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Frequency of recurrence after surgical treatment of cervical intraepithelial neoplasia grade 1-3

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Summary

Purpose of investigation: The objective was to demonstrate the frequency of invasive cervical cancer or recurrent CIN in patients treated by a previous diagnosis of CIN 1-3. Methods: We analyzed 1,397 records colpocytologic and medical records. Recurrence of CIN or invasive neoplasia of the cervix after treatment of CIN was assessed. The chi-square test was used for statistical analysis (significance level set at less than 0.05). Results: We obtained 696 CIN 1, 244 CIN 2, 451 CIN 3, and six squamous carcinoma. Regarding patients who relapsed, there were 6/690 (0.9%) patients had an initial diagnosis of CIN 1, 8/236 (3.4%) CIN 2 and 21/430 (4.9%) CIN 3 (p < 0.0001). Comparing the frequency of relapse among each group, we found: CIN 1 vs CIN 2: p = 0.0073; CIN 1 vs CIN 3: p < 0.0001; CIN 2 vs CIN 3: p = 0.38. Conclusion: Although the number of relapses when comparing CIN 2 and CIN 3 were not significant, the data suggest that CIN 2 has lower recurrence rates, so these patients require more conservative treatment if a desire of future pregnancy is expressed.

Key words: Post-treatment recurrence; Cervical intraepithelial neoplasia; Invasive cervical cancer.

Introduction

The incidence and mortality of cervical malignancy have substantially reduced with cervical screening programs [1]. The risk of recurrence of cervical intraepithelial neoplasia (CIN 2/3) after treatment may be associated with the degree of CIN, the type of treatment, and age [2]. CIN is the precursor lesion of cervical cancer. The triad of colposcopy, cytology and histology have confirmed the diagnosis of cervical premalignant and malignant lesions, and histology remains the gold standard which will define the treatment. The decision on choosing the most appropriate therapy for the treatment of cervical intraepithelial lesions depends on many factors such as location and extent of injury, patient age, the desire for pregnancy and adherence to follow-up [3-5].

Low-grade CINs should be followed up every six months with colposcopy and cytology since the rate of regression is high. Treatment is reserved for persistent lesions. The standard procedure for high-grade CIN is the loop electrosurgical excision procedure (LEEP) and its effectiveness depends on the status of the surgical margin, and extent and presence of endocervical lesions in multiple quadrants. The main techniques for removal of cervical lesions include local destructive treatments (cryotherapy, electrocautery, laser) and excisional treatments, which have the advantage of providing material for histological confirmation of the lesion and the margins [3-5].

The American Society for Colposcopy and Cervical Pathology (ASCCP) recommends for follow-up after treatment of CIN 2 and 3, a single HPV test for six to 12 months after treatment, two consecutive cytological tests with colposcopy or cytology), followed by routine check-ups if the usual screening tests are normal. The range for routine screening is nonspecific, but the guidelines indicate that a high risk of recurrence of CIN or invasive cancer persists for many years after treatment and the follow-up should continue for at least 20 years. Guidelines from the Agency of British Columbia Cancer at this time recommend colposcopy four to six months after treatment for CIN 2/3. If results are normal, cytological follow-up is recommended after 12 months of treatment [2].

The objective of the study was to demonstrate the frequency of invasive cervical cancer or recurrent cervical intraepithelial neoplasia in patients who were treated by a previous diagnosis of CIN 1-3 comparing age, pathological diagnosis and treatment.

Materials and Methods

A retrospective study was conducted at the Federal University of Triângulo Mineiro (UFTM). We analyzed 1,391 records (1994-2004) of Pap smears, colposcopy and epidemiologic data of patients diagnosed with CIN 1, CIN 2 or CIN 3 screened by cytology. These patients were treated and continued follow-up after treatment in the colposcopy clinic of the Discipline of Gynecology and Obstetrics, University Hospital of UFTM (HU-UFTM). The record for each patient was obtained, numbered and cataloged according to diagnosis, age, treatment, and analyzed in conjunction with the Discipline of Special Pathology and IPON (Oncology Research Institute) - UFTM. Recurrence of cervical intraepithelial neoplasia or invasive neoplasia of the cervix after treatment of CIN 1 (when there was treatment), 2 or 3 was assessed. We also evaluated data such as age, parity and initial treatment, duration of follow-up (colposcopy and cytology every 6 months), diagnosis, treatment of relapse, and time after initial diagnosis of their appearance. Records not
pertinent to the work, such as other diagnosis, incomplete data and patients who did not undergo regular monitoring, were excluded.

Most patients who were lost to follow-up had a diagnosis of CIN 1 and were excluded from the study. Regarding patients with CIN 1, 288 women were lost to follow-up, and 143 women underwent follow-up irregularly.

Initially the records were separated according to cytological diagnosis, and when a patient had more than one, we considered it more complex. In our service there is no obligation to perform histology when there is a cytologic diagnosis of CIN 1. After confirmation of CIN 1 post Papanicolaou patients were referred to the Colposcopy Clinic for follow-up every six months and were discharged after three General Outpatient Clinic smears showed no CIN or HPV infection. However, for patients with a cytological diagnosis of CIN 2 or 3, it was compulsory to carry out pathological examination to confirm the diagnosis and direct the treatment. For patients with smear dissociation, we considered it a more complex diagnosis.

Cryotherapy or colpo-cytologic monitoring every six months (until there was no evidence of HPV infection) was the procedure for patients with CIN 1. Patients with CIN 2 and CIN 3 were treated with LEEP, conization, or hysterectomy. Hysterectomy was performed when there was no technical requirement for conization (flat or atrophic cervix).

**Statistical analysis**

χ² test and χ² test for trend were used for statistical analysis with the significance level set at less than 0.05. This research was approved by the Research Ethics Committee of the Federal University of Triângulo Mineiro.

**Results**

After rigorous analysis, 1,391 records of smears with CIN 1, 2 or 3 were selected. We obtained 696 CIN 1, 244 CIN 2, and 451 CIN 3. Thirty-nine patients relapsed after treatment of the initial diagnosis and one died after recurrence.

Table 1 shows diagnosis, initial treatment, lesion in recurrence, recurrence time, treatment of recurrence and death.

Regarding patients who relapsed in each group, there were 6/690 (0.9%) patients with an initial diagnosis of CIN 1, 8/236 (3.4%) CIN 2 and 21/430 (4.9%) CIN 3 (p < 0.0001, χ² for trend; Table 2). Four patients who relapsed had an initial diagnosis of invasive carcinoma.

The ages of patients who relapsed varied from 22 to 72 years, with a prevalence of 20-40 years (56.41%), and 43.58% of patients were over 40 years. Recurrence after initial diagnosis ranged from one to 16 years, and follow-up by Pap smear ranged from one to 18 years. Only one patient did not treat the recurrence of injury and another did not return to the clinic.

Regarding patients with CIN 1 who relapsed, the mean age was 38 ± 10.9 years, mean parity was 2.5 ± 1.9 children, four (66.7%) were white women and two (33.3%) were nonwhite women. In patients with CIN 2 who relapsed, the mean age was 40.1 ± 12.3 years, mean parity was 3.1 ± 2.9 children, two (25%) were white and six (75%) were nonwhite women. In patients with CIN 3, the mean age was 38.6 ± 11.2 years, mean parity was 2.5 ± 1.3 children, 17 (80.9%) were white and four (19.1%) were nonwhite women. Regarding patients with an initial diagnosis of invasive carcinoma, the mean age was 58.7 ± 15.7 years, mean parity was 3.7 ± 2.6 children, and all were white women.

Comparing the frequency of relapse among each group, we found: CIN 1 versus CIN 2: p = 0.0073; CIN 1 versus CIN 3: p < 0.0001; CIN 2 versus CIN 3: p = 0.38.
Frequency of recurrence after surgical treatment of cervical intraepithelial neoplasia grade 1-3

Discussion

Patients with CIN and microinvasive carcinoma usually have no specific symptoms. The careful choice of primary therapy for cervical carcinoma is crucial, because treatment of recurrent disease is more difficult.

In patients over 40 years, most of the initial diagnoses were CIN 2 or 3 (64.7%), and recurrence also occurred in more complex lesions or invasive carcinoma. Melnikow et al. demonstrated that the recurrence of CIN 2/3 increases with age, grade of intraepithelial neoplasia and early treatment of disease [2].

Regarding tumor recurrence, only two cases had recurrence as invasive, and in both the initial diagnosis was invasive carcinoma. Thus, we can not conclude that there is a greater predisposition to invasive neoplasia, if the initial diagnosis was a high-grade CIN. Therefore it confirms the importance of prolonged follow-up since relapse occurred after four and 13 years of colposcopic monitoring.

Some recurrences occurred in the short term, other long-term recurrences occurred during follow-up with Pap smears and colposcopy. Thus it is not possible to predict the ideal time in which the patient should return for routine annual check-up.

Age, CIN grade and type of treatment are important factors related to rates of recurrence of intraepithelial lesions or invasive neoplasia [2]. This is in agreement with our results, which also showed an association between recurrence and grade of CIN. Women with CIN 3 who were not treated had an increased risk of developing cervical cancer [6, 7] while the risk was very low in women treated conventionally [6]. Although wide excision of the transformation zone is an effective treatment in high-grade intraepithelial lesions, approximately 15% of patients will have persistent or recurrent disease at follow-up. Furthermore, patients who tested positive for HPV DNA at follow-up seem to have a considerably higher risk of recurrence of intraepithelial lesions than those with these negative tests [7].

Melnikow et al. demonstrated overall rates of CIN 2/3 declined rapidly for the first two years after treatment, but during the first six years of follow-up, these rates were 14.0% for women treated for CIN 3, 9.3% for CIN 2, and 5.6% for CIN 1 [2]. In our study, these rates were 0.9% for CIN1, 3.4% for CIN 2 and 4.9% for CIN 3. The ASCUS/LSIL Triage Study (ALTS) demonstrated women with initial low-grade squamous epithelial lesions who were referred for early colposcopy had rates of subsequent CIN 2/3 of 8% to 13% during a 24-month follow-up [8]. Other studies demonstrated residual or recurrent disease ranged from 7.6% to 17.9% in women treated for high-grade squamous intraepithelial lesions [9-11].

HPV infections in adolescents have a high rate of spontaneous regression [5]. Some will progress to HSIL or LSIL, but rarely is there a progression to cervical cancer. Excisional techniques could disturb the future pregnancy outcome of these patients, so a conservative approach can be done in these cases [4, 5]. The available data have shown an increased risk of overall preterm delivery, preterm delivery after premature rupture of membranes, and low birth weight infants in subsequent pregnancies [12-16].

When a woman expresses a desire for future pregnancy, even the type of excisional treatment should be thoroughly evaluated. When comparing LEEP with cold-knife conization, obstetric complications such as miscarriages and preterm pregnancies are more frequent with cold-knife conization [17].

Epidemiological evidence has demonstrated that the biological behavior of CIN 2 is closer to CIN 1 than to CIN 3, and the risk of progression to invasive carcinoma in cases of CIN 1 and CIN 2 is low [18, 19]. Castle et al. estimated a fraction of cervical intraepithelial neoplasia 2 (CIN 2) that may regress if untreated using data from the ALTS study and approximately 40% of undiagnosed CIN 2 will regress over two years, but CIN 2 caused by HPV-16 may be less likely to regress than CIN 2 caused by other high-risk-HPV genotypes [20].

Our study showed a low frequency of recurrence of CIN 1 (0.9%). This confirms that follow-up every six months is safe for patients with this diagnosis. When comparing the different degrees of CIN, there was a trend towards increased frequency of recurrence with worsening of the injury. Thus maybe the option of more conservative treatment could be performed in patients with CIN2 or even in adolescents with CIN 2 and CIN 3.

Conclusions

Although it did not significantly affect the number of relapses when compared with CIN 2 and CIN 3, the data suggest that CIN 2 has lower recurrence rates, so patients with CIN 2 who require more conservative treatment, such as those with a desire for pregnancy, may be subjected to less invasive treatment. These patients may be advised to have close follow-up, since the frequency of recurrence is lower, preventing a higher rate of premature rupture of membranes, premature labor and of prematurity.

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

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Summary
In all 88 patients, 23-67 years of age (mean of 34.8 years) with abnormal cytology, lesions in the uterine cervix and presence of DNA corresponding to highly oncogenic HPV, two cycles of uterine cervix cryotherapy and local treatment with povidone-iodine resulted in eradication of the virus six months after detection of the virus.

Key words: HPV eradication; Infection; Cryotherapy; Povidone-iodine.

Introduction
Epidemiologic and molecular studies have proven that over 90% of carcinomas in the uterine cervix are linked to infection with highly oncogenic human papillomavirus (HPV) strains, including 15 HPV types: 16, 18, 45, 31, 33, 52, 58, 35, 59, 56, 39, 73, 68, and 66 [1, 2]. Currently, around 80% of sexually active women are thought to experience a HPV infection at some point in their lifetime, which, in most cases, is eradicated spontaneously. Only in 5%-10% of infections, particularly those involving highly oncogenic HPV strains, does the infection persist, which is defined as a persisting presence of DNA of the same type after a period of at least 6-12 months [3, 4].

In cases with additional risk factors, such as immunological dysfunction, other sexually transmitted infections, smoking, prolonged use of hormonal contraception, multiple deliveries, age of ≥ 42 years, the persisting infection leads to Pap test alterations of atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL) or high grade (HSIL) type and to CIN 1, 2, 3 histologic lesions [5-7].

Observational eight-year cohort studies on 1,001 and 2,404 patients demonstrated that CIN 1 lesions (regression, persistence or progression to higher CIN) can develop within six months while changes in Pap test results in women with positive HPV DNA can undergo regression or progression to HSIL in a period of 7.7 to 88 months, depending on the oncogenic potential of the virus [8, 9]. Other studies have proven that CIN 3 can develop even within two to three years due to exposure to HPV, particularly HPV types 16/18 [10, 11]. Development of an invasive carcinoma in the uterine cervix takes years or decades after HPV infection [12, 13].

Anti-viral vaccination using two prophylactic vaccines, Cervarix and Gardasil is effective not only due to the vaccine activity against HPV 16 and 18, the principal highly oncogenic HPV types, but can also provide cross protection against other highly oncogenic HPV types (31, 33, 45, 52 and 58) [14].

In general, it is not the HPV infection itself which is targeted by the treatment but the virus-induced precancerous and cancerous lesions. Various methods of CIN treatment are employed, sometimes excessively aggressive, although references indicate that even after conization relapses of CIN are encountered [7, 15]. Most probably, this reflects either a persisting HPV infection despite the elimination of the cervical lesion or another infection.

A recent report in 2011 appeared on the treatment of HPV infections using intravaginal administration of a zinc-citrate solution, which significantly eliminated the viral infection [16].

Another original paper pertains to carcinoma of the uterine cervix and the action of arsenic trioxide (As₂O₃) as a potential agent which would sensitize lesions to treatment. The target is supposed to involve zinc finger transcription factor YY1, the expression of which is increased in uterine cervix carcinomas and which plays a significant role in progression of HPV-positive carcinoma [17].

This study aimed to evaluate the effects exerted by repeated cryotherapy and pharmacotherapy, using povidone-iodine for the presence of DNA representing highly oncogenic HPV in female patients with abnormal cytology and lesions in the uterine cervix.

Materials & Methods
The treatment was performed in 88 women, ranging in age from 23 to 67 years, who demonstrated cytological alterations, clinical presence of cervical lesions or erosion type and presence of DNA corresponding to highly oncogenic HPV types.

Cervical cytology was estimated using the Bethesda system. Viral genotyping was performed in the Laboratory of Molecular Genetics using reverse transpiration polymerase chain reaction (RT-PCR), capable of detecting 15 highly oncogenic types and 12 low oncogenic types of HPV.
Following the estimation of HPV type the following procedures were performed:

– cryotherapy for 3 min, using liquid nitrogen applied via a cervical probe the size of which depended on the size of the vaginal portion of the cervix and type of vaginal opening, including lesions on the disc and external opening, including the transient zone and part of cervical canal;

– intravaginal povidone-iodine (betadine) was applied (which also acts as has an antiviral) every day for 14 nights to a depth guaranteeing the drug reached the uterine cervix;

– after two months the cryotherapy and local betadine treatment procedures were repeated;

– two months later tests were made to check for presence of HPV DNA.

Results

Cytological examination disclosed: in 62 cases ASC-US and in 26 patients LSIL with koilocytosis. No differences in cytological results were disclosed between women aged 23 and 47 years (69 patients) and those between 49 and 67 years (19 patients).

In 33 women (37%) individual HPV types were identified:

– HPV 16 (noted most frequently) was found in 11 (33%) patients; HPV 58 in five patients, HPV 31 in four patients, HPV 51 in four patients, HPV 18 in four, HPV 33 in three and HPV 52 in one patient and HPV 45 also in one patient.

In 18 women (20.2%) two types of the virus were detected:

– in five patients types 16+31, in four patients HPV 16+another highly oncogenic type (52, 18, 39, 31, 45).

In nine patients two of the following highly oncogenic types of HPV were detected: 52, 51, 45, 39, 59, 58, 56. In 37 women (42.6%) multiple HPV types were detected, apart from those listed above, also types 68 and 66; with types 16 and 51 being the most frequent among the multiple infections.

Six months after HPV infection detection RT-PCR failed to detect HPV DNA in any of the patients and cytological examination performed three months after the diagnosis was normal in every one of the patients.

In seven women, aged 45 to 67, continuing cervical erosion of the vaginal portion led to patients being subjected to conization and histological evaluation of the excised preparation documented the diagnosis of glandular erosion with no traits of koilocytosis.

