (H&E x 200).
Figure 3. — DFSP spindle cells strongly positive for vimentin (vimentin x 200).
Figure 4. — DFSP spindle cells strongly positive for CD34 (CD34 x 200).
a limited number of cases. In these cases the tumor did not recur, while the recurrence rate in patients treated with non-MMS modalities was 32% [16, 17]. This method requires trained staff and experienced surgeons. Finally, imaging studies such as computed tomography (CT) scans, magnetic resonance imaging (MRI), and X-ray of the thorax are necessary to determine the extent of tumor size because finger-like projections into surrounding tissue increase dangerous recurrences.

In conclusion, vulvar DFSP is an uncommon tumor, with less than 35 cases, that has the tendency to recur locally. The diagnosis is established with the help of immunohistochemistry [CD34 (+)]. Most of the cases are characterized by a reciprocal translocation t (17; 22) (q22; q13).

The treatment of vulvar DFSP is with a wide simple excision. More recently MMS is an effective treatment, but this method requires trained staff and experienced surgeons.

References
A case of occult bowel perforation after a cycle of chemotherapy for advanced epithelial ovarian carcinoma

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Summary

Occult bowel perforation is a rare complication in chemotherapy of advanced epithelial ovarian cancer (EOC). The authors present a case of a 75-year-old woman who had appendectomy due to suppurative appendicitis five years prior, that experienced an occult bowel perforation resulting in continuous decline of electrolytes after a single cycle of nedaplatin (NDP) and paclitaxel during neoadjvant treatment of advanced EOC. To the authors’ knowledge, this is the first reported case of an occult bowel perforation after neoadjuvant chemotherapy (NAC) for ovarian cancer. The complication was highly suggestive of a cell lysis mechanism for the perforation.

Key words: Advanced epithelial ovarian cancer; Occult bowel perforation; Electrolytes; Neoadjuvant chemotherapy; Nedaplatin; Cell lysis; CA 125.

Introduction

Epithelial ovarian cancer (EOC) is the fifth most common malignancy among women and most ovarian cancers are only diagnosed after the disease has spread throughout the peritoneal cavity, when prognosis is poor [1]. More than 80% of these women with late-stage disease will die within five years. Approximately 75% of ovarian cancer patients are initially diagnosed with disseminated intra-abdominal disease and classified according to the International Federation of Gynecology and Obstetrics (FIGO) Stage III or Stage IV [2]. Treatment of ovarian cancer is based on the integration of surgery and chemotherapy [3]. Chemotherapy plays a major role in the adjuvant treatment and in the care of patients with advanced disease [4]. Primary chemotherapy with cytoreduction for advanced ovarian carcinoma has been controversial [5]. However, when clinicians think cytoreductive surgery as an initial step, it is not feasible or might entail excessive perioperative risk for the patient, and may then suggest the patient completes cytoreduction after three cycles of chemotherapy. The complications associated with chemotherapy are well known but bowel perforation is a rare event. There have been reports of bowel perforation in ovarian cancer patients with paclitaxel [6, 7]. In 2007, Carter and Durfee reported a case of bowel perforation with Stage III ovarian cancer after one cycle of adjuvant chemotherapy of carboplatin and paclitaxel; the patient was found to have bowel perforation on the fourth day after chemotherapy because of symptoms of acute peritonitis and identification by computed tomography (CT) scan [8]. Bowel perforation is a known complication associated with the use of bevacizumab [9] and in patients treated for gastrointestinal (GI) lymphomas [10-12]. In this case, occult bowel perforation was found intraoperatively which has not been reported before.

Case Report

A 75-year-old woman, with 26 years of menopause, presented to the hospital with several months of abdominal discomfort, weight loss, and a large abdominal-pelvic mass. She denied urinary urgency and frequency, and minor GI symptoms, including abdominal distension, constipation, and smaller volume stools. She did not have hypertension or high blood sugar. Her past surgical history included significant appendectomy due to suppurative appendicitis at age 71. The mass was fixed and filled the pelvis. She was evaluated by CT scan and abdominal ultrasound (AUS), which both revealed a large cystic mass in the pelvis. AUS indicated no bladder invasion or hydroureteronephrosis. Her CA-125 was 3,389 u/ml, CA-199, CEA, and hCG were normal. No palpable rectal involvement was noted during examination and urine and stool were normal. The mass was percutaneously biopsied under US guidance and pathology showed a poorly-differentiated adenocarcinoma consistent with an ovarian primary. The authors diagnosed an advanced ovarian carcinoma. With the patient’s consent, the first cycle of chemotherapy was initiated one week after the initial diagnosis with nedaplatin (80 mg/m²) and paclitaxel (175 mg/m²) and dexamethasone (10 mg in the morning with chemotherapy, and 10 mg daily for two days), ondansetron was used to prevent vomiting. This patient was discharged the third day after chemotherapy. She had mild vomiting and was able to ingest a small amount of food from the sixth day after chemotherapy. She was provided with a supplement of electrolytes and nutritional support in the local community hospital. Vomiting stopped at the tenth day. She had no fever and did not complain of stomach ache. She returned at the 16th day following chemotherapy. Her electrolyte levels (Na+, K+, Ca²+, and Cl⁻) were low to normal; CA-125 level had dropped to 1,978 u/ml, AUS-measured size of the mass had not clearly decreased (Figure 1). Abdominal examination found that the mass was still fixed and there was no muscle tension. According to her weight, approximately half of her electrolytes were supplemented and potassium oral supplements were used. She could eat some food and the authors did not expect to find low electrolytes. However, some reviews found her electrolyte level was not stable; it always declined, even after oral electrolyte supple-

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Figure 1. — Radiologic image of the chest indicating diffuse shadows within the lung, leading to a suspicion of H1N1 infection.

ments and injection on the first day. She did not complain of stomach ache, diarrhea, or abdominal distention, could eat a little food, and her stools were normal. She had a 38.2°C fever that lasted for four hours then dropped to normal; her blood analysis indicated normal leukocyes. Upon patient consent, the authors decided to perform cytoreductive surgery and found extensive intestinal adhesions upon entrance into the abdomen. The adhesions were separated and found the mass originated from the right ovary; its upper pole reached the transverse colon without ascites. Metastasis to the greater omentum or other organs was not found. The authors did not touch the enlargement of the retroperitoneal lymph node. There were two sites (2 × 3 cm² and 2 × 2 cm²) of invasion of the mass into the bowel wall. After separation of the adhesions, two areas where the bowel was perforated were found. The mass and the bowel had formed sinuses, which were blocked because the mass’ periphery was solid and its center was cystic and contained a large amount of foul-smelling fluid but the patient had no symptoms of acute peritonitis. The authors resected the mass and the perforated parts of the bowel (located in the ileum, with a total length of 20 cm) and performed direct anastomosis for the bowel. The pathologists confirmed the diagnosis of poorly-differentiated ovarian serous cystadenocarcinoma. Her postoperative course included two weeks of parenteral alimentation; after that, she could eat a small amount of food, did not feel abdominal distention, and her stools were normal. The patient was discharged on postoperative day 27. Her CA-125 dropped to 451 u/ml. She completed five further cycles of chemotherapy with nedaplatin and paclitaxel. After the third cycle, her CA-125 was normal. Currently, she is alive without disease 11 months after cytoreductive surgery and is undergoing further follow-up.