Discussion

The highly oncogenic HPV types are linked to the development of precancerous lesions and carcinoma of the uterine cervix. Despite differences in the distribution of HPV types on various continents, seven genotypes (type 16, 18, 45, 31, 33, 52 and 58) have been found in 87.4% of invasive carcinomas worldwide. Studies on precancerous lesions prove that after six months around 49% of women diagnosed with CIN 1 manifest a normal cervix, 45% continue to carry CIN, but in 7% of women progression is noted to higher CIN. Moreover, the treatment of precancerous lesions using loop excision of the transformation zone may be linked to the persistence of the viral infection [7, 18].

Until now, viral infections as such were not treated but the therapy was targeted at lesions resulting from the infection. Kim et al. [16] presented pilot results of conservative local management of HPV infection using zinc citrate solution, which proved to be highly promising.

In conclusion, the method presented above is effective, economic and it fulfils the expectations of patients who want to eradicate the virus and lesions in the uterine cervix instead of waiting for spontaneous eradication or a further unfavourable development.

References


Effect of cryotherapy and povidone-iodine preparation on eradication of DNA corresponding to highly oncogenic HPV in women etc.


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Chemotherapy-related hypersensitivity reaction in Japanese patients with gynecologic malignancy


Department of Obstetrics and Gynecology, Osaka City University, Graduate School of Medicine, Osaka (Japan)

Summary

Purpose of investigation: Chemotherapy-related hypersensitivity reaction seems to be problematic in the safe management of chemotherapy. In this study we investigated chemotherapy-related hypersensitivity reaction in patients with gynecologic malignancy. Methods: Between January 2009 and December 2010, we examined hypersensitivity reaction (≥ grade 2) using the Common Terminology Criteria for Adverse Events (CTCAE) v.4.0. We analyzed the incidence, clinical features, management, and outcome. Results: We administered over 1,057 infusions (24 regimens) to 205 patients. We found a total of four hypersensitivity reactions (≥ grade 2) cases (carboplatin: 2; nedaplatin: 1; docetaxel: 1). Signs and symptoms were varied. In two cases, the same regimen was rechallenged by using anti-allergic drugs. The docetaxel case was successful. The carboplatin case was not successful. Conclusion: Chemotherapy-related hypersensitivity reaction (≥ grade 2) does not occur frequently. In the case of platinum, especially, carboplatin, re-administering after hypersensitivity reaction should be done carefully though platinum is a key drug in patients with gynecologic malignancies.

Key words: Hypersensitivity reaction; Chemotherapy; Gynecologic malignancy.

Introduction

Gynecologic malignancies are usually more sensitive to systemic chemotherapy than other malignancies, and patients receive more kinds of chemotherapy and more frequently per patient than other malignancies. Moreover, new drugs (pegylated liposomal doxorubicin, gemcitabine, etc.) have been used recently in gynecologic malignancies [1, 2].

On the other hand, it is assumed that hypersensitivity reactions of chemotherapy seem to be more problematic in the management of chemotherapy than outpatient chemotherapy, especially more frequently in Japan. Hypersensitivity reaction is a known source of great stress to patients, their families, nurses, other patients, and physicians; 52% of a nursing staff have reported that infusion reactions are draining and frightening to them. Around 88% of outpatient and 62% of inpatient nurses consider infusion reactions frightening to other patients, with the potential to cause anxiety and confusion [3, 4].

It is very difficult to estimate the incidence of hypersensitivity reactions [5]. There are a wide variety of grading scales for hypersensitivity reaction. We examined our reported hypersensitivity reaction (≥ grade 2) using the Common Terminology Criteria for Adverse Events (CTCAE) v.4.0.

In this study, we investigated chemotherapy-related hypersensitivity reaction in patients with gynecologic malignancies.

Materials and Methods

This retrospective study was approved by the Osaka City University, Graduate School of Medicine Institutional Review Board. Using the available electronic medical record data between January 2009 and December 2010, we examined our reported hypersensitivity reaction using the CTCAE v.4.0. We analyzed the incidence, clinical features, management, and outcome of chemotherapy-related hypersensitivity reaction (≥ grade 2) in patients with gynecologic malignancy, and the possibility of rechallenge with the drug.

Results

Incidence of hypersensitivity reaction

We administered over 1,057 infusions (24 regimens) to 205 patients with gynecologic malignancies between January 2009 and December 2010. Median age was 60 years (24-84). Diseases of gynecologic malignancy were as follows: ovarian cancer: 78; endometrial cancer: 53; cervical cancer: 45; peritoneal cancer: 13; uterine carcinosarcoma: 4; vaginal cancer: 3; choriocarcinoma and uterine sarcoma: 2; etc. (Table 1). Courses of chemotherapy performed were as follows: TC (paclitaxel+carboplatin): 479; nedaplatin: 84; cisplatin: 83; DC (docetaxel+carboplatin): 72; doxil: 61; AP (adriamycin+cisplatin): 26; CPT-11+nedaplatin: 25; docetaxel+nedaplatin: 23; etc. (Table 2). We found a total of four hypersensitivity reactions (≥ grade 2) cases. Three cases occurred in patients with ovarian cancer and one case was in a patient with cervical cancer. Three cases were treated by platinum (two cases: carboplatin; one case: nedaplatin) and one case was by taxane (docetaxel).

Clinical features and management of hypersensitivity reaction

All cases were grade 2 hypersensitivity reaction. Signs and symptoms varied: thoracic symptoms: chest tightness, hypotension; respiratory symptoms: dyspnea,
Chemotherapy-related hypersensitivity reaction in Japanese patients with gynecologic malignancy

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lenged by using anti-allergic drugs on the same day following hypersensitivity reaction. The docetaxel case was successful. The carboplatin case was not successful and we changed the regimen. In the other two cases, we changed the regimen without rechallenge of the drug.

Discussion

Hypersensitivity reaction is common in clinical practice, and most cases are mild or moderate. For example, hypersensitivity reaction to paclitaxel has been reported to occur in approximately 10% of patients, however, less than 1% of patients experience severe hypersensitivity reaction [6]. There have been several reports investigating hypersensitivity reaction in patients with gynecologic cancer [6-9]. Most reports included mild hypersensitivity reaction cases. Moreover, new drugs (pegylated liposomal doxorubicin, gemcitabine, etc.) have been used recently in patients with gynecologic malignancy [1, 2].

In this study, we investigated hypersensitivity reaction (≥ grade 2) of chemotherapy performed recently. We administered over 1,057 infusions (24 regimens) to 205 patients with gynecologic malignancies between January 2009 and December 2010. We found a total of four hypersensitivity reaction (≥ grade 2) cases. Three cases occurred in patients with ovarian cancer and one case was in a patient with cervical cancer. Three cases were treated by platinum and one case by taxane. Two cases of platinum were carboplatin (2 of 551 courses) and one case was nedaplatin (1 of 132 courses). One taxane case was docetaxel (1 of 98 courses).

It is very difficult to estimate our incidents of hypersensitivity reaction, although there have been many reports of hypersensitivity reaction [6-9]. There are a wide variety of grading scales for hypersensitivity reaction. The CTCAE itself has undergone several revisions (v.4.0 was

Table 1. — Number of patients with gynecologic malignancies.

<table>
<thead>
<tr>
<th>Gynecologic malignancy</th>
<th>No. of patients</th>
<th>No. of HSR (≥ grade 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian cancer</td>
<td>78</td>
<td>3</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>45</td>
<td>1</td>
</tr>
<tr>
<td>Peritoneal cancer</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Uterine carcinosarcoma</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Vaginal cancer</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Uterine sarcoma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Tubal cancer</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Endometrial stromal sarcoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vaginal melanoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bartholin gland carcinoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ovarian cancer &amp; endometrial cancer (double cancers)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

HSR: hypersensitivity reaction.

Table 2. — Regimen of chemotherapy.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. of courses</th>
<th>No. of HSR (≥ grade 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (PTX+CBDCA)</td>
<td>479</td>
<td>1</td>
</tr>
<tr>
<td>CDGP</td>
<td>84</td>
<td>1</td>
</tr>
<tr>
<td>CDDP</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>CPT+11+CDDP</td>
<td>82</td>
<td>0</td>
</tr>
<tr>
<td>DC (DTX+CBDCA)</td>
<td>72</td>
<td>2</td>
</tr>
<tr>
<td>PLD</td>
<td>61</td>
<td>0</td>
</tr>
<tr>
<td>AP (ADR+CDDP)</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>CPT+11+CDGP</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>DTX+CDGP</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>THP-ADR+CDDP</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>CPT-11</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>PTX</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>EMA/CO (VP-16+MTX+ACD+CPA+VCR)</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>CDDP+S-1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>DTX</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>EP/MEA (VP-16+CDDP+MTX+ACD)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>DAVFeron (DTIC+ACNU+VCR+IFN-beta)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>ADR</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>CPT+11+MMC</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>BEP (BLM+VP-16+CDDP)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>DTX+GEM</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>CAP (CPA+ADR+CDDP)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>TP (PTX+CDDP)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CPT+11+PTX</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>


Table 3. — Clinical features and management of hypersensitivity reaction (HSR).

<table>
<thead>
<tr>
<th>Drug of HSR</th>
<th>Carbolipatin</th>
<th>Carbolipatin</th>
<th>Nedaplatin</th>
<th>Docetaxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen of HSR</td>
<td>DC</td>
<td>TC</td>
<td>Nedaplatin</td>
<td>DC</td>
</tr>
<tr>
<td>Allergy history</td>
<td>Alcohol</td>
<td>Pyrine</td>
<td>(–)</td>
<td>(–)</td>
</tr>
<tr>
<td>Prior chemotherapy</td>
<td>TCx8</td>
<td>CDDP+CPT-11x6</td>
<td>CDDP+CPT-11x6</td>
<td>GEMx1</td>
</tr>
<tr>
<td>Cycle no. of 1st HSR</td>
<td>5</td>
<td>9</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Grade of HSR</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Signs and symptoms</td>
<td>Thoracic (–)</td>
<td>Hypotension (–)</td>
<td>Chest tightness (–)</td>
<td>Dyspnea (–)</td>
</tr>
<tr>
<td>Respiratory (–)</td>
<td>Dyspnea (–)</td>
<td>Desaturation (–)</td>
<td>Wheezing (–)</td>
<td>Desaturation (–)</td>
</tr>
<tr>
<td>Dermatological (–)</td>
<td>Erythema (–)</td>
<td>Flushing (–)</td>
<td>(–)</td>
<td>(–)</td>
</tr>
<tr>
<td>Treatment methods</td>
<td>Diphenhydramine (+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Hydrocortisone (+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Oxygen (+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Rechallenge</td>
<td>not successful</td>
<td>(–)</td>
<td>(–)</td>
<td>successful</td>
</tr>
</tbody>
</table>

wheeling, desaturation; dermatological symptoms: erythema, flushing, etc. The treatment methods for hypersensitivity reaction to each drug were very similar in the four cases, utilizing mainly diphenhydramine (2 cases), and intravenous hydrocortisone (3 cases) and supplemental nasal oxygen (2 cases) (Table 3).

Rechallenge of the drug following hypersensitivity reaction

In two cases (carboplatin and docetaxel) of four hypersensitivity reaction cases, the same regimen was rechallenged by using anti-allergic drugs on the same day following hypersensitivity reaction. The docetaxel case was successful. The carboplatin case was not successful and we changed the regimen. In the other two cases, we changed the regimen without rechallenge of the drug.

Discussion

Hypersensitivity reaction is common in clinical practice, and most cases are mild or moderate. For example, hypersensitivity reaction to paclitaxel has been reported to occur in approximately 10% of patients, however, less than 1% of patients experience severe hypersensitivity reaction [6]. There have been several reports investigating hypersensitivity reaction in patients with gynecologic cancer [6-9]. Most reports included mild hypersensitivity reaction cases. Moreover, new drugs (pegylated liposomal doxorubicin, gemcitabine, etc.) have been used recently in patients with gynecologic malignancy [1, 2]. In this study, we investigated hypersensitivity reaction (≥ grade 2) of chemotherapy performed recently.

We administered over 1,057 infusions (24 regimens) to 205 patients with gynecologic malignancies between January 2009 and December 2010. We found a total of four hypersensitivity reaction (≥ grade 2) cases. Three cases occurred in patients with ovarian cancer and one case was in a patient with cervical cancer. Three cases were treated by platinum and one case by taxane. Two cases of platinum were carboplatin (2 of 551 courses) and one case was nedaplatin (1 of 132 courses). One taxane case was docetaxel (1 of 98 courses).

It is very difficult to estimate our incidents of hypersensitivity reaction, although there have been many reports of hypersensitivity reaction [6-9]. There are a wide variety of grading scales for hypersensitivity reaction. The CTCAE itself has undergone several revisions (v.4.0 was
released in May 2009). In clinical trials, these scales for grading allergic reactions have been used frequently. Some studies graded each sign and symptom of hypersensitivity reaction separately using the CTCAE [5]. We examined our reported hypersensitivity reaction (≥ grade 2) using the CTCAE (v.4.0). This grading scale seemed to be a relatively new and ideal tool for hypersensitivity reaction.

Major drugs as a hypersensitivity reaction-causing agent are platinum and taxane. Using the CTCAE (v.4.0), incidence of hypersensitivity reaction (≥ grade 2) to carboplatin have not been so frequent. Hypersensitivity reaction to carboplatin is considered to be an IgE-mediated immune response. The incidence of hypersensitivity reaction per patient increases with the number of doses given, and in cases of documented occupational exposure to the drug. However, a longer platinum-free interval between courses of drugs has been correlated with an increased incidence of hypersensitivity reaction [7-9]. In our study, the first hypersensitivity reaction was during the 5th course of DC therapy after eight courses of TC therapy and six courses of CDDP+CPT-11 therapy, and in the 9th course of TC therapy after six courses of CDDP+CPT-11 therapy. We could not rechallenge carboplatin by using anti-allergic drugs on the same day following hypersensitivity reaction because of a re-hypersensitivity reaction. This is in accord with previous reports. The number of chemotherapy including platinum have decreased for platinum-resistant cancer. Moreover, new drugs (pegylated liposomal doxorubicin, gemcitabine, etc.) have been used recently in gynecologic malignancy, and seemed to be the reason for lower incidents of hypersensitivity reaction to carboplatin. Our study included one case of hypersensitivity reaction to nedaplatin. There has been no report for hypersensitivity reaction to nedaplatin and some reports included a few hypersensitivity reaction cases [10]. In our case, the first hypersensitivity reaction was during the 2nd course of nedaplatin therapy after three courses of cisplatin therapy, in accord with previous reports.

Hypersensitivity reaction to taxane is considered as a non-IgE-mediated immune response. Therefore, hypersensitivity reaction occurs most frequently during the first or second exposure and is severe only during these administrations. Nearly all patients rechallenged after the first administration were able to tolerate subsequent cycles [6, 11, 12]. Our study included one case of hypersensitivity reaction to docetaxel. There have been many reports of hypersensitivity reaction to docetaxel [12]. In our case, the first hypersensitivity reaction was during the first course of DC therapy without prior chemotherapy. We were able to rechallenge docetaxel by using anti-allergic drugs on the same day following hypersensitivity reaction in accordance with previous reports. There was no hypersensitivity reaction to paclitaxel (0 of 498 courses). In most cases of alcohol allergy, we used docetaxel instead of paclitaxel, for example, DC therapy instead of TC therapy. This seemed to be the reason for no case of hypersensitivity reaction to paclitaxel.

There was no case of hypersensitivity reaction to new drugs, for example, pegylated liposomal doxorubicin, gemcitabine, etc., which may be because of the small number of courses of regimen performed.

In two cases (carboplatin and docetaxel) of four hypersensitivity reaction cases, the same regimen was rechallenged by using anti-allergic drugs on the same day following hypersensitivity reaction. The docetaxel case was successful, whereas the carboplatin case was not successful so we changed the regimen.

In conclusion, chemotherapy-related hypersensitivity reaction (≥ grade 2) does not occur frequently in patients with gynecologic malignancies. In the case of platinum, especially carboplatin, re-administering after hypersensitivity reaction should be done carefully though platinum is a key drug in patients with gynecologic malignancy.

References


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Sentinel node biopsy in male breast carcinoma: is the “female” approach justified?

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Department of Surgery, University Hospital of Patras, Rion (Greece)

Summary

Purpose: Mastectomy with axillary lymph node dissection (ALND) represents the gold standard in the treatment of male breast carcinoma. Recently, data have emerged supporting that sentinel lymph node biopsy (SNB) may be feasible in selected patients. The aim of this study was to analyze the safety and prognostic reliability of SNB in male patients with breast carcinoma and clinically negative axilla. Methods: During a 10-year period (2000-2010), 11 men with mean age 66.1 years (range 34-84) diagnosed with breast carcinoma were retrospectively included to our study. All patients underwent SNB. Regardless of the SNB results, completion axillary clearance was conducted in all cases. Results: SNB detection rate was 100%, while the mean number of sentinel nodes removed was 1.5 ± 0.7 (range 1-2). Frozen section analysis revealed a negative sentinel node in four out of 11 patients (36.4%). Independently of these results, all patients underwent completion ALND. The overall false-negative rate, defined as the percentage of all node-positive tumors in which the SNB was negative, was 0%. Conclusion: The current study indicates that SNB may be feasible in selected male individuals with breast carcinoma. The technique may reduce the morbidity related to dissection of the axilla; prospective multicenter trials are needed in order to define the exact criteria for wider application of this technique.