Discussion

Adjuvant chemotherapy for early stage ovarian cancer is still controversial [3]. The standard care for patients with advanced ovarian cancer is maximal surgical cytoreduction followed by systemic platinum-based chemotherapy and it is reasonable to expect a five-year survival for 40% and 20% of women diagnosed with ovarian cancer at Stage III and IV, respectively [3, 13]. Moreover, because most of recurrence after front-line surgical and medical therapies and secondary cytoreduction of surgery does not improve survival [14], further chemotherapy is required to treat relapse. Recently, a phase III randomized study compared primary cytoreduction followed by chemotherapy to neoadjuvant chemotherapy followed by interval debulking surgery in women with advanced ovarian cancer. No statistical difference in morbidity, mortality, or quality of life was found [5]. These results have stimulated a good deal of discussion between experts in this field. It is believed that upfront maximal cytoreduction is still the standard procedure, although further research should focus on how to select patients that cannot receive optimal cytoreduction and can benefit from a neoadjuvant strategy. Therefore, noninferiority of neoadjuvant chemotherapy followed by interval debulking surgery to primary debulking surgery followed by chemotherapy in patients likely to achieve optimal cytoreduction should be validated. A universally applicable clinical model that can predict which patients should undergo optimal cytoreduction remains elusive [15].

Over the years, experts and research groups have experimented with different combinations of drugs in order to improve the prognosis of ovarian cancer. Nedaplatin is a derivative of cisplatin that induces less nausea, vomiting, and nephrotoxicity. The authors found the combination of nedaplatin and paclitaxel had a beneficial effect on this patient, but this needs to be further investigated in other patients.

Bowel perforation is a rare and life-threatening complication of chemotherapeutic treatment of cancer. Spontaneous perforations of the small and large bowels have been reported during the treatment of various malignancies with chemotherapy alone (23%), in conjunction with corticosteroids (57%), and with corticosteroids alone (20%) [10]. The authors present a case of occult bowel perforation after a single cycle of treatment with nedaplatin and paclitaxel during neoadjuvant treatment of Stage III EOC. The mechanism underlying bowel perforation is uncertain, but it is thought to occur as a result of tumor necrosis with weakening of the bowel wall and poor wound healing that leads to bowel injury. Recently, bevacizumab, the first antiangiogenesis molecule to demonstrate significant anti-cancer activity, has been used for the treatment of cancer, it inhibited the activities of vascular endothelial growth factor (VEGF), induced vasoconstriction, and resulted in ischemic injury [9, 16]. Patients with an intra-abdominal inflammatory process, such as diverticulitis, obstruction, tumor at the site of perforation, abdominal carcinomatosis or history of abdominal radiation might be at higher risk of bowel perforation [17-20]. Of course, most patients have obvious symptoms and need surgery immediately after bowel perforations; but if the patients electrolytes are not stable and no other reason is found, the authors think it might be the result of occult bowel perforation.
References


Adenosarcoma ovarii in a 51-year-old woman: case report

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Summary
This case report presents a 51-year-old woman with an adenosarcoma of ovarian origin which is a very rare tumor. She came for consultation due to abnormal vaginal bleeding. The case also illustrates the difficulty of its correct diagnosis and discusses the possible reasons of wrong preoperative and intraoperative diagnosis.

Key words: Adenosarcoma; Ovary; Diagnosis.

Introduction
Adenosarcomas are tumors characterized by a benign neoplastic epithelial component and a malignant mesenchymal component. Malignant mixed tumors of Müllerian origin are combined mesenchymal and epithelial neoplasms which, in order of increasing malignancy, are labeled: mesodermal (Müllerian) adenosarcoma; carcinosarcoma; malignant mixed mesodermal (Müllerian) tumor [1]. Müllerian adenosarcoma is a rare neoplasm that can arise in both uterine and extrauterine locations. Extraendometrial variants (originating in the ovary, adnexa, or myometrium) are much less common, and they tend to present a more advanced stage due to their location. The sarcomatous portion of Müllerian adenosarcoma can vary from low grade to very high grade and the clinical behavior of the tumors can be indolent or aggressive [2]. Müllerian adenosarcomas of ovarian origin are a rare type of tumors. According to the description by Clement and Scully in 1974, 51 cases of ovarian origin have been described [3-5].

Case Report
The patient was a 51-year-old woman and came for consultation in March 2011 due to abnormal vaginal bleeding over a five month period. Upon physical examination and upon abdominal palpation a painless abdominal pelvic tumor of ~ 20 cm with undefined borders was identified. On vaginal palpation, a tumor was found at the fundus of the sac, which did not allow delimitation of the uterus or ovaries. Among well-recognized risk factors for adenosarcoma of the ovary, she did not use a combination of oral contraceptive pills, but on the other hand, had a first pregnancy at 23 years of age, which decreases the risk of the disease. Pelvic ultrasound examination revealed large uterine myoma, from isthmus to fundus with dimensions 20-55 mm. Cavum uteri was compressed, and eccentric. The endometrium was approximately three mm thick. All myomas were firm, with vortical structure, without necrosis. Right ovary was 20 x 16 x 8 mm, gyrase-like, smooth, with small rupture on the surface; uterine tube was 50 x 5 mm without pathology. Left uterine tube was elongated, with free fimbriae and were 80 x 4 mm. Tumor on the left ovary was encapsulated and had a mass of 1.5 kg and had dimensions of a 220 x 170 x 159 mm. The external surface demonstrated to be smooth and pearly-white. On cutting, subcapsular cystic spaces were appreciated and its walls were solid. The nodular zone of the tumor tissue was very firm, whitish, shiny, and without necrosis.

The processing method included the hematoxylin and eosin (H&E) staining protocol and immunohistochemistry.