Key words: Male breast cancer; Sentinel node; Prognostic reliability.

Introduction

Male breast cancer is a rare clinical entity accounting for approximately 1% of all breast cancers [1]. The prognosis of breast cancer in male patients is comparable to that of female patients [2, 3]. Axillary lymph node involvement represents the most important prognostic factor and it is reported to be found in more than 50% in men with T1-T2 tumors [4, 5]. Modified radical mastectomy remains the standard of care for male breast cancer patients in most institutions. However, morbidity related to axillary lymph node dissection is consistent including increased surgical time, drainage, lymphoedema and functional impotence of the arm. Data have emerged supporting that sentinel lymph node biopsy (SNB) may be feasible in selected patients, in particular those with early-stage cancer, preserving accurate staging of the disease in the axilla [6, 7]. The aim of this study was to analyze the safety and prognostic reliability of SNB in male patients with breast carcinoma and clinically negative axilla.

Materials and Methods

During a 10-year period (2000-2010), 18 men diagnosed with breast cancer were retrospectively identified from the database of our institution. From these patients, 11 men with mean age 66.1 years (range 34-84) had preoperatively clinically negative axilla and were finally included in the study. Preoperatively complete staging to rule out distant metastases had been performed. All patients underwent sentinel lymph node biopsy intraoperatively. Sentinel node identification was performed after injection of 2-3 ml diluted isosulfan blue dye in the subareolar and peritumoral area. To perform the SNB the tissue of the axillary region was explored in search for the sentinel lymph node using an axillary incision. The sentinel node specimen was sent for frozen section examination. According to the standard procedure adopted in the department, all patients underwent axillary lymph node clearance independently of the results of frozen section diagnosis. Follow-up included a physical examination of the breast and axilla at three to six month intervals. Statistical analysis was carried out using MedCalc version 10.2. Statistics are descriptive.

Results

Breast surgery consisted of modified radical mastectomy in nine patients (81.8%) and partial mastectomy with axillary lymph node clearance in two patients (18.2%). SNB detection was successful in all cases while the mean number of sentinel nodes removed was 1.5 ± 0.7 (range 1-2). Frozen section analysis revealed a negative sentinel node in four out of 11 patients (36.4%), corresponding to T1N0 in three cases and T4N0 in one case. The SNB was positive in seven patients (63.6%) and of those, five corresponded to Stage II breast cancer (2 patients with IIA and 3 with IIB). The overall false-negative rate, defined as the percentage of all node-positive tumors in which the SNB was negative, was 0% (Table 1). Ten patients were diagnosed with invasive breast ductal carcinoma and one with mucopidermoid breast carcinoma. The majority of patients (45.5%) were diagnosed with Stage II breast cancer, while three patients (27.3%) had a Stage I breast cancer (Table 1). Postoperatively two patients developed autolimited bleeding. The mortality rate was 0%.

Discussion

Male breast carcinoma is an uncommon condition and many of the management recommendations are based on those used for women with breast carcinoma. Innovations
in management for men follow those of women. The lifetime risk of having breast cancer is calculated as 0.11% in men versus 13% in women [4]. In general, male breast cancer has a less favorable outcome than its female counterpart, and crude survival is 55% for males versus 67% for females at five years [8]. The reasons for the worse prognosis are unclear, though a delayed diagnosis due to absence of screening and diagnostic attention for such a rare disease in men is conceivable.

Modified radical mastectomy remains the standard treatment of male breast cancer [5]. As in women, the involvement of axillary lymph nodes represents the most important prognostic factor. In a multivariate analysis study by Borgen et al., axillary lymph node status was the most powerful predictor of outcome with 80% and 35% 10-year overall survival rates in the case of pN0 and pN+, respectively. Axillary lymph node dissection allows optimal staging and ensures approximately 98% of local control although the benefit on survival remains controversial [5]. Recent studies report that SNB may also be feasible in selected male individuals and may reduce the morbidity related to dissection of the axilla [9, 10]. Sentinel lymph node positivity is reported in 33-55% of the cases, suggesting the need for a rigorous patient selection, generally corresponding to T1NO [6, 7, 10, 11]. SNB could be recommended in male patients with breast cancer and clinically negative axilla as a component of the surgical management [9, 10]. In our study 63.6% of the patients had positive sentinel lymph nodes and of those the 71.4% had a Stage II breast cancer. The false-negative rate was 0%. In this study SNB accurately staged the axilla in all patients and may be considered for axillary staging in patients with clinically negative axilla.

The histologic subtypes in men are comparable to those of invasive carcinoma in women. However, lobular invasive carcinoma is very rare in men [12]. In our study ten (90.9%) patients were diagnosed with invasive breast ductal carcinoma and one with a high-grade mucoepidermoid breast carcinoma with axillary lymph node metastasis. Mucoepidermoid carcinoma of the breast is a very rare type of neoplasm [13].

This study effectively evaluated the prognostic reliability of the SNB. The limitation of this study is the evaluation of the safety of the SNB. The procedure of SNB was safely performed in all the patients without any intraoperative complications and accurately staged the axilla. Postoperatively two patients developed autolimited bleeding. Since all patients underwent axillary lymph node clearance independently of the results of the SNB, the morbidity related to the SNB itself cannot be evaluated.

Conclusion

SNB is considered the gold standard for staging female patients with early-stage breast carcinoma. The current study indicates that this technique may also be feasible in selected male individuals. SNB may be considered for axillary staging in patients with clinically negative axilla. Prospective multicenter trials are needed to define the exact criteria for wider application of this technique.

References

Comparison of the efficacy and complications of different surgical methods for cervical intraepithelial neoplasia

S.Y. Zeng¹, M.R. Liang¹, L.Y. Li¹, Y.Y. Wu¹

¹Department of Oncology, Maternal and Child Health Hospital of Jiangxi Province, Nanchang (China)

Summary

Objective: The aim of this study was to offer some reference for the treatment of cervical intraepithelial neoplasia (CIN) by comparing complication rates and treatment failure rates of different surgical methods of CIN. Methods: 1,256 cases of CIN diagnosed by punch biopsy and pathological confirmation of postoperative specimens between January 2002 and June 2007 were reviewed and analyzed, in which 74 cases underwent the loop electrosurgical excision procedure (LEEP), 869 patients adopted cold knife conization (CKC), 49 patients received vaginal enlarged amputation of cervix, and 264 patients accepted extrafascial hysterectomy. The chi-square test was used to compare the rate of complication and treatment failure of different surgical methods. Results: The rates of surgical complications for LEEP, CKC, vaginal enlarged amputation of cervix and extrafascial hysterectomy were, respectively, 8.1% (6/74) 6.2% (54/869) 6.1% (3/49) and 2.3% (8/264), but this difference was not statistically significant. The treatment failure incidences for LEEP, CKC, vaginal enlarged amputation of cervix and external fascia hysterectomy were, respectively, 4.1% (3/74), 0.2% (2/869), 0.0% (0/49) and 0.4% (1/264). When comparing among the groups, the treatment failure incidence was higher in LEEP than that in CKC (p = 0.004) and extrafascial hysterectomy (p = 0.034); there was no statistically significant difference between CKC and extrafascial hysterectomy, and no significant difference was revealed between vaginal enlarged amputation of cervix and any other group. Conclusion: LEEP, CKC, vaginal enlarged amputation of cervix and extrafascial hysterectomy are all secure and effective procedures for patients with CIN, and patients can make their own individual choice depending on different conditions.

Key words: Cervical intraepithelial neoplasia, Gynecologic surgical procedures; Treatment outcome.

Introduction

In the past decades, great changes have taken place in the therapy of cervical intraepithelial neoplasia (CIN) from radical hysterectomy or radiotherapy at the beginning to trachelectomy. And in the past ten years cryotherapy or laser ablation and even pharmacotherapy have been put forward by some scholars to offer conservative management [1]. There are many types of management for CIN; however, the literature is inconsistent as to the efficacy and complications of different therapies, and the best treatment remains controversial [2, 3]. To offer some reference for the treatment of CIN, 1,256 inpatients with CIN diagnosed between January 2002 and June 2007 at the Maternal and Child Health Hospital of Jiang Xi were enlisted. These cases were classified by therapies to compare the rate of complications and treatment failure of different surgical methods.

Material and Method

Patients

Patients (average age 36.8, range 18-69) with CIN diagnosed between January 2002 and June 2007 in the Maternal and Child Health Hospital of Jiang Xi were enlisted in the study; 602 cases had CIN and 654 cases had cervical carcinoma in situ. Revised manuscript accepted for publication July 28, 2011

Screening standard

In order to exclude vulvar intraepithelial neoplasia (VIN) vaginal intraepithelial neoplasia (VAIN), glandular intraepithelial neoplasia, invasive cancer of the uterine cervix and other invasive carcinomas, all the patients with CIN were required to undergo physical examination, cytology, colposcopy, punch biopsy and endocervical curettage before the operation. All patients were confirmed both by punch biopsy under colposcopy and postoperative pathology, and the most serious result was considered as the diagnosis.

Evaluation standard

Surgical complications can occur at any time during surgery or afterwards. Intraoperative hemorrhage means blood loss more than 500 ml during the operation, and postoperative hemorrhage means that gauze and even sutures or hysterectomy are required to ensure homeostasis. Menstrual abnormalities include mild menses, menostaxis and menstrual irregularity. Any persistent lesion or recurrence or any unsuspected invasive carcinoma confirmed by histology are treatment failures. A persistent lesion of CIN is any grade of CIN detected within the first year after the operation [4]; while the recurrence of CIN is any grade of CIN or invasive carcinoma detected one year or later after the operation [5]. A persistent lesion or recurrence after extrafascial hysterectomy is VAIN or when invasive carcinoma occurs on the vaginal stump.

Surgical methods

Young patients with CIN who want to preserve the uterus and/or reproductive function receive the loop electrosurgical excision procedure (LEEP) or cold knife conization (CKC).
Vaginal enlarged amputation can be adopted when the lesion size observed under colposcope is more than 3/4 of the cervix or the lesion involves the glandular structure. When patients with positive surgical margins after CKC desire uterine and reproductive function preservation, vaginal enlarged amputation is also an option. Extravascial hysterectomy is chosen when patients with CIN also have ovarian cysts or myoma or prolapse of the uterus and other diseases while reproductive function has been completed. Extravascial hysterectomy can also be a complementary treatment for patients with positive surgical margins after CKC, for which there are two surgical methods - transabdominal or transvaginal. All our patients were classified into four groups: LEEP, CKC, vaginal enlarged amputation of cervix and extravascial hysterectomy.

Clinical and pathological data

Seventy-four (5.9%) patients with CIN adopted LEEP, in which one patient had a positive margin after operation and four patients with excisional margins were cauterized unduly; 869 (69.2%) patients received CKC, and two patients had positive margins after surgery; 49 (3.9%) patients accepted vaginal enlarged amputation of cervix, and 264 patients underwent extravascial hysterectomy. Clinical and pathological data of the four groups are summarized in Table 1.

Follow-up method

All patients were followed up at 3-month intervals during the first year after surgery. During the first year pelvic examination and cytology were done at each follow-up visit, while colposcopy was performed every six months, and human papilloma virus (HPV)-DNA was screened 8-12 months after the operation. From the second year after surgery cytology and colposcopy were done once a year. Follow-up was continued as long as a persistent lesion or recurrence was detected. If no persistent lesion or recurrence was detected, the terminal time for follow-up was June 2008. During the follow-up, 82 (6.5%) patients were lost.

Continuous data are shown as average ± standard deviation (\(\bar{x} \pm s\)). The difference of numeration datas was performed using Pearson \(\chi^2\) or Fisher probabilities in a 2 x 2 table; a value of \(p < 0.05\) was considered statistically significant.

Results

Comparison of surgical complications

The total incidence of surgical complications was 5.5% (69/1256). Rates of surgical complications for LEEP, CKC, vaginal enlarged amputation of cervix and extravascial hysterectomy were, respectively, 8.1% (6/74) 6.2% (54/869) 6.1% (3/49) and 2.3% (8/264), but this difference was not statistically significant (\(\chi^2 = 7.155\), \(p = 0.067\)). The surgical complications of all treatments are summarized in Table 2.

Comparison of treatment efficacy

The follow-up time for surviving patients ranged from 12 to 78 months, mean 29 months. Among these patients four had persistent lesions, two had recurrences and the overall rate of treatment failure was 0.5%. The treatment failure incidences for LEEP, CKC, vaginal enlarged amputation of cervix and extravascial hysterectomy were 4.1% (3/74), 0.2% (2/869), 0.0% (0/49) and 0.4% (1/264). When comparing between the groups, the treatment failure incidence of LEEP was higher than that of CKC (\(\chi^2 = 6.676\), \(p = 0.034\)) and extravascial hysterectomy (\(\chi^2 = 6.676\), \(p = 0.034\)), and there was no statistically significant difference between CKC and extravascial hysterectomy. Also no statistically significant difference was revealed between vaginal enlarged amputation of the cervix and any of the other three groups. Cases with persistent lesions or recurrence after surgery are summarized in Table 3.

Table 1. — Clinical and pathological data of four groups.

<table>
<thead>
<tr>
<th>Patient data</th>
<th>LEEP</th>
<th>CKC</th>
<th>Vaginal enlarged amputation</th>
<th>External fascia hysterectomy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=74)</td>
<td>(n=869)</td>
<td>(n=49)</td>
<td>(n=264)</td>
<td>(n=1256)</td>
</tr>
<tr>
<td>Age</td>
<td>(33.7 \pm 6.3)</td>
<td>(35.5 \pm 6.6)</td>
<td>(34.8 \pm 5.9)</td>
<td>(42.4 \pm 7.5)</td>
<td>(36.8 \pm 7.4)</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Parity</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Cervical size</td>
<td>(\leq 3)</td>
<td>(3.1-3.5)</td>
<td>(3.6-4.0)</td>
<td>(&gt;4.0)</td>
<td>(5.6)</td>
</tr>
<tr>
<td>Lesion size</td>
<td>Smooth</td>
<td>0 (0)</td>
<td>6 (0.6)</td>
<td>0 (0)</td>
<td>3 (1.1)</td>
</tr>
<tr>
<td>Vaginal extension</td>
<td>yes</td>
<td>3 (4.1)</td>
<td>13 (16.0)</td>
<td>12 (24.5)</td>
<td>65 (24.6)</td>
</tr>
<tr>
<td>Glandular extension</td>
<td>yes</td>
<td>3 (4.1)</td>
<td>145 (16.7)</td>
<td>15 (30.6)</td>
<td>138 (52.3)</td>
</tr>
</tbody>
</table>

Continuous data are shown as average ± standard deviation; enumeration data are shown in the form of \(n (%)\).

Table 2. — Surgical complications of all treatments.

<table>
<thead>
<tr>
<th>Complications</th>
<th>LEEP (n=74)</th>
<th>CKC (n=869)</th>
<th>Vaginal enlarged amputation (n=49)</th>
<th>External fascia hysterectomy (n=264)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraoperative bleeding</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Intraoperative bleeding</td>
<td>5</td>
<td>27</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Abnormal menstruation</td>
<td>1</td>
<td>24</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal incision infection</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal incision</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Abdominal incision

Consistent pain

Postoperative ankylenteron

Total

6 (8.1%) 54 (6.2%) 3 (6.1%) 6 (2.3%)
Comparision of the efficacy and complications of different surgical methods for cervical intraepithelial neoplasia

Table 3. — Six cases with persistent lesions or recurrence after surgery.

<table>
<thead>
<tr>
<th>Case</th>
<th>Surgical method</th>
<th>Age</th>
<th>Preoperational Lesion size</th>
<th>Preoperational vagina extension</th>
<th>Postoperative surgical margin</th>
<th>Time to persistent lesion or recurrence</th>
<th>Diagnosis of persistent lesion or recurrence</th>
<th>Retreatment method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LEEP</td>
<td>42</td>
<td>&lt; 1/3</td>
<td>Yes</td>
<td>Negative</td>
<td>8</td>
<td>CIN III</td>
<td>Hysterectomy</td>
</tr>
<tr>
<td>2</td>
<td>LEEP</td>
<td>37</td>
<td>&gt; 2/3</td>
<td>No</td>
<td>Negative</td>
<td>9</td>
<td>CIN II</td>
<td>CKC</td>
</tr>
<tr>
<td>3</td>
<td>LEEP</td>
<td>49</td>
<td>&lt; 1/3</td>
<td>Yes</td>
<td>Not clear</td>
<td>6</td>
<td>CIN III</td>
<td>Hysterectomy</td>
</tr>
<tr>
<td>4</td>
<td>CKC</td>
<td>40</td>
<td>&gt; 2/3</td>
<td>No</td>
<td>Negative</td>
<td>52</td>
<td>CIN II</td>
<td>Hysterectomy</td>
</tr>
<tr>
<td>5</td>
<td>CKC</td>
<td>31</td>
<td>&lt; 1/3</td>
<td>No</td>
<td>Negative</td>
<td>8</td>
<td>CIN I</td>
<td>LEEP</td>
</tr>
<tr>
<td>6</td>
<td>Extrafascial hysterecmy</td>
<td>42</td>
<td>&lt; 1/3</td>
<td>No</td>
<td>Negative</td>
<td>17</td>
<td>Invasive carcinoma</td>
<td>Radiotherapy</td>
</tr>
</tbody>
</table>

Discussion

With the increasing incidence of CIN and younger age of onset, the surgical resection extent and operative technique of CIN has come into focus. LEEP has been extensively applied in treating CIN because it will not destroy reproductive function; however, it is still controversial as to whether LEEP will become the alternative method of CKC in treating CIN [6].

It is reported that there is no obvious difference between the surgical efficacy of LEEP and CKC, but more than 50% of postoperative specimens of LEEP will be cauterized unduly. As a result, a definite pathological diagnosis can be determined in approximately 7%-8.3% of specimens [4]. Meanwhile the postoperative specimen of LEEP is smaller than that of CKC, and the positive surgical margin rate is increased correspondingly; especially in treating cervical carcinoma in situ the recurrence rate for LEEP is 29%, while only 6% for CKC [7]. In our study 69.2% patients with CIN opted for CKC, and 5.9% patients with CIN received LEEP. There was an apparent difference in surgical complications between them, but the surgical failure rate of LEEP was higher than that of CKC. It was reported by Kietpeerakool et al. [8] that more patients with CIN have become younger, and some studies have shown that to some extent use CKC, and patients without endocervical canal extension who want to preserve reproductive function can choose LEEP.

Vaginal enlarged amputation of cervix was put forward by Maltez [9]. Its recurrence rate is relatively low as part of the vaginal wall attached to the cervix will be excised in this operation. In the literature Souen et al. [10], reported the recurrence rate of vaginal enlarged amputation of cervix to be the lowest among the different methods in treating 334 patients with cervical carcinoma in situ. Since 2002 we have recommended vaginal enlarged amputation of cervix to patients with squamous cervical carcinoma Staging IA1, CIN patients with large lesion size (when lesion size observed with the help of colposcopy is more than 3/4 of the cervix), patients with CIN and VAIN, recurring CIN II - patients, patients with residual lesions and positive surgical margins, and patients who want to preserve uterine and reproductive function.

The surgical efficacy was 100% during a recent follow-up and no surgical complication was observed [11]. The median follow-up in our study was 29 months. The results showed that the surgical complication rate was 6.1%, and that no patient had residual lesion or suffered recurrence. There was no statistically significant difference of surgical failure when compared to others; however, four patients in the group of vaginal enlarged amputation of cervix were those who were found to have CIN after LEEP (three of them were carcinoma in situ) and required additional surgery. The ratio of cases with a lesion size larger than two-thirds of the cervix was higher than that of other groups. Therefore, we advocate that patients with extensive lesion size, suffering recurrence after LEEP or CKC, or with residual lesions can choose vaginal enlarged amputation of cervix when reproductive function is desired. There was no statistically significant difference regarding surgical failure of vaginal enlarged amputation of cervix when compared to that of others. The small sample capacity of this group may account in part for that, and studies with a larger sample capacity and a longer follow-up time still need to be carried out.

From 2002 to 2007, the cases of extrfascial hysterectomy accounted for 21% of all cases, and the surgical complications were similar to LEEP, CKC and vaginal enlarged amputation of cervix. The surgical failure rate was lower than that of LEEP, but there was no statistically significant difference when compared to CKC and vaginal enlarged amputation of cervix. Recently patients with CIN have become younger, the number of patients with a desire to save uterine and or reproductive function has increased, and some studies have shown that to some degree extrafascial hysterectomy is an over-treatment for CIN III. Therefore extrafascial hysterectomy is not the preferred therapy for CIN. However, when patients with CIN also have ovarian cysts, uterine myomas, uterine prolapse, or when patients with an atrophic cervix after menopause can not choose CKC and the patient also has a positive surgical margin after CKC, extrafascial hysterectomy can be chosen.

To sum up, LEEP, CKC, extrafascial hysterectomy, vaginal enlarged amputation of cervix can all be a safe and available treatment for CIN if surgical indications are strictly controlled. For patients who desire fertility, LEEP
can be used if the lesion is smaller than one-third of the cervix and there is no cervical canal extension, while when the lesion is larger than that CKC is the preferred treatment. Patients with extensive lesion size, with recurrence after LEEP or CKC, or with residual lesion can choose vaginal enlarged amputation of cervix when reproductive function is still desired. Extrafascial hysterectomy is a choice for patients without the desire to save reproductive function, when CKC is not suitable for the patients with an atrophic cervix after menopause and/or when patients also have a gynecologic benign cyst.

Reference


“Low-grade positivity” of HPV viral load after atypical squamous cells of undetermined significance (ASC-US) cytology identifies women at low-risk for cervical intraepithelial neoplasia grade 2 and 3

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2Preventive Gynecology Unit, European Institute of Oncology (IEO), Milano (Italy)

Summary
The correlation between high-risk HPV-DNA viral load, expressed as relative light units (RLU) values obtained from the Hybrid Capture 2 (HC2) test, and the prevalence of CIN2/CIN3 was investigated and statistically analyzed in 614 ASC-US consecutive cases. Cases were categorized into three groups according to RLU values: “low-grade positivity”, “intermediate positivity” and “high-grade positivity”, and the prevalence of CIN2/CIN3 was evaluated in the single groups and compared among them. CIN/CIN3 rates demonstrated a significant (p < 0.001) increase with a direct correlation with increasing RLU values: 4.6% (RLU from 1.0 to 10.0), 9.1% (RLU from 11.0 to 100.0) and 32.2% (RLU > 100.0) respectively. The prevalence of CIN2/CIN3 between the group with RLU < 10.0 (4.6%) and the group with RLU > 10 (24.2%) showed statistical significance (p = 0.0002). Increasing hrHPV viral load significantly correlates with increasing prevalence of CIN2/CIN3 in ASC-US cases.

Key words: ASC-US; HPV; HPV-DNA; viral load; HC2; Cervical Intraepithelial Neoplasia; CIN.

Introduction
Uterine cervical cancer is the second most common malignancy among women worldwide [1], and its incidence has demonstrated a dramatic decrease in response to widespread cytology-based (Pap test) screening programs [2]. Since the introduction of the 2001 Bethesda system (TBS) for cervical cytology, the category of atypical squamous cells of undetermined significance (ASC-US) represents the commonest abnormal cytological result in many countries [3-5], accounting for almost two million cases per year in the United States. According to the well documented cause-effect role of high-risk human papillomaviruses (hrHPV) in cervical oncogenesis [6], hrHPV-DNA detection has been demonstrated to significantly improve the low sensitivity of conventional cytology and has been clearly identified as the highest performance option for the triage of ASC-US cases [7]. In this setting, the sensitivity of hrHPV-DNA testing for the detection of high-grade cervical intraepithelial neoplasia (CIN2/CIN3) is reported as 83%-100%; this high sensitivity is however often correlated with low specificity (63%) and low positive predictive value (PPV) [8], determining too high referral rates to second-level colposcopy and biopsy compared to the low prevalence of true lesions. In fact, the rate of CIN2/CIN3 in ASC-US cases is reported as high as 15-17% [9]. For this reason, many attempts have been made to improve the tests specificity and optimize the performance of the ASC-US triage with the viral tests, the goal being the identification of the subgroup of patients with an underlying histology-proven CIN2/CIN3 lesion; in this field, hrHPV viral load quantification represents an interesting issue and has been diffusely investigated in different settings [10-12], the consistent results of these studies being the significant direct correlation between increasing hrHPV viral load and increasing risk of worsening cervical lesions. The detection of the viral load in biological samples is achievable by the use of different biomolecular laboratory techniques (real-time PCR, bDNA, NASBA) with most of them being particularly sophisticated, expensive, and high-expertise correlated; due to this, these approaches have very little or no use in clinical practice. Hybrid Capture 2 (HC2) is one of the most widely adopted hrHPV-DNA tests worldwide. Together with the positivity/negativity result in a qualitative fashion, the test also allows an indirect quantification of the hrHPV-DNA copies detected in the collected cervical sample. Assuming that qualitative hrHPV-DNA testing is validated as the most effective triage option of ASC-US cytology, in this study, we tested the hypothesis that different levels of hrHPV-DNA viral load in these cases might correlate with different risks of an underlying CIN2/CIN3. In particular we investigated if low levels of hrHPV-DNA viral load might reduce the risk of high-grade CIN.
Materials and Methods

The study population is represented by 614 consecutive and unselected patients from the greater Milan area, screened at San Raffaele Scientific Institute and European Institute of Oncology (IEO) in a 2-year period, who were cytologically diagnosed with ASC-US. All cases underwent high-risk HPV-DNA (hrHPV) testing using the commercially available HC2 assay (Qiagen Inc. Corporation, Germany) following the manufacturer’s instructions for sample collection and result interpretation.

Testing for the DNA of oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 was performed on 4 ml aliquots of the PreservCyt samples. The test involves denaturation, hybridization of HPV target DNA with a cocktail of full-length HPV type specific RNAs followed by capture of DNA/RNA hybrids on a solid phase and amplification of the signal by the binding of hybrids to multiple conjugated antibodies that specifically recognize DNA/RNA hybrids. The reaction is chemoluminescent and is detected by a luminometer, which provides relative quantification of each individual sample compared with the mean of a series of positive controls containing 1.0 pg/ml HPV DNA; the latter is identified as the cut-off of positivity (CO). Results are quantified as the ratio between relative light units (RLU) of the tested sample and the positivity cut-off, (RLU/CO). Accordingly, a RLU/CO value equal or greater than 1.0, corresponding to 5000 or more HPV-DNA copies per test is considered positive.

RLU/CO values from each sample were collected and recorded and, for the purpose of the study, categorized in three groups of increasing values: samples with ratios ranging from 1.0 to 10.0 were defined as “low-grade positivity”, cases with ratios from 11.0 to 100.0 RLU/CO were defined “intermediate positivity”; and cases with RLU/CO > 100.0 as “high-grade positivity”.

According to guidelines for the triage of ASC-US cytology, hrHPV-DNA positive cases (RLU/CO > 1.0) were referred to second-level colposcopy and, if indicated by aceto-whitening areas or unsatisfactory colposcopy, a biopsy/endoendocervical curetage was performed and histologically examined. In the event of a negative and satisfactory colposcopy, a repeat Pap test was immediately obtained and considered diagnostic.

According to pathological diagnosis, cases were classified as negative, low-grade squamous intraepithelial lesions (HPV/CIN 1) or high-grade squamous intraepithelial lesions (CIN2/CIN3).

Correlation between final diagnosis and RLU/CO groups was summarized in Table 1.

Results

Of the 614 ASC-US cases that underwent HPV-DNA testing, 338 (55%) had a negative result (hr-RLU/CO < 1). The remaining 276 (45%) patients with a positive HC2 test were colposcopically triaged and represent the study group.

Mean and median age of the studied cases were 38.3 and 37.6 years, respectively. Time from HPV-DNA testing to outcome final diagnosis ranged from 0 to 12 months, with a mean of 3.4 months.

Among the 276 cases with a clinical follow-up, 92 (33.3%) had a negative final diagnosis, 134 (48.5%) were classified as low-grade lesions (HPV/CIN1) and 50 (18.1%) as high-grade lesions (CIN2/CIN3).

According to RLU/CO ratio results of HC2 and to the study purpose, 86 (31.1%) patients were classified as “low-grade positivity” (>1.0 ≤ 10.0), 66 (23.9%) as “intermediate positivity” (≥11.0 ≤ 100.0) and 124 (44.9%) as “high-grade positivity” (>100.0) of RLU/CO ratios.

Out of the 86 “low-grade positivity” cases, 38 (44.2%) were negative, 44 (51.2%) had CIN1 and four (4.6%) had CIN2/CIN3; in detail, these four cases showed an RLU ratio ranging from 4.9 to 6.9. The corresponding prevalence of CIN2/3 in the other two groups of RLU ratios was 9.1% and 32.2% for “intermediate” and “high-grade positivity”, respectively (chi-square: p < 0.001 – CI 95%); combining these latter two groups (RLU/CO from 11.0 to 100.0 and over 101.0), the cumulative risk of CIN2/3 was 24.2% (46/190), which was a significantly greater risk than that of women with 1.0 to 10.0 RLU/CO ratios (4.6% - 4/86) (chi-square and Fisher exact test: p = 0.0002 – CI 95%).

For patients with a negative follow-up outcome (92 cases) or affected by CIN1 (134 cases), no statistically significant differences were observed among the three groups of RLU/CO (chi square: p = 0.29 - CI 95%) and between women with “low-grade positivity” (RLU/CO from 1.0 to 10.0) and women with > 10.0 RLU values (chi square: p = 0.73 - CI 95%). Results are graphically summarized in Table 1.

Table 1. — RLU/CO groups and clinical outcome.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. of cases</th>
<th>RLU/CO groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-10</td>
</tr>
<tr>
<td>Negative</td>
<td>92</td>
<td>38 (44.2%)</td>
</tr>
<tr>
<td>CIN1</td>
<td>134</td>
<td>44 (33.2%)</td>
</tr>
<tr>
<td>CIN2/CIN3</td>
<td>50</td>
<td>4 (4.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>262</td>
<td>86</td>
</tr>
</tbody>
</table>

Discussion

The overall prevalence of histologically confirmed CIN2/CIN3 lesions in our study group of hrHPV-DNA positive ASC-US women (50 cases - 18.1%) is only apparently beyond the expected rates of a screening setting that, accordingly with reported data from large series, should range from 10-17% [3, 9]. In our opinion this can be explained by the age characteristics of our study population; in fact, mean and median age were 38.3 and 37.6 years, respectively. This age group can be easily recognized as a high-risk group for viral persistence.
and/or precancerous progression [13], with a significant increase of CIN2/CIN3 detection rate compared to younger age groups [10, 13]. Moreover, in young patients a significantly higher percentage of spontaneous viral clearance and intraepithelial neoplasia (CIN) regression to normality is well documented [14]. Accordingly, our study group appears to be particularly suitable to be investigated from the virological side.

HPV viral load detected by the use of HC2 has already been reported by several studies as directly correlated with CIN2/CIN3 or cervical cancer frequency [10, 12, 15, 16]. However, the ASC-US cytological category has been poorly investigated since the validation of hrHPV-DNA testing is the optimal triage option in these cases compared to repeat cytology and immediate colposcopy [7]. In fact, despite the high sensitivity of the viral tests in identifying the real negative cases, the specificity remains poorly satisfactory and leads to high referral rates to second-level colposcopy and histology [3, 9, 17]. The first noteworthy result of our study is consistent with our tested hypothesis: increasing HPV viral load expressed as RLU/CO ratios in ASC-US cases is significantly correlated with increased prevalence of CIN3 lesions (p < 0.001). In our opinion, this is particularly relevant because it can be seen both in terms of stratifying the risk of CIN2/CIN3 and as a viable option to improve hrHPV-DNA testing specificity. The issue of improving the test specificity has already been considered by previous papers, suggesting and testing higher RLU/CO cut-off points of positivity: the majority of these experiences concluded that raising the cut-off of positivity (e.g., to 2.0 RLU/CO) determined a loss of sensitivity [18, 19]. One of the major positive characteristic of HC2 is reproducibility, also for low levels of positivity. However, several studies suggested that RLU/CO levels around the cut-off of positivity may be considered false-positives due to cross-contamination of samples or chemiluminescent signals in adjacent wells [20, 21]. For this reason the manufacturer’s recommendations include retesting samples with borderline results (RLU/CO between 1.0 and 2.5) and reporting as definitely positive a sample with a retest of 1.0 or above. In case of a retest with less than 1.0 RLU/CO a second retest should be performed, with the results of the third test being the final result.

In the current study we demonstrated that ASC-US cases with RLU/CO ratios below 10.0 are associated with a significantly lower rate of histologically proven CIN2/CIN3 compared to RLU/CO ratios > 10.0 (4.6% vs 24.2% - p = 0.0002); we did not demonstrate any difference in CIN1 prevalence according to RLU/CO ratios. Our results are consistent with those of Jarboe et al. [22], who recently reported 3.2% and 17.3% (p = 0.047) in the same groups respectively, and no difference in CIN1 prevalence; this is, to our knowledge, the one and only paper that approached ASC-US cases in this fashion. The authors concluded that in weakly hrHPV-DNA positive ASC-US cases a modification of the management algorithm may be justified. In particular, referral to colposcopy could be hypothesized for cases with RLU/CO above 11.0. This appears to be a very interesting proposal, and our results reinforce the validity of this option. Moreover, differently from the cited paper in which no information about patient age was available, our results came from a high-risk subgroup of patients from the age-related standpoint (mean age 38.3 yrs), and thus seem to be bias-free. We do agree that a modification of the standard algorithm for the triage of ASC-US [7] based on quantitative results from the HC2 assay would need to be carefully considered [22], and that other options such as HPV genotyping [23] or the use of new biomarkers (p16^INK4A/Ki-67) [24] deserve further investigation in the same field of application. Nonetheless we feel that the biomolecular viral aspects of cervical cancer precursors in terms of early detection are particularly noteworthy and promising.

Conclusion

The detection of high-risk human papillomavirus viral load expressed as RLU results of the HC2 test correlates with the prevalence of histologically confirmed CIN2/CIN3 lesions after ASC-US cytology. These results may allow modifications of the triage algorithm for these cases.

References


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Correlation of cancer risk evaluation and early detection (CADET) scores with abnormal ultrasonographic ovarian findings

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Departments of ¹Obstetrics and Gynecology and ²Internal Medicine “H”, Tel-Aviv Sourasky Medical Center
Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv (Israel)

Introduction

Ovarian cancer is the most lethal form of malignancy of the female reproductive tract and the fifth cause of cancer-related deaths among women, following the breast, lung, colon and pancreas [1]. The incidence of the disease is around 17:100,000 (corrected by age), and 23,000 women in the USA are newly diagnosed with the disease and 14,000 die each year. One of the main reasons for the high death ratio in ovarian cancer is the difficulty in diagnosing it at an early stage [2]. Failure to diagnose ovarian cancer in early stages is currently attributed to the fact that there are no efficient screening tests for early detection of the disease and to the lack of significant clinical symptoms at early stages, which are characteristically non-specific [3]. The rate of complete cure of early diagnosed ovarian cancer is about 90%, but most women are not diagnosed until the disease reaches advanced stages (Stage III or IV) where the estimated cure rate drops to 20%.