Macroscopic description
The specimen obtained through surgery was an enlarged and deformed uterus with recognizable right adnexa, elongated left uterine tube, and a large tumor on left ovary. The uterus mass was 290 grams and its dimensions 120 by x 95 by x 65 mm. The interval orifice was closed and the endocervix presented cysts. The portio and vaginal mucosa was smooth, shiny and whitish deformed by numerous intramural and subserous myoma, from isthmus to fundus with dimensions 20-55 mm. Cavum uteri was compressed, and eccentric. The endometrium was approximately three mm thick. All myomas were firm, with vortical structure, without necrosis. Right ovary was 20 x 16 x 8 mm, gyrase-like, smooth, with small rupture on the surface; uterine tube was 50 x 5 mm without pathology. Left uterine tube was elongated, with free fimbriae and were 80 x 4 mm. Tumor on the left ovary was encapsulated and had a mass of 1.5 kg and had dimensions of a 220 x 170 x 159 mm. The external surface demonstrated to be smooth and pearly-white. On cutting, subcapsular cystic spaces were appreciated and its walls were solid. The nodular zone of the tumor tissue was very firm, whitish, shiny, and without necrosis.

Microscopic description
On the cervix, ecrition with metaplastic squamous epithelium were seen without atypia. In the endocervical wall there were numerous retention cysts. The endometrium showed signs of proliferation of stroma and epithelium, without atypia. The myomas were all moderately cellular with mitosis under 2 / 10HPF and no necrosis or hemorrhage could be seen. The right ovary was with scars, and the cellular cortex had rare inclusion cysts. There was a hemorhagic zone, without cysts. The left ovary included a fibroma that was slightly to moderately cellular, with high collagenisation of interstitum; mitoses were very rare, less than 1 / 10HPF. In subcapsular location there were cystic and papillary spaces, with biphasic pattern characterized centrally by medium or small glands and covered by endometrial epithelium that was not atypical and that externally covered the papillary formations. Atypical spindle cell stroma, generally low grade, proliferated around the glands, focally of intermediate grade with anisonucleosis and multinucleated cells of histiocytic aspect.

The histopathological diagnosis was adenosarcoma of the ovary, intermediate grade (FIGO Stage IA).
Discussion

The World Health Organization (WHO) classification of ovarian tumors includes also those classified as endometrioid tumors. In these tumors, epithelial or stromal elements or both are demonstrated, and they resemble those neoplasias found more frequently in the endometrium. As with other neoplasias of the superficial epithelium of the ovary, they can be benign, borderline or malignant [6]. Included among the malignant neoplasias are adenosarcoma, mixed mesodermic tumor (Müllerian) or carcinosarcoma and stromal endometroid sarcoma. The first two could be homologous or heterogeneous. With regards to homologous tumors, the mesenchymatous structures are normal for the organ, whereas heterogeneous refers to the presence of mesodermal structures not usual for the organ (cartilage, bone, fat, striated muscle, etc.). There are few possible explanations of preoperative and intraoperative misdiagnosis in this case.

The described large benign tumor of the ovary, contained only peripheral / subcapsular mixed epithelial stromal tumor with malignant stromal component and the capsula was intact (Figure 1).

Through immunohistochemistry we confirmed homolog-type adenosarcoma with the presence of stromal cells of endometrioid type that showed CD10 positivity (Figure 2), estrogen and progesteron receptor positivity (steroid receptors were positive in stromal and epithelial cells) but also, sarcoma cells showed smooth muscle differentiation and were desmine, smooth muscle actin and panactin positive. Ki 67 proliferative index was between 5 and 8%, with irregular pattern of positivity, with typical periglandular location, where an atypical mitosis was found.

The patient was discharged from the hospital with the diagnosis of ovarian fibroma, but the pathohistological tumor diagnosis was homologous adenocarcinoma of the left ovary. The tumor was classified as clinical Stage IA with sarcomatous overgrowth. It was decided not to treat the patient with any other additional or adjuvant therapy, by oncology advisory consilium. The patient is presently disease free six months after follow-up.

Figure 1. — Ovarian adenosarcoma with benign epithelial and malignant stromal component. H&E, x20.

Figure 2. — CD10 intracellular positivity in sarcomatous component of tumor. Immunohistochemistry, x40.

Figure 3. — Estrogen receptor (ER) positivity in benign epithelial and malignant stromal compartment of tumor. Immunohistochemistry, x400.
From the description by Clement and Scully of the histological pattern of uterine adenosarcoma in 1974 and the first extraterrane presentation by Clement and Scully in 1978 [7], multiple sites of origin for these tumors have been described [8-10], with the ovary being second in frequency, up to 18% of the cases [4]. However, to the authors’ knowledge, this only represents 52 cases reported, including the present one. This is the reason why the authors did not assume this kind of outcome.

Another study referred to diagnostic imaging problems associated with the diagnosis adenosarcoma of the ovary. Adenosarcoma should be considered in patients with a predominantly solid pelvic mass on ultrasound imaging [11] like in this case, but differential diagnosis includes uterine myoma, which can resemble the adenosarcoma of the ovary; therefore, more meticulous ultrasound exam is stipulated in all cases of pelvic tumor masses.

Even macroscopic finding on laparotomy did not raise suspicion of adenosarcoma. Ovarian tumor was considered as ovarian fibroma. Macroscopically adenosarcomas of the ovary measure approximately 10 cm on average in diameter, some with smooth external surface. The present case, in contrast, was much bigger, with 22 cm in the biggest diameter, which can also be the reason of misdiagnosis. On cutting, ovarian adenosarcomas are medium brown in color with zones of necrosis and hemorrhage and with the presence of small cysts. The cut surface is spongy and multicystic with clear or yellowish-colored fluid. The largest tumors could present hemorrhagic zones. The authors did not cut the specimen during laparotomy because it could have raised suspicion of adenosarcoma, as fibromas have a different appearance. Fibromas are usually large at presentation with a mean diameter of over 10 cm and an average weight of 1 kg. They are solely or predominantly solid masses with well defined lobulated borders. Cystic degeneration, oedema and even hemorrhage occur, especially in large tumors. Calcification is present in less than 10% of cases [12].

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Enlarged Virchow’s node as an initial complaint of serous ovarian adenocarcinoma

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Summary

Background: An enlarged Virchow’s node or left supraclavicular lymph node is a classic precursor to the diagnosis of metastatic cancer, usually originating from an abdominal organ. It is rarely found in ovarian carcinoma. Case report: A 49-year-old woman presented a painless mass in her left supraclavicular fossa. A histopathological examination of the same mass was consistent with a serous adenocarcinoma of ovarian origin. The patient was initially asymptomatic, even with the disease in an advanced stage. Left supraclavicular adenopathy has not been previously reported as a presenting complaint of ovarian carcinoma. Conclusion: Ovarian carcinoma in contemporary with a Virchow’s node is an isolated finding.

Key words: Virchow’s metastasis; Ovarian carcinoma.