Routine transvaginal ultrasound examination, a widely used, noninvasive and inexpensive examination, has been studied extensively as a screening test for the detection of ovarian cancer. False-positive results are common and particularly problematic in pre-menopausal women since ovarian morphology depends on the menstrual cycle phase. Most women with positive screening by pelvic transvaginal ultrasonography will turn out to be disease free of the disease, and the positive predictive value (PPV) of a screening test among women in an average risk group is 2%. In other words, 98% of women with positive findings will actually be disease free, thus discouraging its application as a screening test in the general population [4].

The serum marker, CA-125, commonly used to follow-up an already diagnosed patient, has also been proven to be inefficient as a screening tool due to its high percentage of both false positive and false negative rates [5]. Thus, in 1994 the National Institute of Health (NIH) declared that there is no role for screening tests for early detection of ovarian cancer in the general population [6], a position subsequently supported by other organizations [7-9].

On the one hand, reports on early detection of the disease based on clinical signs and symptoms failed to identify a reliable pattern of clinical presentation [10, 11]. On the other hand, a large proportion of ovarian cancers are known to already be symptomatic even at an early stage [7, 12-16]. The problem lies in discovering a way to unify potential symptoms and suggestive clinical findings into a tool for enhancing the yield of a screening process.

The CADET (cancer risk evaluation and early detect-
tion) software was developed to assist physicians to assess the risk for cancer in a specific patient, based on a detailed self-reported questionnaire. This study aimed to assess the correlation between positive CADET scores and abnormal ultrasonographic ovarian findings in order to establish the utility of the CADET score as a screening tool for ovarian cancer to be used by general practitioners in the community. We hypothesized that if such a correlation did exist, the CADET questionnaire might become a screening tool to be used by general practitioners who do not have routine access to ultrasound (US) examination of their patients. Thus, the CADET score may identify patients who require a more specific investigation for the presence of the disease.

Materials and Methods

Approval for this study was obtained from the “Maccabi” Health Care Services ethics committee. The study population included all peri- and postmenopausal women who saw their community gynecologist for a routine check-up between January 2008 and June 2008, women who filled in the CADET questionnaire pertinent to ovarian cancer before being examined by their gynecologists and who were subsequently referred to routine transvaginal pelvic sonography, were eligible for study entry after signing an informed consent form. Excluded were women who were not examined routinely, not peri- or post-menopausal or did not fill in the questionnaire. All the relevant clinical information on each woman was provided to us by the computerized data base of the health service. The data retrieved from the questionnaire were processed by the CADET software, and a specific score was assigned to each woman based on her responses to the items included in the questionnaire. The treating gynecologists were unaware of the patients’ CADET scores when they later interviewed and examined them.

The CADET software is based on an algorithm which integrates data on signs, symptoms and risk factors from medical, surgical and oncological textbooks, national cancer organizations and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletins, MEDLINE publications and epidemiological reports. These data apply to the different stages and NIH statistical bulletin. The diagnostic transvaginal pelvic sonographic scans were reviewed by US specialists who are highly qualified in interpreting ovarian pathology. We compared their findings with the CADET scores in order to assess the correlation between positive CADET scores and abnormal ovarian findings on sonography.

Statistical analysis

The Fisher’s Exact test was used for assessing proportions and the Student’s t test was applied for continuous variables. A two-sided p value < 0.05 was considered as being significant.

Results

A total of 181 peri- or postmenopausal women who went to their gynecologists for routine check-ups were eligible for study recruitment. The final study group consisted of 154 women who were referred for routine US evaluation and whose scan results and CADET scores were available. All the relevant data including patients’ age, age at menopause, duration of menopause, US scan results and CADET scores were reviewed.

Thirty-eight of the 154 women (24%) had abnormal ovarian findings on their US examinations (30 simple cysts and 8 complex adnexal findings, Group A), while the other 116 women (76%) had normal US findings (Group B). Demographic characteristics were similar in both groups (Table 1). Thirteen of the 38 Group A women (34%) and 52 of the 116 Group B women (45%) had positive CADET scores (p = NS). The difference in the average CADET score of each group also did not reach a level of significance (0.8 ± 1.7 for Group A and 1.7 ± 2.5 for group B, p = NS). The scores of three of the 13 women in Group A (23%) and 25 of the 52 women in Group B (48%) were in the higher end of the CADET score (≥ 2.0). This difference was not significant (Table 2). Although the CADET score was higher in the sub-group of women with complex adnexal findings (1.95 ± 3.2), it did not reach statistical significance when compared to the score of women with either simple cysts or normal US adnexal findings.

Discussion

The rate of complete cure of early diagnosed ovarian cancer is about 90%, and thus the lack of efficient screening tools for early diagnosis of ovarian cancer is inarguably the major reason for ovarian cancer being the leading cause of death from all cancers of the female reproductive tract. To date, studies which focused on strategies to diagnose ovarian cancer at an early stage failed to identify a specific and reliable symptom pattern. The early clinical signs and symptoms of ovarian cancer are loss of weight, bloating, and abdominal discomfort which are non-specific and it is still unclear if and how it would be possible to incorporate them into a screening tool for expediting the detection of ovarian cancer.

Table 1. — Demographic characters of women with normal and abnormal ultrasonographic findings.

<table>
<thead>
<tr>
<th></th>
<th>Group A = abnormal (n = 38)</th>
<th>Group B = normal (n = 116)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.8 ± 7.0</td>
<td>59.3 ± 6.4</td>
<td>NS</td>
</tr>
<tr>
<td>Age at menopause (years)</td>
<td>51.2 ± 4.5</td>
<td>50.3 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of menopause (years)</td>
<td>7.8 ± 7.6</td>
<td>8.7 ± 6.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD; NS, non-significant.

Table 2. — CADET scores of women with normal and abnormal ultrasonographic findings.

<table>
<thead>
<tr>
<th></th>
<th>Group A = abnormal (n = 38)</th>
<th>Group B = normal (n = 116)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive CADET score, n (%)</td>
<td>13 (34)</td>
<td>52 (45)</td>
<td>NS</td>
</tr>
<tr>
<td>CADET score ≥ 2.0, n (%)</td>
<td>3 (23)</td>
<td>25 (48)</td>
<td>NS</td>
</tr>
<tr>
<td>CADET score*</td>
<td>0.8 ± 1.7</td>
<td>1.7 ± 2.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Values are presented as mean ± SD; NS, non-significant.
Appendix A

The CADET questionnaire

Please answer all the questions even if your answer is NO.

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT Have you ever given birth?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTb Have you ever undergone infertility treatments?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F7c Have you ever used oral contraception pills?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you ever been diagnosed with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F18 Endometrial (uterine) cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F18b Ovarian cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F18c Breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F18d Colon/rectal cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F22 Multiple cysts in the ovaries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has anyone in your family (parent, sibling, aunt/uncle) ever been diagnosed with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F19b Ovarian cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F19c Breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F19d Colon/rectal cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If you were examined for the presence of the breast/ovarian cancer gene was it mutated?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F20b BRCA-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F20c BRCA-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you feel that you:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B8 Lost more than 5 kg during the last 3 months without intention to do so</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B8b Have an unusual lack of energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B8c Have an abnormal degree of constipation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B8d Have an unusual lack of appetite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B8e Have an abnormal degree of diarrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B8f Have an abnormal degree of nausea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B85 Have had an unusual feeling of fullness in the rectum or anus during the past year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If you have any abdominal pain or discomfort, which of the following applies:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J1 Pain that gradually increased over the past few months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J5 Pain in the lower abdomen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J9 Abdominal pain that wakes you up at night</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J11 Any other type of abdominal pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you feel:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J15 An unexplained feeling of fullness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J15b Bloating, fullness and/or an unusual pressure in the abdomen or pelvic region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J16 Feeling bloated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J17 That your meals are not digested well enough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J18 Pressure in the lower abdomen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J21 Over-activity of your bowel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did you recently notice any of the following:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K4 Urgency in urination (difficult or impossible to control the urge to urinate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K4b Pain during urination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K5 More frequent urination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If you have back pains, which applies to you:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1 Pain which is only during night time, or more severe pain at night</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L3 Back pain combined with abdominal pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L3b Unusual lower back or abdominal pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L4 Pain that is aggravated upon lying down and relieved upon sitting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L5 Recent onset of pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L6 Pain which has recently become more severe</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We assessed the correlation between the replies to a self-reported computer-analyzed questionnaire (CADET software) and US findings in peri- and postmenopausal women who underwent a routine gynecological check-up. We hypothesized that a high CADET score might correlate with abnormal ovarian findings on sonography, and thus identify women at higher risk for developing ovarian cancer. Our results did not bear out the existence of such a correlation. The same proportion of women with abnormal and those with normal ovarian findings on US scans had a positive CADET score. Both groups also had a similar mean CADET score. The differences in the proportion of women in both groups with the higher CADET scores were also not significant.

One explanation for the lack of correlation may lie in the items that were chosen for the patients’ questionnaire and the relative weight they were assigned in the CADET score analysis. It should be borne in mind that the CADET score is aimed at identifying women who are at higher risk of developing ovarian cancer and not to predict those who will have abnormal US findings. Women with abnormal ovarian findings on sonographic scans will not necessarily develop ovarian cancer, and women with normal US findings are not exempt from developing ovarian cancer. This is clearly supported by the fact that most of the abnormal ovarian findings in our current study were, in fact, simple cysts which were not suspicious for an existing or future ovarian malignancy. The group of women with a high CADET score (resulting from a higher reported incidence of early signs and symptoms) may be at higher risk of developing ovarian cancer, even though no abnormal ovarian findings were discovered on their scans. The lack of correlation we found between the CADET scores and abnormal sonographic ovarian findings discourages the use of this tool to replace sonographic scans in the general population. Transvaginal US remains, non sensitive and specific as it is, the only acceptable tool for discovering ovarian abnormalities which require further investigation.

Kim et al. used a symptom index as a screening tool to compare ovarian cancer patients and healthy controls [19]. This type of screening has the disadvantage of a recall bias since patients are more likely to recall the appearance of symptoms before the diagnosis of a disease, whereas healthy controls do not pay such attention to temporary inconveniences. Pavlic et al. also attempted to use a symptom index as a screening tool [20]. Their study has the disadvantage of enlisting only women who underwent transvaginal US rather than general patients attending the clinic. Another inherent bias in screening by means of a symptom index is the limited number of items in a questionnaire which is based on patient symptoms alone. We attempted to overcome this bias by extending the questionnaire items and by including the complete CADET algorithm incorporating personal and family history and cancer risk factors.

In conclusion, we found that the CADET software could not identify peri- and postmenopausal women who had abnormal US findings. However, a longer follow-up
period should be undertaken in order to disclose the accurate risk of developing ovarian cancer in women with a high CADET score when compared with women with low CADET scores.

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References

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Normal serum CA125 half-life and normal serum nadir CA125 level in patients with ovarian cancers

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3Department of Obstetrics and Gynecology, Nishisaitama-Chuo National Hospital, Tokorozawa, Saitama (Japan)

Summary

The normal serum CA125 half-life and distribution of the normal serum nadir CA125 value in patients with epithelial ovarian carcinoma (EOC) have not been determined yet. Among patients with EOC, 41 patients met the inclusion criteria of the present study: the patients that underwent complete cytoreductive surgery and six cycles of platinum-containing chemotherapy, and who had no recurrent disease more than five years. Serum CA125 half-life (T_{1/2}) during primary surgery and primary chemotherapy was calculated and serum nadir CA125 level was evaluated by logarithmic-transformed serum CA125. Median value of nadir CA125 was 7 U/ml (range 3-20 U/ml), and the mean ln (serum nadir CA125) was 1.96 ± 0.45. Mean T_{1/2} was 10.4 days in all patients, and T_{1/2} value was associated with the preoperative serum levels of CA125. Predicted slope of CA125 regression curve was also influenced by the preoperative CA125 value. The present study provides fundamental information with regard to normal half-life time and normal nadir of CA125 in EOC patients.

Key words: Ovarian cancer; CA125; Tumor marker; Half-life; Nadir.

Introduction

Since RECIST (response evaluation criteria in solid tumors) was published in 2000 [1], many clinical studies used these criteria in the assessment of treatment outcomes. In patients with epithelial ovarian carcinoma (EOC), however, many patients develop clinical features of advanced disease such as peritoneal carcinomatosis and advanced disease that are not suitable to be measured by these criteria. Serum level of CA125 is a reliable tumor marker for measuring response, as the marker was elevated in more than 80% of women diagnosed with EOC [2]. CA125 serum concentration is also adopted to evaluate the clinical situation such as response or relapse in EOC patients. In addition, serum CA125 regression during early chemotherapy which is mainly represented by serum half-life seems to be an important predictive and prognostic factor for advanced EOCs [3-10]. On the other hand, the normal CA125 half-life value which was defined as the half-life period observed in cases who achieved optimal cytoreduction and complete remission has not been determined, varying from 4.8 to 12.1 days [4, 5, 11, 12]. These unfixed values might be derived from a small number of the patients analyzed in previous reports (1 to 13 patients), or heterogeneity of the patients. Additionally, it has been reported that the serum nadir CA125 level within normal range (< 35 U/ml) could be a prognostic factor for advanced EOCs [13-16]. However the normal values of serum nadir CA125 and distribution in ovarian cancer patients still remain unresolved. This study was conducted to evaluate the normal serum CA125 half-life and distribution of the normal serum nadir CA125 values during primary surgery and first-line chemotherapy in patients with EOC who underwent complete cytoreductive surgery and achieved complete remission.

Patients and Methods

Between January 1998 and May 2005, 148 patients with EOC were treated with primary cytoreductive surgery (PCS) followed by platinum-based chemotherapy at the National Defense Medical College Hospital. Forty-one patients who met the inclusion criteria were enrolled in this investigation: (a) patients who received no prior chemotherapy before any surgical therapy; (b) patients who underwent macroscopically complete cytoreductive surgery with complete surgical staging including hysterectomy, bilateral salpingo-oophorectomy, peritoneal washing, partial omentectomy, pelvic lymphadenectomy, and paraaortic lymphadenectomy; (c) patients treated with six cycles of platinum-containing chemotherapy after PCS; (d) patients whose serum CA125 levels were more than 35 U/ml; (e) patients who had no recurrent disease more than five years after PCS.

In all cases, a platinum-based combination therapy such as cyclophosphamide and doxorubicin and cisplatin (CAP), etoposide and cisplatin (EP), irinotecan and cisplatin (CPT-P), paclitaxel and carboplatin (TC), and docetaxel and carboplatin (DC), was used for the first-line chemotherapy after PCS. The CAP regimen consisted of 500 mg/m² of cyclophosphamide, 50 mg/m² of doxorubicin, and 50 mg/m² of cisplatin on day 1. The EP regimen consisted of 50 mg/m² of etoposide during days 1-5 and 50 mg/m² of cisplatin on day 1. The CPT-P regimen consisted of 22.5 mg/m² of irinotecan during days 1-5 and 10 mg/m² of cisplatin during days 1-5. The TC regimen consisted of 180 mg/m² of paclitaxel and AUC = 5 of carboplatin on day 1. The DC regimen consisted of 70 mg/m² of docetaxel and
AUC = 5 of carboplatin on day 1. All regimens were given every three to four weeks. In the present analysis, all regimens were categorized into two groups: “platinum-based therapy” and “taxanes plus platinum”. Platinum-based therapy included CAP, EP, and CPT-P regimens, and taxanes plus platinum therapy included TC and DC regimens.

Patients were routinely monitored as follows: month 1 to month 6, physical examination and serum tumor marker estimation including serum CA125 level on the day or one day prior to each cycle of chemotherapy, computed tomography (CT) or magnetic resonance imaging (MRI) every three cycles of chemotherapy; month 7 to year 2, physical examination and serum tumor marker estimation including CA125 every one to two months, CT or MRI every six months; year 3 through year 5, physical examination and serum tumor marker estimation including CA125 every three to five months, CT or MRI annually; year 6 and over, physical examination and serum tumor marker estimation including CA125 annually, CT if indicated. Additional clinical assessments were performed as indicated clinically. For example, CT or MRI were usually recommended when serum CA125 was elevated more than 70 U/ml. The serum tumor marker including CA125 every one to two months, CT or MRI every six months; year 3 through year 5, physical examination and serum tumor marker estimation including CA125 every three to five months, CT or MRI annually; year 6 and over, physical examination and serum tumor marker estimation including CA125 annually, CT if indicated. Additional clinical assessments were performed as indicated clinically. For example, CT or MRI were usually recommended when serum CA125 was elevated more than 70 U/ml. The serum tumor marker including CA125 before PCS was obtained within four days before PCS.

Progression of the disease (PD) was defined as the appearance of a new lesion evaluated by a CT of the chest/abdomen or pelvic MRI. The serum levels of tumor markers including CA125 were not used for the definition of PD.

Serum CA125 half-life (T1/2) during PCS and primary chemotherapy was calculated according to Buller’s formula [12] and Gadducci’s formula [7] derived from van der Burg et al’s report. We set C1 as the serum level of CA125 before PCS, and C2 as the first CA125 level < 35 U/ml, and t1 and t2 as the corresponding time in days; s is the slope of the regression of serum CA125.

\[ s = \ln(C1/C2) / (t2 - t1) \]
\[ T1/2 = \ln2 / s \]

In addition, we evaluated the correlation between s and ln(C1). The serum nadir CA125 level was also evaluated by logarithmic-transformed serum CA125 to evaluate if these values showed a normal distribution which was observed in healthy women [17].