Introduction

Serous ovarian carcinoma, which represents 60% of ovarian epithelial carcinomas, is the most lethal gynecologic malignancy, with a five-year survival rate of less than 30%. It is frequently diagnosed at an advanced stage because the initial symptoms of bloating, fatigue, early satiety, and constipation are nonspecific; peritoneal metastasis occurs as an early event [1]. Furthermore, there is no available screening test to date. The most common pattern of spread involves seeding in the peritoneal cavity, and nodal spread via the pelvic and para-aortic nodes also occurs [2]. The disease may rarely spread to a supraclavicular lymph node. This report describes a 49-year-old Japanese woman with ovarian serous adenocarcinoma who initially presented an enlarged left supraclavicular node or Virchow’s node. This is the first reported case of ovarian carcinoma in contemporary with supraclavicular lymphadenopathy.

Case Report

The patient, a 49-year-old female had consulted with her gynecologist for leg edema. Prior to this, her only complaint had been a painless left supraclavicular mass of two months duration. Pelvic magnetic resonance imaging (MRI) revealed a bilateral, multilocular ovarian tumor with multiple lymph nodes metastasis. This was confirmed with ultrasound and computed tomography (CT). CT images (Figure 1) show the bilateral ovarian tumors, multiple lymph nodes metastasis, bilateral hydrenephrosis, ascites, and a 3 cm mass in the left supraclavicular fossa. There was no evidence of pulmonary, hepatic, pancreatic, splenic, adrenal, or brain metastasis. The patient’s CA125 level was 387 u / ml. The biopsy of the supraclavicular lymph node showed metastatic adenocarcinoma. An exploratory laparotomy and bilateral salpingo-oophorectomy were performed. The right and left adnexa measured 5 x 3.5 x 3.5 cm and 7 x 3 x 3 cm, respectively. Both fallopian tubes included metastatic nodules. A 1.5 x 1 x 1 cm omental nodule was also resected. The patient was diagnosed with Stage IV high-grade serous ovarian carcinoma (Figure 2). Systemic chemotherapy with conventional platinum and taxane chemotherapy (Carboplatin AUC5, Paclitaxel 175 mg/m²) was initiated. After six months, histopathological examination of another cervical lymph node biopsy was positive for poorly differentiated carcinoma. Treatment was continued with second line chemotherapy (CPT-11 or Gemcitabin) but to no avail; carcinoma progressed and the patient died from her cancer two years after diagnosis.

Discussion

An enlarged left supraclavicular lymph node (Virchow’s node or Troisier’s sign) leads to a diagnosis of metastatic cancer, usually originating from an abdominal organ [3]. Rudolf Virchow (1821-1902), a German pathologist, first described the gland and its association with gastric cancer in 1848 [4]. The French pathologist Charles

Figure 1. — CT image showing bilateral ovarian tumors.
Enlarged Virchow’s node as an initial complaint of serous ovarian adenocarcinoma

Emile Troisier noted in 1889 that other abdominal cancers also spread to this node [5]. This node is on the left side of the neck where the lymphatic drainage of most of the body (from the thoracic duct) enters the venous circulation via the left subclavian vein. It is thought that the metastasis blocks the thoracic duct, which leads to the reflux of lymph into the surrounding nodes i.e. Virchow’s node. Another explanation is that the Virchow’s node corresponds to the terminal node along the thoracic duct and is therefore the end repository for metastatic nodal disease [6].

Malignancies of the abdominal viscera frequently remain asymptomatic until they reach an advanced stage. Gastric cancer, for example, is frequently asymptomatic even when metastatic. One of the first areas of metastasis for gastrointestinal (GI) tumors is the supravacularilary lymph node. In the present case, the patient was initially asymptomatic when she noted the enlarged supravacularilary lymph node. Unlike GI cancers, ovarian cancers only rarely present with isolated lymphadenopathy.

Ovarian serous carcinoma is the most common ovarian epithelial malignancy (60% of cases). There are two different types of ovarian serous carcinomas in terms of morphology and molecular genetics: low-grade serous carcinoma and high-grade serous carcinoma. The low-grade serous carcinomas account for about 10% of all ovarian serous cancers [7] and demonstrate a relatively indolent clinical course. The five-year survival rate is approximately 40%-56%. The more common high-grade serous carcinomas are extremely aggressive, with a five-year survival rate of 10%-20%. They constitute 90% of all serous carcinomas and often manifest at an advanced stage, with up to 85% of patients presenting widespread peritoneal metastases. Mediastinal and supravacularilary lymphadenopathy, and metastases to the brain at the time of initial presentation have been documented in rare instances [8].

Serous carcinomas are frequently characterized by vague, nonspecific symptoms that are frequently ascribed to GI disorders, such as an irritable bowel. Even with a timely evaluation, most women are diagnosed with disseminated intra-abdominal disease. This is a reflection of the tumor’s propensity to spread early during its course by seedling in the peritoneal cavity. Less common are lymphatic metastases and hematogenous spread is also rare. In early stage (Stage I) disease, a mobile but irregular pelvic mass can often be palpated through pelvic examination, especially if the tumor is more than five cm in size and has not protruded above the pelvic brim. As the disease spreads into the pelvic cavity, nodules may be found in the cul-de-sac, particularly through bimanual examination (Stage II). Ascites may occur in all stages, but become more evident when the tumor involves the upper abdomen (Stage III). The omentum is a frequent site of metastatic disease and its removal is recommended as part of the complete cytoreductive procedure. Pelvic and para-aortic lymph nodes are involved in approximately 40-70% of Stage III tumors. The malignant cells may spread via lymphatic channels that follow the ovarian blood supply along the infundibulopelvic ligament, terminating in the para-aortic lymph nodes to the level of the renal vessels. Other lymphatics pass laterally through the broad ligament and parametrium to the external iliac, obturator, and internal iliac nodal chains. Infrequently, metastases may also follow the round ligament to the inguinal nodes [9]. Finally, the disease may spread to the supraclavicular lymph nodes and into the pleura, causing a malignant effusion (Stage IV).

Survival of serous carcinomas is a function of disease stage. Five-year survival rates for early stage disease (Stage I or II) range from 80% to 95%, whereas patients with advanced disease (Stage III or IV) have lower survival rates of 10%-30% [10]. The present patient’s disease course is in keeping with these statistics. The present case highlights an unusual presentation of ovarian cancer. The initial asymptomatic presentation of this patient emphasizes the need for an effective means of screening for this devastating malignancy.

Figure 2. — Histopathological image. (A) Metastatic foci of adenocarcinoma in the left supraclavicular lymph node; (B) Solid nests of serous adenocarcinoma in the ovary.
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Malignant primary peritoneal mesothelioma: report of two cases and review of literature

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Summary

Introduction: Malignant primary peritoneal mesothelioma is a rare and highly aggressive tumor. This tumor can be misdiagnosed as ovarian carcinoma. Case: Two cases of malignant primary peritoneal mesothelioma that were misdiagnosed as ovarian carcinoma were operated in our institution. Patients were 74 and 45 years-old at admittance. Conclusion: Malignant primary peritoneal mesothelioma is a problem for gynecologic oncologists because of the close similarity with epithelial ovarian cancer. Diagnosis and treatment of these patients are still under debate.

Key words: Peritoneal mesothelioma; Diagnosis; Epithelial ovarian cancer.

Introduction

Malignant peritoneal mesothelioma (MPM) is an uncommon and very aggressive tumor. The first report of this disease was by Miller and Wynn in 1908 [1]. Since then many treatment methods as surgery, chemotherapy, and radiotherapy were proposed and used but none seemed to alter the natural progression of this disease [2-4]. Treatment methods and survival figures for this malignancy are limited and ineffective. Oncogenetic mechanisms and tumor behavior which cause aggressiveness and poor response to therapy are still under investigation. Aggressive cytoreductive surgery and adjuvant chemotherapy are accepted as the definitive treatment method. Adjuvant chemotherapy method is accepted as hyperthermic intraperitoneal chemotherapy (HIPEC). Patients who suffer from this cancer type usually present with abdominal pain or distention. Many of these patients are operated by gynecologic oncologists because of very similar symptoms and findings with epithelial ovarian cancer. The differential diagnosis between this rare but aggressive tumor and both serous papillary carcinoma of the ovary and peritoneum is a problem [5-7].

Case Reports

Patient 1

A 74-year-old woman was admitted to our clinic with complaints of abdominal distention and gastrointestinal (GI) problems. On examination her abdomen was distended and no obvious mass was palpated. An abdominal ultrasonography (US) revealed diffuse ascites, peritoneal implants, and a tumoral mass of 14 cm in diameter located on the transverse colon and omentum. Abdominal computed tomography (CT) scan showed a large mass on the transverse colon (15 x 15 cm). A serologic survey including CA125, CA 19-9, CA 15-3, carcinoembryonic antigen (CEA), and alpha fetoprotein (AFP) levels were studied and were all found to be in normal ranges. A laparotomy was carried out and total abdominal hysterectomy with bilateral salpingooophorectomy (TAH+BSO), infracolic omentectomy, bilateral pelvic and paraaortic lymphadenectomy, and segmental colon resection were performed and there were many peritoneal implants. Pathologic evaluation of the specimen was reported as well-differentiated papillary peritoneal mesothelioma (WDPPM). Pathologic differential diagnosis was made by the immunohistochemical analysis results; and tumor was found to be positive for calretinin, CK7, CD56, meizothelin, and CA125 while negative for CK20, CDX2, chromogranin, synaptophysin, and CD15 (Figure 1). The patient was referred to the medical oncology department for adjuvant chemotherapy after an uncomplicated recovery period.

Patient 2

A 45-year-old woman was admitted to a gynecology clinic with the complaint of abdominal distension and pain. Abdominal US in that clinic revealed a pelvic cystic mass and the patient was operated. She was found to have frozen pelvis which was determined to be inappropriate for surgery. Peritoneal and tumoral biopsies were done and surgery was completed. The pathology result was reported as primary peritoneal mesothelioma (positively stained for Calretinin, CK7, HBME-1, and negative for CEA). Abdominal and thorax CT scans were done and the thorax was found to be normal. An abdominal scan revealed that the pelvis was full of tumoral mass as “frozen pelvis”, omental caking, retroperitoneal enlarged lymph nodes, and ascites. The patient was referred to our gynecologic oncology clinic for further evaluation and treatment.

A second laparotomy was decided together with the general surgeons. The gynecologic oncology team was invited because of the frozen pelvis. Patient had a second laparotomy and TAH+BSO, infracolic omentectomy, bilateral pelvic and paraaortic lymphadenectomy, appendectomy and peritoneal debulking were carried out. Pathologic evaluation reported a primary epitheloid mesothelioma with decidual variant

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Figure 1. — Tumor having solid (A; HE x100) and papillary growth (B; HE x100) of cells with large cytoplasm, vesicular nuclei and positively stained with CK7 (C), calretinin (D), mezothelin (E), and CD56 (F) (x400).

Figure 2. — Tumor on the serosal surfaces of ovaries (A; HE x 40) and uterus. Tumor cells arranged in solid, tubular, or papillary patterns (B; HE x 200 and C; HE x 400). On higher magnification vesicular nuclei can be seen with prominent nucleoli and large polygonal cytoplasm reminiscent of desidual cells (C). Immunohistochemical studies show calretinin and CK5/6 positivity, with negative CEA, ER, and PR.
Malignant primary peritoneal mesothelioma: report of two cases and review of literature

(Figure 2). The patient was referred to the medical oncology department for adjuvant chemotherapy after an uncomplicated postoperative period.

Conclusion

Mesotheliomas are tumors usually arising from the pleural cavity, and some from peritoneal surfaces. This tumor type usually runs a rapid and fatal course [8] and occurs mostly in the fifth and seventh decades of life [9]. The most common admittance symptoms of these patients are abdominal pain and increasing abdominal girth. The classic knowledge includes asbestos exposure for the etiologic risk factors of this disease, but only 58% of peritoneal mesothelioma cases had this exposure in the past. Decreasing usage and control of asbestos exposure also render this etiopathologic relation more suspicious today. Many of these patients are diagnosed as having ovarian carcinoma and are operated by gynecologic oncologists in a similar method with ovarian carcinoma. The main differential diagnosis is primarily made on the basis of immunohistochemistry that yields positive staining of calretinin, Wilm’s tumor-1 antigen, mesothelin, and antimesothelial cell antibody-1 [6]. Definitive surgical therapy is the main treatment and should include cytoreductive surgery, the same as for ovarian carcinoma. Gynecologic oncologists are becoming more important for the disease diagnosis and treatment because of their experience in late stage epithelial ovarian carcinoma treatment. Gynecologic oncology consultation should be the main evaluation in these patients that are admitted to general surgery and gastroenterology clinics.