Statistical analyses were performed using StatMate III software (ATMS Co. Ltd., Tokyo, Japan). Values are shown as mean ± SEM when applicable. Comparisons were evaluated with the Fisher’s exact probability test or the chi-square test when appropriate. Parameters were evaluated with the two tailed unpaired Student’s t-test or compared by one-way analysis of variance (ANOVA). The correlation between s and ln(C1) was analyzed by Pearson’s correlation test. The prediction of s from ln(C1) was analyzed by a linear regression test. Values of p < 0.05 were considered significant.

Results

A total of 41 EOC patients were enrolled in the present study. The characteristics of patients are shown in Table 1. Median age was 53 years (range: 35-71), and median follow-up period of the cases from PCS was 58 months (range, 65-109 months). Median interval between the date of PCS and the first chemotherapy was 18 days (range: 12-28 days). Twenty-three patients underwent a second-look laparotomy, and pathological complete remission was confirmed.

The number of patients was 24 (59%) in Stage Ic, ten (24%) in Stage II, and seven (17%) in Stage III according to the International Federation of Gynecology and Obstetrics (FIGO) staging methods. Histological subtype was serous type in 12 (29%), endometrioid type in 12 (29%), clear-cell type in 11 (27%), and mucinous type in six cases (15%). Platinum-based therapy was used for 25 cases: 11 cases by CAP, five cases by EP, and nine patients by CPT-P. EP regimen was mainly used five patients with mucinous type, and CPT-P regimen was

<table>
<thead>
<tr>
<th>FIGO stage</th>
<th>Number of the patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ic</td>
<td>24 (59%)</td>
</tr>
<tr>
<td>II</td>
<td>10 (24%)</td>
</tr>
<tr>
<td>III</td>
<td>7 (17%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residual disease</th>
<th>Number of the patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No residual</td>
<td>32 (78%)</td>
</tr>
<tr>
<td>Microscopic residual</td>
<td>9 (22%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primary chemotherapy</th>
<th>Number of the patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platinum-based therapy</td>
<td>25 (61%)</td>
</tr>
<tr>
<td>Taxanes plus platinum</td>
<td>16 (39%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Follow-up period, months</th>
<th>Median 58 (range: 65-109)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>C1, Preoperative CA125 (U/ml)</th>
<th>Number of the patients</th>
<th>Mean T1/2 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35-100</td>
<td>11</td>
<td>14.2</td>
</tr>
<tr>
<td>100-200</td>
<td>7</td>
<td>12.3</td>
</tr>
<tr>
<td>200-1000</td>
<td>8</td>
<td>11.9</td>
</tr>
<tr>
<td>1000-5000</td>
<td>9</td>
<td>6.3</td>
</tr>
<tr>
<td>5000-</td>
<td>6</td>
<td>6.3</td>
</tr>
<tr>
<td>All patients</td>
<td>41</td>
<td>10.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CA125 (U/ml)</th>
<th>s</th>
<th>T1/2 (days)</th>
<th>CA125 percentage reduction/4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.047</td>
<td>14.8</td>
<td>73%</td>
</tr>
<tr>
<td>100</td>
<td>0.058</td>
<td>12.0</td>
<td>80%</td>
</tr>
<tr>
<td>200</td>
<td>0.069</td>
<td>10.0</td>
<td>86%</td>
</tr>
<tr>
<td>500</td>
<td>0.084</td>
<td>8.3</td>
<td>90%</td>
</tr>
<tr>
<td>1000</td>
<td>0.095</td>
<td>7.3</td>
<td>93%</td>
</tr>
<tr>
<td>2000</td>
<td>0.106</td>
<td>6.5</td>
<td>95%</td>
</tr>
<tr>
<td>5000</td>
<td>0.121</td>
<td>5.7</td>
<td>97%</td>
</tr>
</tbody>
</table>
The correlation between \( s \) and \( \ln(C_1) \) also showed significant relationship when \( C_1 > 100 \text{ U/ml} \) (\( r = 0.65, p < 0.001 \)). The predicted slope of CA125 regression curve \( s \), \( T_{1/2} \), and CA125 percentage reduction after four weeks according to preoperative CA125 value are shown in Table 3.

The slope of serum CA125 regression curve \( s \) and preoperative CA125 \( (C_1) \) according to clinicopathologic variables are shown in Table 4. There were no significant relationships between \( s \) and clinicopathologic variables of age, FIGO stage, histology, residual disease status, and chemotherapeutic regimen.

The mean value of the serum CA125 half-life was 10.4 days in all patients. Notably, \( T_{1/2} \) was associated with preoperative serum level of CA125, and the correlation between \( s \) and \( \ln(C_1) \) showed significant relationship. A summary of reported \( T_{1/2} \) and analyzed patient distribution was shown in Table 5. Canney et al. reported mean \( T_{1/2} \) was 12.1 days in a cohort of seven patients who had complete resection of early stage tumor [11]. Buller et al. showed mean \( T_{1/2} \) was 10.4 days among 13 patients with Stage II-IV tumors that had complete resection [12]. Yedema et al. reported a mean \( T_{1/2} \) of 10.7 days by van den Burg’s formula in nine patients who had complete resection of Stage I-II tumors and that of 9.8 days by Buller’s formula in five patients with Stage I-II tumors with complete resection [5]. However, these reports were not based on the serum CA125 values before PCS or chemotherapy. We would stress there have been very few reports on the correlation between serum CA125 regression and serum CA125 values before PCS. Peters-Engl et al. implied that the patients with low serum CA125 level before chemotherapy would have a low decline in CA125; however, the detailed results were not shown [18]. Reidinger et al. evaluated the serum CA125 half-life in only cases with serum CA125 more than 100 U/ml before chemotherapy, and they suggested low CA125 level \( (< 100 \text{ U/ml}) \) before chemotherapy was too low to enable half-life calculations [9]. Our study demonstrates a significant correlation between \( T_{1/2} \) and preoperative CA125 values when the serum CA125 > 35 U/ml. Buller et al. reported that serum CA125 regression fit the exponential model most, when \( C_1 \) was set as the level of CA125 before PCS, and \( C_2 \) as the first CA125 level < 35 U/ml [12]. Yedema et al. reported that the cytoreductive surgery itself might cause a transient CA125 rise, so we did not estimate the CA125 values within two weeks after PCS [19]. An investigation during the first-line paclitaxel/platinum chemotherapy showed that patients with the serum CA125 half-life \( \leq 14 \)
days and mono-exponential decay had better outcome than patients with that ≤ 14 days and bi-exponential decay [20]. It is assumed that patients with an initially low CA125 had mono-exponential decay. The serum CA125 values cannot have bi-exponential decay to be less than 35 U/ml when the serum CA125 is near to 35 U/ml. The correlation coefficients between the exponential model and serum CA125 regression were very high (r = 0.95-0.98) [9, 12]. Tsuda et al. reported that CA125 regression in a paclitaxel-containing regimen was slower than that in a non-paclitaxel regimen [21]. In their report, there was difference of the mean value in initial serum CA125 lev-

Figure 1. — The correlation between the slope of the regression of serum CA125 (s) and ln(preoperative CA125 value (C1)) in all the patients. The correlation between s and ln(C1) showed a significant relationship: r = 0.71, p < 0.001. In the simple linear regression analysis, it could be expressed as the following equation, s = 0.01617 x ln(C1) – 0.01647.

First, the median value of the serum nadir CA125 was 7 U/ml (range, 3-20 U/ml) in the present study. The distribution of ln(serum nadir CA125) was normal shaped (95% range, 2.9-17.3 U/ml), as was observed in the distribution of serum CA125 of healthy women [17]. Median serum CA125 value was 14.2 U/ml (95% range, 6.0-41.0 U/ml) in healthy postmenopausal Caucasian women, and 9.0 U/ml (range, 4.0-26.0 U/ml) in African women, and 9.0 U/ml (range, 4.0-26.0 U/ml) in Asian women [17]. The normal serum nadir CA125 values investigated in our study seem to be lower than the reported serum CA125 values of healthy postmenopausal women. This might be explained by the fact that serum CA125 values were influenced by hysterectomy and bilateral salpingo-oophorectomy. Alagoz et al. suggested that serum CA125 value of the patient who underwent hysterectomy and bilateral salpingo-oophorectomy (TH/BSO) was lower than that of the normal population [23]. According the report, serum CA125 values of two-thirds of patients treated with TH/BSO were less than 10 U/ml, and 95% of the cases had CA125 levels lower than 20 U/ml. Recently, Santillan et al. reported that the medi-

Second, the median value of the serum nadir CA125 was 7 U/ml (range, 3-20 U/ml) in the present study. The distribution of ln(serum nadir CA125) was normal shaped (95% range, 2.9-17.3 U/ml), as was observed in the distribution of serum CA125 of healthy women [17]. Median serum CA125 value was 14.2 U/ml (95% range, 6.0-41.0 U/ml) in healthy postmenopausal Caucasian women, and 9.0 U/ml (range, 4.0-26.0 U/ml) in African women, and 9.0 U/ml (range, 4.0-26.0 U/ml) in Asian women [17]. The normal serum nadir CA125 values investigated in our study seem to be lower than the reported serum CA125 values of healthy postmenopausal women. This might be explained by the fact that serum CA125 values were influenced by hysterectomy and bilateral salpingo-oophorectomy. Alagoz et al. suggested that serum CA125 value of the patient who underwent hysterectomy and bilateral salpingo-oophorectomy (TH/BSO) was lower than that of the normal population [23]. According the report, serum CA125 values of two-thirds of patients treated with TH/BSO were less than 10 U/ml, and 95% of the cases had CA125 levels lower than 20 U/ml. Recently, Santillan et al. reported that the medi-

Figure 2. — The distribution of serum nadir CA125 (a) and distribution of ln(serum nadir CA125) (b). The median value of the serum nadir CA125 was 7 U/ml (range, 3-20 U/ml). The distribution of the serum nadir CA125 was not normal shaped. The distribution of the ln(serum nadir CA125) was normal shaped. The mean value of ln(serum nadir CA125) was 1.96 ± 0.45, corresponding to a 95% reference range from 2.9 to 17.3 U/ml.
an CA125 nadir level at the time of complete clinical and radiographic response (CR) was 6 U/ml (range 3-16 U/ml) in the recurrence group, and 11 U/ml (range 4-17 U/ml) in the non-recurrence group [24]. Markman et al. revealed that patients with baseline CA125 values before initiation of maintenance chemotherapy ≤ 10 U/ml had a superior progression-free survival compared with the cases with higher levels of CA125 [15]. Clinically, the upper limit of the normal serum CA125 values after initial treatment in patients with ovarian cancer is usually defined as 35 U/ml. Our results are in agreement with those reports where the upper limit of the normal serum CA125 values was lower than 20 U/ml. It is possible that the upper limit of normal serum CA125 values is lowered excessively as we determine the recurrence risk after CR only with the serum nadir CA125 value. However, we believe that comprehensive evaluation of the distribution of non-recurrence patients would be most important, although our study had a limitation based on a relatively small number of patients. In addition, the distribution of logarithmic-transformed serum CA125 showed normal shaped more than absolute value of CA125. Further investigation including a large number of cases is necessary to evaluate the clinical usefulness of our results.

Conclusion

The present study provides novel and fundamental information on the normal serum CA125 half-life and distribution of the normal serum nadir CA125 values during first-line chemotherapy in patients with EOC who showed completed remission after primary therapy.

References


Expression of P-Akt, NFκB and their correlation with human papillomavirus infection in cervical carcinoma

C. X. Du, Y. Wang

Department of Gynecology, The First Affiliated Hospital of He’nan, University of Science and Technology, Luoyang (China)

Summary

Purpose: To investigate the expression of P-Akt and NFκB and their correlation with human papillomavirus (HPV) infection in cervical carcinoma. Material and Methods: Expression of P-Akt and NFκB was detected by an immunohistochemical SP technique with HPV DNA detection by PCR in 26 cases of cervical carcinoma tissues, 18 cases of cervical intraepithelial neoplasia tissues (CIN I/n = 5, CIN II/n = 3, CIN III/n = 10) and 19 cases of chronic cervicitis tissues. The different expressions of P-Akt and NFκB were compared in different pathological types of cervical carcinoma (cervical squamous cell carcinoma, cervical adenocarcinoma), different pathological grading (high, medium, poorly differentiated) and different clinical stage (FIGO I to IV). The relationships between P-Akt and NFκB, respectively, with HPV infection in cervical carcinoma were analyzed. Results: The positive expression rate of P-Akt in chronic cervicitis tissues, CIN and cervical carcinoma tissues was 21.05%, 66.67%, and 92.31%, respectively. There was no obvious difference in the expression of P-Akt in cervical carcinoma in different pathological types or in pathological grading and no obvious difference in different clinical stages. The positive expression rate of NFκB in chronic cervicitis tissues, CIN and cervical carcinoma tissues was 10.52%, 72.22% and 96.15%, respectively; there was no statistically significant difference among the groups for different pathological types and there was no obvious difference in different pathological grading or different clinical stage. There was an obviously positive correlation between P-Akt and NFκB expression rate and degree of disease (r = 0.998, p < 0.05). Cervical carcinoma and CIN cases totaled 44; the positive expression rate of P-Akt was 87.55% in 32 cases of positive HPV-DNA of the 44 cases, and the positive expression rate of P-Akt was only 16.70% in 12 cases of negative HPV-DNA of the 44 cases. The positive expression rate of NFκB was obviously higher in the HPV DNA positive than in the HPV-DNA negative cases. There was a statistically significant difference among the groups (p < 0.05). Conclusion: The positive expression rate of P-Akt and NFκB was closely related with cervical disease extent, and closely related with HPV infection in cervical carcinoma. This study suggests that P-Akt and NFκB more probably play an important role in the occurrence of cervical carcinoma.

Key words: Cervical carcinoma; P-Akt; NFκB; Human papilloma virus.

Introduction

Cervical carcinoma is the second most common malignant neoplasm among woman around the world [1]. At present, its morbidity and mortality are still high and there has been a younger trend in our country in the last 20 years [2]. Although human papillomavirus (HPV) infection is known to be a major risk factor for cervical carcinoma, its specific pathogenesis is still not very clear. The ubiquitously expressed serine/threonine kinase Akt and the transcription factor of the nuclear factor (NF)-κB family are both involved in cell proliferation and apoptosis. Akt is a serine/threonine protein kinase in the PI3K/Akt signal transduction pathway at the hub site. The phosphorylation of protein kinase B (P-Akt) is the activated form of Akt. The NFκB family controls expression of genes which promote cell growth, survival, and neoplastic transformation by a phosphatidylinositol 3 (PI3)-kinase to the Akt/protein kinase B (PKB) pathway and this signaling pathway has been shown to activate NFκB [3]. Furthermore, the activation of Akt and/or NFκB has been suggested to be associated with occurrence and development of human tumors. In this study the expression of P-Akt and NFκB was detected by the immunohistochemical SP technique with HPV DNA detection by PCR in cervical carcinoma tissues to explore its role in the development of cervical carcinoma, and the relationship to HPV infection.

Materials and Methods

Study

This study was conducted in the Department of Gynecology at the First Affiliated Hospital of He’nan University of Science and Technology. Patients who underwent surgery for cervical disease in the period from September 2009 through April 2010 were enrolled. The research project was approved by the Institution Research Ethics Committee and all patients signed an informed consent. The mean age of the patients was 38.73 years with a range from 28 to 64 years. Cervical biopsy pathological examination confirmed that highest level at the final pathological diagnosis of the patients; there were 26 cases of cervical carcinoma tissues, 18 cases of cervical intraepithelial neoplasia tissues (CIN I/n = 5, CIN II/n = 3, CIN III/n = 10) and 19 cases of chronic cervicitis tissues. Three months elapsed where none of the patients were treated by radiotherapy, chemotherapy or any other special therapy until this study was initiated.

HPV DNA detection

HPV DNA of all samples was detected by PCR. Cervical cells were collected as a sample from each patient. The HPV GenoArray Test Panel included: 1) high-risk: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68; 2) low-risk: HPV 6, 11, 42, 43, 44, CP830. The HPV GenoArray DNA testing...
method makes use of both DNA amplification and Hybribio’s proprietary flow-through hybridization technology (US patents 5,741,647 & 6,020,187) to genotype the samples with specific DNA probes. Extracted samples of DNA amplified the amount of DNA through PCR amplification. Based on the principle of double-stranded DNA complementary, the flow-through hybridization technology works by directing the flow of the targeted molecules towards the specific probe pre-fixed on low-density gene chips and hence enables rapid hybridization to occur and finally specific hybridization results. HPV testing: A total of 24 hybrid membrane points, including biotin and Ic for the normal control point. Other points appeared as blue-purple dots, compared with the corresponding HPV genotype - positive or negative.