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A case of primary squamous cell carcinoma of the endometrium associated with extensive “ichthyosis uteri”

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Summary

Background: Ichthyosis uteri is an uncommon entity in which the entire endometrium is replaced by stratified squamous epithelium. Though the condition often is considered as benign, dysplastic changes have been reported. Case: We describe herein an exceedingly rare case of primary squamous cell carcinoma of the endometrium (PSCCE) associated with extensive ichthyosis uteri with chronic pyometra, who presented with blood-stained vaginal discharge of six-seven months duration. Although repeated endometrial biopsies revealed only strips of stratified squamous epithelium showing moderate to severe dysplastic changes, the tumor markers and magnetic resonance imaging strongly suggested advanced uterine body malignancy. Exploratory laparotomy was performed, and histologic findings of the superficial layer were consistent with ichthyosis uteri; in contrast the lesion of invasive squamous cell carcinoma was located in the deeper layer and lymph nodes. No dysplastic changes of the cervix were noted. Conclusions: It is suggested that PSCCE could be associated with pre-existing ichthyosis uteri and deeper biopsies should be performed for the accurate pre-operative diagnosis of cases with chronic pyometra.

Key words: Squamous cell carcinoma; Endometrial carcinoma; Ichthyosis uteri.

Introduction

Primary squamous cell carcinoma of the endometrium (PSCCE) is an uncommon entity and has been rarely reported to be associated with ichthyosis uteri, where the entire endometrium is replaced by stratified squamous epithelium, which is originally considered as a benign condition. In this report the authors present the unique clinical and pathological findings of a case of PSCCE in association with extensive ichthyosis uteri.

Case Report

A 66-year-old gravida 2, para 2, healthy woman was referred to our hospital with a several months’ history of purulent vaginal discharge with foul odor. Several cytologic examinations of the cervix and endometrium had been performed and classified as negative. She had attained menopause about 15 years before. Endometrial curetting revealed strips of stratified squamous epithelium showing partly well differentiated squamous cell carcinoma, while cervical smear and curetting showed no malignant cells. Magnetic resonance (MR) imaging revealed a well circumscribed intramural mass of predominantly low to intermediate signal intensity on T2-weighted images resembling degenerated leiomyoma and an ill-demarcated mass of mixed signal intensity in the uterine cavity. Extravasation of the adnexa or pelvic lymph node was suspected. No invasion to the cervix was detected. The patient’s tumor marker profile was as follows: SCC (13.7 ng/ml; normal < 3 5 ng/ml), CEA (4.3 ng/ml; normal < 2.5 ng/ml), CA 125 (25.1 U/ml; normal < 35 U/ml). From these findings, squamous cell carcinoma originating in the endometrium was highly suspected. At exploratory laparotomy, the uterus was remarkably enlarged and densely adherent to the left tube, which was enlarged 3 x 3 x 4 cm. The pelvic peritoneum appeared thick and inflamed. Radical hysterectomy, bilateral salpingo-oophorectomy, pelvic lymph node dissection with pelvic washings and omentectomy were carried out.

Macroscopically, the uterine cavity was markedly distented and filled with foul-smelling purulent keratin debris, and pus occluding the whole entire endometrial cavity (Figure 1). Sectioning revealed stromal invasion of the tumor to almost 100% of the uterine wall thickness with direct invasion to the left fallopian tube. The cervix was examined entirely and showed no gross mucosal lesions. Microscopically, the polyoid mass was covered externally by cornified squamous epithelium and had fibrovascular stroma inside. The entire endometrium was replaced by stratified squamous epithelium showing areas of heavy keratinization, koilocytic changes, nuclear hyperchromasia and moderate increase in nuclear-cytoplasmic ratio indicating low grade dysplastic changes in the underlying ichthyosis uteri (Figure 2). In contrast the invasive squamous cell carcinoma lesion was located in the deeper layer (Figure 3). No dysplastic changes of the cervix were noted. Metastatic lesions measuring 3 x 3 x 2 cm were observed in the left pelvic lymph nodes.

The patient was treated with concurrent chemoradiation therapy. Six months after radiation, SCC level slightly increased, and positron emission tomography and computed tomography suggested recurrence in the small pelvis. She received adjuvant treatment with six courses of weekly carboplatin and paclitaxel administration. She is alive and well with no evidence of disease 22 months after surgery.

Discussion

The case that we report here showed PSCCE in pre-existing extensive ichthyosis uteri with dysplasia. To cor-
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Histopathological examination, extensive ichthyosis uteri with dysplasia and squamous cell carcinoma with areas of multifocal invasion into the myometrium was present. With these reports and from our results, it might be speculated that chronic inflammation of the endometrium plays an important role in the development and progression of PSCCE, in terms of the metaplasia-hyperplasia-dysplasia sequence.

Survival data for PSCCE are scanty. The presence of a malignant squamous cell component in endometrial carcinoma are said to worsen the prognosis [6]. The prognosis of squamous cell carcinoma of the endometrium is related to the stage at diagnosis. In a review of reported cases, 80% of Stage I patients survived whereas survival for patients with Stage III disease was only 20% [7]. Diagnosis of primary squamous cell carcinoma of the endometrium should be considered in a postmenopausal elderly female presenting with pyometra, and deeper biopsies should be performed for an accurate preoperative diagnosis.

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Peritumoral allergic response in epithelial ovarian carcinoma

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Summary

Aims: Desmoplasia, intratumoral lymphocyte infiltration, and calcification within the tumor and peritoneum are quite common in advanced epithelial ovarian carcinoma. Peritumoral inflammatory reactions associated with hematologic paraneoplastic syndrome are extremely rare. Materials and Method: We describe in detail two cases of epithelial ovarian carcinoma associated with proliferation of Russell bodies and Mott cells in desmoplastic tumor stroma. Results: In the first case, monoclonal proliferation was diagnosed with intranuclear inclusions (Dutcher bodies) in plasma cells. Dutcher bodies were seen both in the tumor tissue and bone marrow. Monoclonal gammapathy of undetermined significance (MGUS) was diagnosed with an IgM level less than 3 g/l and clonal paratrabecular lymphoplasmocytoid infiltrate less than 10% in the bone marrow. There were no light chain restrictions. Elevated beta 2 microglobulin level and anemia complicated the patients’ survival. In the second case, because of desmoplasia the tumor volume did not decrease after the standard chemotherapy although a significant portion of the carcinomatous tissue disappeared. In this study, we checked the role of peritumoral allergic response prospectively in five epithelial ovarian carcinoma cases by actin, desmin, and lambda light chain immunohistochemical staining of the omentum. Conclusions: Further studies are necessary to show the relation between peritumoral hypersensitivity reaction and HLA 1 antigen processing machinery to improve disease-specific survival in epithelial ovarian carcinoma.

Key words: Epithelial ovarian carcinoma; Beta 2 microglobulin; MGUS; Russel body; Dutcher body; Mott cell.

Introduction

Proliferation of non-fibrous connective tissue within the tumor and peritoneum is quite common in the cases of advanced stages of ovarian carcinoma. Paraneoplastic syndromes, including endocrinopathies, neurologic abnormalities, skin lesions, and coagulation abnormalities may occur with tumors as a result of hypersensitivity reactions. The response to the therapy differs across patients despite the standardization of surgical therapy and chemotherapy. Epithelial ovarian carcinomas account for nearly 90% of all ovarian malignancies. Intratumoral lymphocyte infiltration varies significantly among benign, borderline, and malignant epithelial ovarian tumors [1]. The absence of intratumoral T cell infiltration has a negative effect on the survival of patients with ovarian carcinoma [2]. Matrix metalloproteinases (MMP) are proteolytic enzymes implicated in ovarian cancer progression and metastases [3]. The overexpression of stromal MMP-9 and membrane type 1-MMP is found to be independently associated with shorter survival rate, which is specific to the type of the disease in epithelial ovarian carcinoma [4]. Hoskins et al. [5] demonstrated that the effect of secretory leukocyte protease inhibitor (SLPI) is amplified in serous ovarian cancer. SLPI and MMP-9 expression are strongly correlated in serous ovarian cancers. Degradation of tumor matrix and normal tissue result in the release of tumor-derived DNA into the circulation. Kamat et al. [6] demonstrated that preoperative plasma cell free DNA is an independent predictor of death from disease in ovarian carcinoma. Although the origin of cell free DNA is unclear, physiochemical investigations suggest that it may originate from internucleosomal cleavage of the chromatin, a hallmark of the apoptotic process. Ebstein-Barr virus DNA is also detected in the cell-free DNA of nasopharyngeal carcinoma patients’ serum. In addition to all of these studies, actin bundling protein fascin expression patterns are found to be a tumor phenotype, which does not change despite standard treatment procedures in epithelial ovarian carcinoma [1-4].

The aim of the study was to evaluate peritumoral allergic responses by histopathologic examination of the omentum in seven epithelial ovarian carcinoma cases.

Case Report

Case 1

A 61-year-old woman was admitted to Osmangazi Hospital because of postmenopausal bleeding and a pelvic mass. Initial laboratory test results were as follows: hemoglobin; 11.9 g/dl, leucocytes 6940/ml, and platelets 220,000/ml. HbsAg, anti HCV and HIV tests were negative. Alpha fetoprotein: 3.19 U/ml, hCG: 2.69 mIU/ml, CA-125: 21.6 U/ml. The patient was diagnosed with advanced-stage ovarian carcinoma and underwent surgical staging. Type II hysterectomy, bilateral salpingo-oophorectomy, lymphadenectomy, partial omentectomy and multiple biopsies from the peritoneum and Douglas pouch were performed. The postoperative course was uneventful and the patient was discharged from hospital. The pathologic examination revealed biphasic malignant tumoral involvement of the ovaries, lymph nodes, omentum, peritoneum, and Douglas pouch. Mixed pleomorphic tumor infiltrate containing lymphoid cells, plasma cells, atypical pleomorphic large cells containing Dutcher bodies, clusters of Russell bodies and Mott cells, and focal serous ovarian carcinoma were seen in the ovaries, and six metastatic lymph nodes and peritoneum (Figure 1-2). Binucleated or multinucleated plasma cells were observed in the desmoplastic tumoral stroma. Multiple intranuclear eosinophilic inclusions were seen in the nucleus of atypical pleomorphic large cells. Kappa or Lambda light cell restrictions

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IgG: 13.6 g/l, IgM: 0.777 g/l, IgA: 0.29 g/l, beta 2 microglobulin: 4.22 mg/dl (N: 1.16-3.21 mg/dl). Histopathological classification and laboratory evaluation revealed high-grade, Stage IIIC serous ovarian carcinoma with MUGUS. Two months after the surgery the patient was readmitted to the hospital and was administered the standard adjuvant chemotherapy for serous ovarian carcinoma. The patient died of disease after the first dose of chemotherapy because of acute renal failure.

Case 2

A 62-year-old female patient was referred to Osmangazi Hospital with unresectable pelvic tumor. Needle biopsy was performed. Tumor cells had hyperchromatic pleomorphic nuclei, scant cytoplasm and formed irregular nests in the desmoplastic stroma. Plasma cells, clusters of Russell bodies and Mott cells were observed in the sections from ovaries and pelvic para-aortic lymph nodes. After the operation, laboratory test results were as follows: myoglobulin: 659.36 ng/ml (N = 10-92 ng/ml), troponin I: 0.11 ng/ml (N = 0-0.1 ng/ml), LDH: 539 U/l (N = 240-480 U/l). Bone marrow biopsy examination revealed parabascular lymphoplasmacytoid infiltrate and some atypical monocytoid cells. Dutcher bodies in plasma cells showed monoclonal proliferation of lymphoplasmacytoid cells in the bone marrow (Figure 4). The protein electrophoresis was as follows: gamma: 20.2 g/l (N: 7-12 g/l), alpha 1: 8.2 g/l (N: 2-3 g/l), alpha 2: 12.1 (N: 4-10 g/l), albumin: 48.2 g/l (N: 35-50 g/l), beta 1: 5.9 g/l (N: 5-11 g/l), beta 2: 5.4 g/l, immunoglobulin levels: IgG: 13.6 g/l, IgM: 0.777 g/l, IgA: 0.29 g/l, beta 2 microglobulin: 4.22 mg/dl (N: 1.16-3.21 mg/dl). Histopathological classification and laboratory evaluation revealed high-grade, Stage IIIC serous ovarian carcinoma with MUGUS. Two months after the surgery the patient was readmitted to the hospital and was administered the standard adjuvant chemotherapy for serous ovarian carcinoma. The patient died of disease after the first dose of chemotherapy because of acute renal failure.

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The right ovary was 18 x 13 x 8 cm in dimension. The left ovary was 3.5 x 1.5 x 1.2 cm in size. High-grade serous ovarian carcinoma was diagnosed in the right and left ovary, Douglas pouch and peritoneum. Infarction was observed in the right ovary. Blastomyces hyphae were seen in the necrotic areas of the right ovary. In the left ovary benign Brenner tumor focus associated with serous carcinoma was observed. The stroma of the tumor was desmoplastic. Actin and desmin positive myofibroblastic cells, and multinuclear giant cells were seen in the stroma and peritoneum. Cytokeratin 18 immunohistochemical stain revealed decreased tumor cell volume after chemotherapy. Collagen-1 binding proteoglycan, syndecan-1 (CD138) immunohistochemical staining decorated the surface of serous carcinoma cells with papillary architecture and plasma cells. There were no light chain restrictions by kappa or lambda immunohistochemical staining.