**Immunohistochemical SP technique**

Representative specimens were fixed in 10% dehydrated formalin. All tumor specimens were fixed in formalin and embedded in paraffin. All the above-mentioned samples were obtained from each patient, and were classified according to the World Health Organization (WHO) classification (2003). Hematoxylin and eosin stained slides were reviewed to confirm histological diagnoses. Representative specimens were selected for immunohistochemistry-SP. Immunoperoxidase staining for P-Akt and NF-κB was performed in 4.0-µm-thick tissue sections from all specimens. The BioGenex Automatic Staining System (Santa Cruz, CA) was used. In brief, tissue sections were deparaffinized, rehydrated, and soaked in 0.6% hydrogen peroxide for 30 min in order to block endogenous peroxidase activity. Microwave antigen retrieval in citrate buffer with pH 6.0 (Santa Cruz, CA) for 25 min followed. Tissue sections were incubated with the polyclonal rabbit anti-P-Akt1/2/3 antibody (Santa Cruz, CA) for 25 min followed. Tissue sections were incubated with the polyclonal rabbit anti-P-Akt1/2/3 antibody (Santa Cruz, CA) at a dilution of 1:50 and the polyclonal rabbit anti-NF-κB/p50 antibody (Maixin, China) respectively, for 30 min. Incubation with a peroxidase-streptavidin conjugate for 20 min followed. Diaminobenzidine tetrahydrochloride was then used as a chromogen and sections were counterstained with hematoxylin, dehydrated and mounted. Tissue sections from testicle tissue with strong membranous and cytoplasmic staining for P-Akt and NF-κB were used as a positive control. For evaluation of immunohistochemical data a scoring system was used, as described previously [4]. In brief, staining intensity was evaluated for P-Akt and NF-κB. Immunoperoxidase staining for P-Akt and NF-κB was performed in 4.0-µm-thick tissue sections from all specimens. The BioGenex Automatic Staining System (Santa Cruz, CA) was used. In brief, tissue sections were deparaffinized, rehydrated, and soaked in 0.6% hydrogen peroxide for 30 min in order to block endogenous peroxidase activity. Microwave antigen retrieval in citrate buffer with pH 6.0 (Santa Cruz, CA) for 25 min followed. Tissue sections were incubated with the polyclonal rabbit anti-P-Akt1/2/3 antibody (Santa Cruz, CA) at a dilution of 1:50 and the polyclonal rabbit anti-NF-κB/p50 antibody (Maixin, China) respectively, for 30 min. Incubation with a peroxidase-streptavidin conjugate for 20 min followed. Diaminobenzidine tetrahydrochloride was then used as a chromogen and sections were counterstained with hematoxylin, dehydrated and mounted. Tissue sections from testicle tissue with strong membranous and cytoplasmic staining for P-Akt and NF-κB were used as a positive control. For evaluation of immunohistochemical data a scoring system was used, as described previously [4]. In brief, staining intensity was evaluated for P-Akt and NF-κB.

**Statistical analysis**

Statistical analysis of experimental data was carried out by SPSS17.0 software of which the level of significance was \( p < 0.05 \). The chi-square test, Fisher exact test and Pearson correlation test were used for statistical analysis.

**Results**

Table 1 and Figure 1 present the expression of P-Akt. The positive expression of P-Akt was mainly located in the cytoplasm, while a small part was located in the nucleus; it was cytoplasm-nucleus-type expression in cervical carcinoma cells. According to the staining intensity of expression, the color was brown to light yellow. The positive expression rate of P-Akt in chronic cervicitis tissues, CIN tissues and cervical carcinoma tissues was 21.05%, 66.67%, and 92.31%, respectively (\( p < 0.05 \)). There was no obvious difference among the expressions of P-Akt in cervical carcinoma in different pathological types and in pathological grading, and no obvious difference among different clinical stages.

Table 2 and Figure 2 present the expression of NF-κB. NF-κB proteins were located in the cervical nucleus and cytoplasm (brown nucleus or/brownish yellow cytoplasm). It was regarded as strong expression when the nucleus was brown or/brownish yellow. The positive expression rate of NF-κB in chronic cervicitis tissues, CIN and cervical carcinoma tissues was 10.52%, 72.22% and 94.74%, respectively. There was no obvious difference among the groups in different pathological types and different clinical stages.

With the increase of cervical histology and expression of NF-κB and P-Akt the color deepened. The results showed low expression in cervicitis, increased expression in CIN, and the strongest expression in cervical carcinoma. The Pearson correlation method used for statistical analysis showed that P-Akt and NF-κB expression in cervical lesions had a linear correlation (Pearson correlation index \( r = 0.998; p < 0.05 \)).
Table 3 shows the 44 cervical carcinoma and CIN cases. The positive expression rate of P-Akt was 87.55% in 32 cases of positive HPV-DNA of the 44 cases, and the positive expression rate of P-Akt was only 16.70% in 12 cases of negative HPV-DNA of the 44 cases ($\chi^2 = 16.78, p < 0.05$). The positive expression rate of NFκB was obviously higher in the HPV-DNA positive than in the HPV-DNA negative cases. There was a statistically significant difference among the groups ($\chi^2 = 9.65, p < 0.05$).

Discussion

The ubiquitously expressed serine-threonine kinase Akt and the transcription factor NFκB are both involved in cell proliferation and apoptosis. Furthermore, the activation of Akt or NF-kappaB has been suggested to be associated more with human tumors [5]. Akt is known to be involved in the PI3-kinase/AKT signaling pathway; activated Akt (P-Akt) modulates the function of numerous substrates involved in the regulation of cell survival, cell cycle progression and cellular growth. In recent years, it has been shown that the PI3K/Akt signalling pathway components are frequently altered in human carcinomas [6]. Amplification of chromosome arm 3q is the most consistent aberration in cervical carcinoma, and it is implicated in the progression of dysplastic uterine cervical cells into invasive carcinoma. The results of comparative genomic hybridization show that the 3q26.3 amplification was the most consistent chromosomal aberration in primary tissues of cervical carcinoma, and a positive correlation between an increased copy number of PIK3CA (detected by competitive PCR) and 3q26.3 amplification was found in tumor tissues and in cervical carcinoma cell lines [7-9]. In this study, the expression of P-Akt in CIN and cervical carcinoma rates were respectively, 66.67% (12/18) and 92.31% (24/26), significantly higher than that in the chronic cervicitis expression rate 21.05% (4/19). The experiments also suggest that the overexpression of P-Akt was significantly correlated with advanced stage disease, but did not reach statistical significance.

PI3K/Akt activity was analyzed by phosphatidylinositol trisphosphate production and phosphorylated Akt (p-Akt) expression, and also increased NFκB activity [10]. The larger NFκB family of proteins is composed of two...
Expression of P-Akt, NFκB and their correlation with human papillomavirus infection in cervical carcinoma

subfamilies: the NFκB (p50) proteins and the RelA (p65) proteins. It be able to regulate gene expression in a variety of protein molecules. Under normal circumstances, it exists in the cytoplasm in a non-active state. When there is an infectious virus or other factors, the NFκB protein is highly expressed in the tumor, with regulating gene transcription and expression, inhibits tumor cell apoptosis and promotes tumor development [11-13]. This experiment showed that with increased levels of cervical pathology, the extent of NFκB expression was enhanced, and was positively correlated with expression of P-Akt, indicating the incidence of cervical carcinoma development. NFκB and P-Akt may form a network for each other.

HPV infection is the most important factor, and an important initiation factor has been recognized in cervical carcinoma. HPV DNA was detected in more than 90% of patients with cervical carcinoma. The most common HPV types in cervical carcinoma were HPV type 16, 58 and 18. HPV infected patients have a higher risk of developing cervical carcinoma, which is 75.79 times more than non-infected people [14]. The E5 oncoprotein of HPV 16 plays an important role in early cervical carcinogenesis. Vascular endothelial growth factor (VEGF) plays a central role in switching on the angiogenic phenotype during early cervical carcinogenesis. E5-mediated epidermal growth factor receptor (EGFR) activation was accompanied by P-Akt and ERK1/2, which are also involved in VEGF expression. Furthermore, the mRNA stability of VEGF was not affected by which are also involved in VEGF expression. Further-activation was accompanied by P-Akt and ERK1/2, E5-mediated epidermal growth factor receptor (EGFR) activates NFκB in murine lymphoma cell lines. Kim et al’s results suggest that HPV 16 E5 increases VEGF expression by activating EGFR, MEK/ERK1/2 and PI3K/Akt [15]. The early gene product E7 from high-risk HPV is considered probably by activating signaling pathways PI3K/Akt/ NFκB and their correlation with human papillomavirus infection in cervical carcinoma.

better understanding of this result can help to fully exploit the potential benefits of cervical cancer diagnosis, treatment and prevention.

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Preoperative serum leptin levels in patients with endometrial cancer and its correlation with prognostic variables

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Summary

Purpose of investigation: Since leptin is believed to be a key player in carcinogenesis, a study has been designed to investigate the relationship between leptin levels and endometrial cancer. Methods: A study including 30 patients with endometrial cancer and 30 healthy controls was carried out between November 2008 and July 2009 in Hacettepe University Hospital. All patients with endometrial cancer underwent a complete surgical staging procedure including lymphadenectomy. Preoperative leptin levels of endometrial cancer patients and healthy controls were compared. The relationships between leptin levels and stage, grade, histological type and lymph node status of endometrial cancer cases were evaluated. Results: The mean serum leptin levels were 16.9 ng/ml among endometrial cancer cases and 19.0 ng/ml among controls (p = 0.32). Of endometrial cancer cases, the mean leptin level was found to be 15.8 ng/ml for Stage I and 18.5 ng/ml for Stage II-IV disease (p = 0.34). The figure was 17.7 ng/ml for endometrioid and 13.2 ng/ml for non-endometrioid type of tumor (p = 0.24). The mean leptin levels of 16.3 ng/ml for grade 1 and 19.9 ng/ml for grade 2-3 tumors were observed (p = 0.07). The cases with positive and negative lymph nodes had leptin levels of 20.2 ng/ml and 16.1 ng/ml, respectively (p = 0.30). Conclusions: Serum leptin levels in endometrial cancer patients were similar to healthy controls. Leptin did not show any significant correlation with stage, grade, histological type and node metastases in endometrial cancer.

Key words: Leptin; Endometrial cancer; Obesity.

Introduction

Obesity has long been known to be associated with an increased risk of endometrial cancer [1]. Various bioactive substances produced by adipose tissue such as estrogens, insulin and insulin-like growth factors are believed to be involved in the association of obesity and endometrial tumorigenesis [2]. Leptin, which is a prominent type of adipokine produced by adipose tissue, is positively correlated with obesity, food intake and energy balance. Leptin is also responsible for hyperinsulinemia by reducing tissue sensitivity to insulin [3]. There is increasing evidence that leptin has an impact on development of several obesity-related cancers [4, 5]. Furthermore, leptin appears to be involved in angiogenesis and regulation of cancer progression by stimulating tumor cell migration [6, 7]. Leptin could also promote endometrial thickness and promote proliferation of endometrial cells [8, 9]. Although leptin is believed to be a key player in carcinogenesis, its role in endometrial cancer is mostly unclear. Thus, a study has been designed to investigate the relationship between leptin levels and endometrial cancer.

Materials and Methods

A study including 30 patients with endometrial cancer and 30 healthy controls was carried out between November 2008 and July 2009 in Hacettepe University Hospital. All patients with endometrial cancer underwent a complete surgical staging procedure including lymphadenectomy. Preoperative leptin levels of endometrial cancer patients and healthy controls were compared. The relationships between leptin levels and stage, grade, histological type and lymph node status of endometrial cancer cases were evaluated.

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The research protocol was approved by the institutional ethics committee and written informed consent for the utilization of serum samples and personal information was obtained from all subjects. Consecutive patients with newly diagnosed and histologically confirmed endometrial cancer were enrolled in this study. All patients with endometrial cancer underwent a complete surgical staging procedure including abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and pelvic and paraaortic lymphadenectomy. Control subjects were simultaneously recruited from the menopause clinic of the same hospital during the study period.

Preoperative leptin levels of endometrial cancer patients and healthy controls were compared. The relationships between leptin levels and stage, grade, histological type and lymph node status of endometrial cancer cases were also evaluated. Leptin concentrations in serum were measured with enzyme-linked immunosorbent assay. Statistical analysis was carried out using SPSS 14. The categorical variables were analyzed using Pearson’s chi-square test. Student’s t-test was used to assess the significance of differences in continuous variables between the two groups. P values of less than 0.05 were considered to be statistically significant.

Results

The demographic characteristics of the endometrial cancer cases and controls are summarized in Table 1. There were no statistically significant differences between the mean values of the endometrial cancer cases and controls in terms of the gravidity, para, height, weight and body mass index (BMI). However, the endometrial cancer group had a statistically higher mean age than the control group (p = 0.01).
Preoperative serum leptin levels in patients with endometrial cancer and its correlation with prognostic variables

The mean serum leptin levels were 16.9 ng/ml among endometrial cancer cases and 19.0 ng/ml among controls (Table 2). There was no statistically significant difference between mean values of endometrial cancer cases and controls.

Of the 30 patients with endometrial cancer, 18 (60%) had Stage I disease (Table 3). Pathological examination revealed grade 1 tumor in 17 cases. While 25 cases had endometrioid type of tumor, nonendometrioid histologic subtype cases included serous papillary in three, clear cell in one and undifferentiated tumor in one case. The mean number of nodes removed during lymphadenectomy was found to be 33.4 ± 8.7. Of the ten cases with lymph node metastases, five had only pelvic lymph node metastases, three had only paraaortic lymph node metastases and in two patients both pelvic and paraaortic lymph node metastases were observed.

Of the endometrial cancer cases, the mean leptin level was found to be 15.8 ng/ml for Stage I and 18.5 ng/ml for Stage II-IV disease (Table 4). The figure was 17.7 ng/ml for endometrioid and 13.2 ng/ml for nonendometrioid type of tumor. Mean leptin levels of 16.3 ng/ml for grade 1 and 19.9 ng/ml for grade 2-3 tumors were observed. The cases with positive and negative lymph nodes had leptin levels of 20.2 ng/ml and 16.1 ng/ml, respectively. None of the parameters studied had a statistically significant correlation with serum leptin level.

Discussion

Endometrial cancer is a hormone-dependent neoplasm and obesity is a well known risk factor for it. Peripheral aromatization of androstenedione to estrone takes place largely in the adipose tissue and has been alleged to be responsible for development of endometrial cancer. However, the epidemiological association between obesity and endometrial cancer risk cannot be fully explained by obesity-related changes in serum levels of sex hormones. Leptin was found to be involved in neoplastic processes of hormone-dependent tumors such as breast cancer [4, 5]. Possible mechanisms resulting in the development of neoplasms are positive correlation with obesity, stimulation of angiogenesis, inducing production of cytokines, hyperinsulinemia and activation of aromatase [4, 6, 8].

Several studies investigated the correlation of leptin with endometrial cancer. Petrیدou et al. noted significantly higher leptin levels among 84 endometrial cancer patients when compared to controls [10]. Yuan et al. studied the expression leptin receptors in endometrial cancer cells and found higher serum concentrations of leptin in patients with endometrial cancer but the difference was not significant after normalization of body mass index [11]. Cymbaluk et al. found that serum concentrations of leptin in endometrial cancer and hyperplasia were higher than controls [12]. The difference was significant in all BMI groups. Ashizawa et al. suggested that the leptin-adiponectin ratio was independently associated with an increased risk for endometrial cancer development [13].

We have found similar serum leptin concentrations in endometrial cancer patients and controls. Thus, the present study results do not support the previous studies in terms of the role of increased leptin in endometrial carcinogenesis. It possibly results from the similar BMI values of cancer cases and controls in the current study. Higher leptin levels show a stronger correlation with obesity than endometrial cancer. Furthermore, leptin did not show any significant correlation with stage, grade, histological type and node metastases in endometrial cancer.

Table 1. — Demographic characteristics of cases.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>56.8 ±10.3</td>
<td>50.2 ± 8.2</td>
<td>0.012</td>
</tr>
<tr>
<td>Gravida</td>
<td>3.6 ± 1.8</td>
<td>3.9 ±1.3</td>
<td>0.636</td>
</tr>
<tr>
<td>Para</td>
<td>2.9 ± 1.5</td>
<td>3.1 ± 1.1</td>
<td>0.325</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155 ± 0.08</td>
<td>1.54 ± 0.04</td>
<td>0.542</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.2 ± 20.1</td>
<td>80.5 ± 20.4</td>
<td>0.933</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.7 ± 6.03</td>
<td>32.9 ± 5.8</td>
<td>0.625</td>
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Table 2. — Serum leptin levels.

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Mean</td>
<td>16.9 ± 7.5 ng/ml</td>
<td>19.0 ± 8.24 ng/ml</td>
</tr>
<tr>
<td>Median</td>
<td>15.5 ng/ml</td>
<td>18.4 ng/ml</td>
</tr>
<tr>
<td>Range</td>
<td>4.6-32.0 ng/ml</td>
<td>6.5-35.5 ng/ml</td>
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Table 3. — Pathological characteristics of endometrial cancer cases.

<table>
<thead>
<tr>
<th>Stage</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>I</td>
<td>18</td>
<td>60.0</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>20.0</td>
</tr>
<tr>
<td>III-IV</td>
<td>6</td>
<td>20.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>56.7</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>30.0</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>13.3</td>
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<table>
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<tr>
<th>Histology</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid</td>
<td>25</td>
<td>83.3</td>
</tr>
<tr>
<td>Nonendometrioid</td>
<td>5</td>
<td>16.7</td>
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<table>
<thead>
<tr>
<th>Lymph node status</th>
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<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>20</td>
<td>66.7</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>33.3</td>
</tr>
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</table>

Table 4. — Correlation of leptin levels with prognostic parameters in endometrial cancer cases.