**Control cases**

The control patients’ age ranged between 44-75 years. Omental biopsies of five cases of grade 3 epithelial ovarian carcinoma were examined prospectively to check the presence of hypersensitivity reaction. Among the histopathologic types of cases, four were serous and one was endometrioid type. There were three patients in Stage IA, one patient in Stage 3C, and one patient in Stage 4. Among the cases with histopathologic examination of the omentum, two cases had tumoral involvement, one case had fat necrosis, the other cases had mild to moderate degrees of fibrosis with capillary proliferation and mononuclear inflammatory cells in the adipose tissue or around the vessels. Immunohistochemical stains were performed for actin, desmin and lambda light chain by using the avidin-biotin peroxidase complex method.

**Results**

Desmin immunohistochemical stain only stained periarteriolar myoid cells. Actin immunohistochemical stain highlighted desmoplastic fibrous tissue with small capillary proliferation in four patients. Lambda immunohistochemical stain was partially positive in plasma cells in three cases. The staining pattern highlighted the polyclonal composition of lymphoplasmacytic infiltrate. One case of Stage IA, grade 3 serous carcinoma had non-specific strong lambda light chain staining in the dense, homogenous and hyalinized connective tissue. The other case of Stage IA serous carcinoma had minimal inflammation and fibrous tissue proliferation in the omentum.

**Discussion**

Waldenström and Pedersen demonstrated the presence of large quantities of a high molecular weight globulin in the plasma of a group of patients and differentiated the electrophoretic patterns of monoclonal and polyclonal gammapathies. The patterns of gamma globulin in a chronic viral disease were very broad whereas in that of myeloma they were sharp with a narrow spike designated as monoclonal gammopathy [7]. The presence of monoclonal B-cell proliferation does not necessarily imply malignancy. Monoclonal gammapathies can be complicated as a variety of cutaneous diseases, Sjogren syndrome, scleromyxedema, necrobiotic xanthogranuloma with paraproteinaemia and POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M protein and skin lesions) [7-9]. Waldenström’s macroglobulinemia (WM) associated with hepatic carcinoma, lung carcinoma, conjunctival in situ carcinoma, mucinous carcinoma of urinary bladder, larynx carcinoma, and some other malignancies were reported [10-13]. Most of these solid malignancies were diagnosed during the course of paraproteinaemia. The median survival is five years and approximately 10% of the patients are still alive after 15 years [14].

Lymphoplasmacytic lymphoma (LPL) is a rare low-grade lymphoma that is often associated with WM, which is defined as a subset of plasmacytoid lymphocytic lymphoma. The presence of a large IgM paraprotein in the absence of LPL is no longer considered as WM, and LPL in the absence of IgM paraprotein is not WM. Diagnostic criteria for asymptomatic myeloma are M-protein in the serum at myeloma levels less than 3 g/l and/or less than 10% of clonal plasma cells in bone marrow. This disease is called IgM MGUS (= monoclonal gammapathy of undetermined significance) and the patient has no signs of anemia, constitutional symptoms, lymphadenopathy or hyperviscosity [15]. Our first case had a pelvic mass, multiple abdominal lymphadenopathies, anemia, and high levels of beta 2 microglobulin. However, other laboratory results and the bone marrow biopsy were compatible with MGUS. In this case, hematologic examination was performed ten days after the cytoreduction surgery for ovarian carcinoma. Our patient had two poor prognostic criteria (anemia and high beta 2 microglobulin level) for WM [15]. The presence of Dutcher bodies containing plasma cells and/or atypical cells in both bone marrow and peritoneal tissue demonstrated that the proliferation of plasma cells were monoclonal, however there was no light chain restriction.

Satoskar et al. [16] reported 24 patients with cardiac amyloidosis. Six patients in this series were diagnosed as MGUS related to AL amyloidosis. We could not find any case of amyloidosis associated with ovarian neoplasia. However, Han et al. [2] reported beta 2 microglobulin expression of 63.3% for epithelial ovarian carcinoma. In the first case, high levels of plasma beta 2 microglobulin and anemia were associated with poor prognostic factors for WM [15]. Defects in HLA class I antigen processing machinery (APM) might create a mechanism for tumor cells to escape from immune recognition. Han et al. [2] demonstrated heterogeneous or positive expression of TAPI, TAPII, HLA-class I heavy chain and beta 2 microglobulin in epithelial ovarian carcinoma. Down-regulation of the APM component has been found to have a negative effect on the survival of patients with ovarian carcinoma.

Chronic inflammatory infiltrate in the peritoneum displays perivascular or diffuse patterns containing lymphocytes, macrophages, plasma cells, and eosinophils. The fibrous component is characterized by myofibroblasts within a type I collagen matrix. Metastatic tumor cells within the retroperitoneum may cause an exuberant
desmoplastic response. Plasma cells may be positive for IgG4 isotype [17-20].

In this study, we have presented two rare cases of serous ovarian carcinoma associated with special type desmoplastic reaction with peritumoral proliferation of Mott cells and Russell bodies. Mott cells, specific forms of plasma cells that contain multiple intracytoplasmic inclusions called Russell bodies, are occasionally observed in hematopoietic tumors with plasmacytic differentiation, as in plasma cell myeloma [8]. Shinozaki et al. [21] described two cases of Epstein-Barr virus associated-gastric carcinoma with prominent reactive Mott cell proliferation. The tumor volume did not decrease in our second case after six doses of standard chemotherapy and there were actin and desmin positive myofibroblasts in the tumoral stroma, however an important portion of carcinomatous tissue had disappeared. There were no Ducther bodies in the peritumoral tissue. In the second case, Mott cell and Russell body proliferation were reactive. Rampisela et al. [8] described an unusual self-limited clonal Mott cell proliferation with lymphoplasmacytic lymphoma-like features in an inguinal lymph node of a child who was previously diagnosed with Wiskott-Aldrich syndrome and Von Recklinghausen's neurofibromatosis. The child spontaneously recovered in less than a year, however Burkitt's lymphoma developed in the parapharyngeal space four years later. Mott cell proliferation in this case was also reactive because there were no Ducther bodies containing plasma cells in the infiltrate.

The presence of monoclonal proliferation of Mott cells and Russell bodies are extremely rare peritumoral allergic responses in epithelial ovarian carcinoma. In the first case, we demonstrated the defective antigen processing machinery component by elevation of beta-2 microglobulin in the plasma [2]. All our cases were treated with standard therapy. The apparent differences in disease-specific survival among epithelial ovarian carcinoma cases showed the role of peritumoral allergic response in epithelial ovarian carcinoma. Further studies on defects of HLA class I antigen processing machinery are necessary for the improvement of disease-specific therapy in advanced stages of epithelial ovarian carcinoma.

References


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