<table>
<thead>
<tr>
<th>Endometrial cancer</th>
<th>Mean leptin level (ng/ml)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>15.8 ± 7.4</td>
<td></td>
</tr>
<tr>
<td>II-IV</td>
<td>18.5 ± 7.6</td>
<td>0.349</td>
</tr>
<tr>
<td>Cell type</td>
<td></td>
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<tr>
<td>Endometrioid</td>
<td>17.7 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>Non-endometrioid</td>
<td>13.2 ± 9.0</td>
<td>0.245</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16.3 ± 7.6</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>19.9 ± 5.1</td>
<td>0.075</td>
</tr>
<tr>
<td>Lymph node status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>16.1 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>20.2 ± 8.3</td>
<td>0.303</td>
</tr>
</tbody>
</table>
References


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Expression of tumor associated antigens CA 15-3 and CA 19-9 in trophoblast of the normal human placenta

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¹Institute for the Application of Nuclear Energy, INEP, University of Belgrade
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Summary

Mucin 1 (MUC1) is abundantly expressed by various organs, including human placenta and endometrium. Since glycan modifications of MUC1 are potentially relevant for physiological as well as pathological processes, this study was aimed at establishing an expression profile of two MUC1 glycopeptides, CA 15-3 and CA 19-9, in trophoblast throughout pregnancy. Immunohistochemical analysis of normal placenta demonstrated that trophoblast cells express both mucin antigens throughout gestation with a distinct staining pattern. The staining of villous trophoblast was non-uniform for both antigens, and stronger for CA 15-3. Only a proportion of extravillous trophoblast of the cell column, in decidual stroma or lining blood vessels was also stained. Whether the studied MUC1 glycoforms can be linked to trophoblast cells invasion remains to be established.

Key words: CA 15-3, CA 19-9; Glycosylation; Trophoblast.

Introduction

Mucins are large, heavily glycosylated proteins produced by secretory epithelia, lining the luminal surfaces of gastrointestinal, respiratory and reproductive tracts [1]. One of the mucin glycoproteins, mucin 1 (MUC1), is abundantly expressed by various organs, including acini and ducts of salivary and mammary glands, and prostate gland epithelium [2]. In the reproductive tract of women, MUC1 is expressed at the apical surface of the uterine epithelium, where it has been proposed to regulate embryo attachment [3-5], and in the placenta throughout pregnancy [6, 7]. Studies on human placenta showed that MUC1 is mainly expressed at the fetomaternal interface, by syncytiotrophoblast, fetal epithelium in contact with maternal blood, and by extravillous trophoblast cells invading the decidualized endometrium [6, 7].

Altered glycosylation is significant for the beginning, development and outcome of different human diseases [8, 9]. Remarkable diversity in the carbohydrate composition of MUC1 molecules between normal and tumor tissues has been shown [10]. Glycan modifications of MUC1 in normal and cancer cells are potentially relevant for physiological as well as pathological processes and could be significant for clinical practice.

MUC1 is a carrier of two differently glycosylated antigens, CA 15-3 and CA 19-9, both known as effective serum markers currently used in clinical practice for breast (CA 15-3), colon, and pancreas (CA 19-9) carcinoma [11, 12]. In contrast to the investigation of MUC1 molecules in human trophoblast, expression of different MUC1 glycoforms throughout pregnancy remains poorly understood so far. Previous investigation of CA 19-9 expression in placental tissue and amniotic fluid, revealed presence of this glycoprotein in decidual and amnion epithelial cells, but not in trophoblast subpopulations [13, 14]. Therefore, the aim of this work was to investigate expression of CA 15-3 and CA 19-9 antigens in normal placenta during gestation.

Materials and Methods

Tissue samples and immunohistochemical analysis

For this study material from the first (11 cases) and second trimester (5 cases) of pregnancy and at term (5 cases) was used. Tissue samples were obtained from the Institute of Obstetrics and Gynecology, Clinical Centre of Serbia, Belgrade, in accordance with ethical standards. Tissue sections were analyzed using monoclonal antibodies to CA 15-3 (clone M411149) and CA 19-9 (clone M602207) (Fitzgerald Industrial International - MA, USA). Trophoblast and decidual cells were identified by immunostaining using monoclonal antibody to cytokeratin-18 (CK-18, Dako Cytomation, Denmark) and to vimentin (clone V6, Sigma, MO, USA), respectively. Endothelial cells were identified by monoclonal antibody to CD34 (Serotec, Oxford, UK; not shown). Primary antibodies were used in the respective dilutions: 1:100 for anti-CA 15-3, 1:40 for anti-CA 19-9, 1:6000 for anti-CK-18, 1:1000 for vimentin and 1:25 for anti-CD34. Antigen unmasking (for CK-18 staining) was performed by boiling the slides in 10 mM citrate buffer, pH 6.0 for 1 min. Immunohistochemistry for all used antibodies was performed as previously described [15], using DAB or Nova Red as chromogen (Vector Laboratories, Burlingame, CA, USA). Omission of the primary antibody resulted in complete absence of staining. Slides were counterstained with hematoxylin, examined and photographed using a Carl Zeiss Axio Imager 1.0 microscope (Jena, Germany), with a Canon A640 Digital Camera System (Tokyo, Japan).

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cytokeratin staining was used to identify trophoblast (arrows in Figure 1A, D, G) and vimentin staining to identify decidual cells (Figure 2E). Immunohistochemical analysis of normal placenta demonstrated that trophoblast cells express both mucin/tumor antigens throughout gestation with a distinct staining pattern. In

Results

Expression of mucin-related glycoepitopes was studied on sections from the first and second trimesters of pregnancy, and at term pregnancy using monoclonal anti-CA 15-3 and anti-CA 19-9 antibodies. In parallel sections, cytokeratin staining was used to identify trophoblast (arrows in Figure 1A, D, G) and vimentin staining to identify decidual cells (Figure 2E). Immunohistochemical analysis of normal placenta demonstrated that trophoblast cells express both mucin/tumor antigens throughout gestation with a distinct staining pattern. In

Figure 1. — Immunohistochemical localization of mucin-like epitopes in the first (B, C) and second trimester (E, F, H, I) trophoblast and the decidua. Arrows point to cytokeratin-positive trophoblast cells identified in first (A) and second trimester placentas (D, G), also stained with CA 15-3 in the first (B) and second trimester (E, H), and with CA 19-9 in the first (C) and second trimester (F, I). Decidual cells (DEC) also stained for CA 15-3 (B, E, H) and CA 19-9 (C, F, I). Scale bar represents 20 µm.
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the first trimester of pregnancy, in all analyzed samples, non-uniform staining with anti-CA 15-3 was detected in trophoblast villous (Figure 1B), ranging from negative to moderate positive staining. Strong staining with CA 15-3 was observed in some, but not all, cytokeratin-positive cells of the cell column, and invasive trophoblast (Figure 1B), while surrounding decidual stromal staining was relatively weak. Regarding CA 19-9 in the first trimester of pregnancy, moderate and non-uniform staining was present in trophoblast villous and in the cell column (Figure 1C).

In second trimester placentas staining with CA 15-3 and CA19-9 was detected in the same trophoblast cell types as in the first trimester, as can be observed in panels E and H for CA 15-3, and in F and I for CA 19-9. In addition, trophoblast lining blood vessels were also found to express CA 15-3 (Figure 1H) and CA 19-9 (Figure 1I). At term, there was strong CA 15-3 staining of syncytiotrophoblast (Figure 2A), chorion, and trophoblast of placental septae (Figure 2D). Immunohistochemical analysis demonstrated weak or absent staining for CA19-9 in syncytiotrophoblast (Figure 2B), and weak to moderate staining of chorion and placental septae (placental septae, Figure 2D). Our results also showed that decidual stromal cells express both mucin antigens throughout pregnancy (Figure 1 B, C, E, F, H, I).

Discussion

Mucin MUC1 expressed by human uterine epithelium has been proposed to act as one of the glycoproteins involved in blastocyst attachment [16]. In recent years, studies detected MUC1 in human villous and extravillous trophoblast [6, 7]. Jeschke et al. [6] reported using immunohistochemistry strong expression of MUC1 in both first and second trimester placentas, and to a lesser degree in the third trimester, while Shuy et al. [7] found weak expression in the first trimester, which increased with gestational age, at mRNA and protein levels. Reported findings of MUC1 expression are somewhat controversial, which might result from the techniques or antibodies used. It is interesting to note that the same finding was obtained for both peptide core and glycopeptide specific antibody [7]. In the present study, localization of two differently glycosylated human MUC1 forms, CA 15-3 and CA 19-9 tumor antigens, in normal placentas during gestation was investigated. We observed that both antigens were present in human trophoblast, but the staining for CA 15-3 was more pronounced than for CA 19-9. On the other hand, decidual stroma was considerably more stained for CA 19-9, and negative to moderately stained for CA 15-3. Invasive trophoblast in the first
and the second trimester of pregnancy were found to consistently express CA 15-3, which is in keeping with the report of Shuy et al. [7] with respect to percent of trophoblast stained and relatively weak staining. On the other hand, in our study endovascular trophoblasts were also found to express this MUC1 glycoform (CA 15-3), which differs from a previous report by Jeschke et al. [6]. Our finding regarding CA 19-9 expression by trophoblast cells is novel, and differs from the previous reports that did not find CA 19-9 antigen in trophoblast cells [13, 14]. There are several glycotopes comprised of short sugar chains which can be found on MUC1, and they include Thomsen-Friedenreich antigen (TF or T antigen), Tn (N-acetylgalactosamine) and sialyl-Tn antigen [2]. The results of immunohistochemical studies in general critically depend on the reactivity of antibodies used. The specific anti-CA 15-3 and CA 19-9 antibodies used here have not been previously used. Data presented in the literature do not relate to the same antibodies, and there is a possibility that the other reported antibodies detected different stages in MUC1 processing.

Overexpression of MUC1 contributes to the malignant phenotype [1]. However, in contrast to the breast and intestinal mucins [17], glycan of placental MUC1 are incompletely known [18]. It has been shown that MUC1 extracted from term placental tissue contains a short glycans structure, T and Tn antigens, similar to tumor carbohydrate antigens [18]. Furthermore, the T antigen associated with MUC1 may play a critical role in cancer cell adhesion to endothelium, through interaction with galectin-3 [19]. Our previous study has shown that gestational trophoblastic diseases are associated with increase in galectin-1 and galectin-3 expression compared to the normal trophoblast [20]. There is a possibility that oligosaccharides linked to MUC1 are potential ligands for trophoblast galectins and could thus mediate cell interactions of trophoblast. At present, there is no evidence to show that altered expressions of CA 15-3 or CA 19-9 antigens characteristic for neoplastic tissue are in any way responsible for the development of transformed trophoblast phenotype. However, the presence of MUC1 [7] and CA 15-3 antigen shown here in some, but not all cytokeratin-positive cells of the cell column, invasive and endovascular trophoblast, raises a possibility that this mucin could have an active role in trophoblast cell invasion. It is interesting to note that overexpression of MUC1 was found to decrease invasion of chorionicarcinoma cell line JAr [7]. Whether the studied MUC1 glycoforms can be linked to trophoblast cells invasion or not remains to be established.

References
Regulation of radiosensitivity by HDAC inhibitor trichostatin A in the human cervical carcinoma cell line Hela

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Summary
Histone deacetylase (HDAC) inhibitors play an important role in inducing growth arrest, differentiation, and/or apoptosis in cancer cells. Given their ability to disrupt critical biological processes in cancer cells, these agents are emerging as potential therapeutics for cancer. Recently, it has been identified that HDAC inhibitors can also efficiently enhance the radiation sensitivity of cells, both in vitro and in vivo. In this study, we investigated whether the potent HDAC inhibitor, Trichostatin A, modulates the radiation sensitivity of the human cervical carcinoma cell line Hela under hypoxic conditions. We concluded that TSA could significantly inhibit the proliferation of Hela cells in a dose- and time-dependent manner under normoxic and hypoxic conditions. Hypoxia results in the cervical carcinoma Hela cells resistant to TSA. The findings from clonogenic survival assays indicate that incubation with TSA for 24 hours prior to irradiation enhances the radiation sensitivity of Hela cells under hypoxic conditions. More generally, we found Hela cells under hypoxic conditions treated with TSA could significantly down-regulate the expressions of HIF-1α and VEGF proteins. Taken together, our results demonstrated that TSA acts as a powerful radiosensitizer in Hela cells under hypoxic conditions probably by down-regulated expression of HIF-1α and VEGF proteins.

Key words: Tumor cell; Hypoxia; Radiosensitivity.

Introduction

Hypoxic regions in tumors are a major cause of radiotherapy and chemotherapy failure in solid tumors (chemotherapy and radiotherapy resistant) [1-5]. The hypoxic cells account for 10%-50% in solid tumors and their tolerance to radiation and chemotherapy is 2.5-3 times stronger than that of aerobic cells, which becomes one of the important factors making cancer difficult to cure, and easy to recur and metastasize [6]. Therefore, utilizing cytotoxic drugs in combination with chemoradiotherapy is an important regimen for cancer treatment [7, 8].

Histone acetylation is balanced by a regulation between histone acetyltransferase (HAT) and histone deacetyltransferase (HDAC) [9]. While HAT activity relaxes chromatin and promotes transcription by allowing access of transcription factors to DNA, HDAC activity condenses chromatin, leading to transcriptional repression. Both HAT inactivity and HDAC overactivity have been associated with tumorigenesis, presumably because of transcriptional repression of tumor suppressor genes. In the last few years, therapeutic interest on histone deacetylase (HDAC) inhibitors has been rapidly increasing and it has been demonstrated that these drugs, including trichostatin A (TSA), induce growth arrest, differentiation, and/or apoptosis in cancer cells [10-13]. Similar to other anticancer drugs, HDAC inhibitors selectively induce apoptosis in tumor cells; in vitro, the studies show that these cells may be tenfold more sensitive to HDAC inhibitors compared with normal cells. Trichostatin A (TSA), initially isolated as fungistatic antibiotics from streptomycyes hygroscopicus, is a potent inhibitor of HDAC activity at nanomolar concentrations [14]. TSA has been suggested to block the catalytic reaction by chelating a zinc ion in the active site pocket through its hydroxamic acid group [15]. Although TSA has been demonstrated to induce apoptosis of various cancer cells and enhance their chemoradiotherapy [16-19], but their effect on the human cervical carcinoma cell line Hela under hypoxic conditions is not clearly known yet. In this study, the human cervical carcinoma cell line Hela was used as an in vitro model to explore the expression differences of hypoxia inducible factor Iα (HIF-1α) and vascular endothelial growth factor (VEGF) in Hela cell lines under normoxic and hypoxic conditions and the impact of TSA on the expression. In addition detection of the radiation sensitizing effects of TSA on Hela cell lines under hypoxic conditions by clonogenic survival assays was undertaken so as to provide a new sensitizing means for clinical radiotherapy of cervical carcinoma.

Materials and Methods

Drugs and reagents
TSA was purchased from Sigma Chemical Co. (St Louis, MO, USA); methylthiazolyl tetrazolium (MTT) and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co.; RPMI-1640 medium was purchased from Gibco (Invitrogen, CA, USA); calf serum from ShangBao Bio-Engineering Co. (Shanghai, China); Mouse anti-human vascular endothelial growth factor monoclonal antibody and KIT-9710 kit from Maixin Bio- Development Co., Ltd. (Maixin, Fuzhou, China); Rabbit anti-human hypoxia-inducible factor-1 alpha polyclonal antibody from Boster Bio-Engineering Co., Ltd. (Boster, Wuhan, China).

Cells and culture conditions
The human cervical carcinoma cell line Hela was kindly provided by Tongji Medical College, Huazhong University of
Science and Technology. Cells were cultured in RPMI 1640 culture medium containing fetal calf serum (10%), penicillin (100 u/ml) and gentamicin (40 u/ml) under conventional conditions (37, 5% CO₂), for hypoxic culture, conventionally cultured cells were placed in a hypoxic chamber containing 99.9999% N₂. Cells were preconditioned in hypoxic conditions according to the experimental group. All experiments were carried out in the cell exponential growth phase.

**Proliferation assays**

Cells under normoxic and hypoxic conditions in the exponential growth phase were collected, adjusted to a cell concentration of 2 × 10⁴/ml with RPMI-1640 medium containing 10% fetal calf serum, and were inoculated onto a 96 well plate, 0.1 ml/well. After cell adherence, different concentrations of TSA (a final concentration of 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 µmol/l) were added, adding the same amount of dimethyl sulfoxide (DMSO) in the control group; the drug of each concentration was inoculated into five holes. After 12, 24, 48 and 72 h under normoxic and hypoxic conditions, 20 µl MTT (5 mg/ml) was added, supernatant was discarded after a 4 h incubation at 37°C, and added with 150 µl DMSO. After full blending, the Multi-Skan Ascent enzyme-labeled instrument was used to measure the absorbance value at 492 nm wavelength and the cell viability was calculated by the following formula using application software of SPSS13.0 to calculate the 50% inhibition concentration (IC50) and 10% inhibition concentration (IC10). Each experiment was repeated three times.

\[
\text{Cell viability} = \frac{(A_{\text{test group}} - A_{\text{blank group}})}{(A_{\text{control group}} - A_{\text{blank group}})} \times 100\%.
\]

**Clonogenic survival assays**

Cells under normoxic and hypoxic conditions in the exponential growth phase were collected and inoculated in a dish 60 mm in diameter, sub into the normoxic irradiation group, hypoxic irradiation group, and hypoxic drug-added irradiation group. After 12 h cell adherence, with a dose rate of 2.5 Gy/min 6MV X-ray irradiation under room temperature, radiation doses were 0, 1, 2, 4, 6 and 8 Gy. After irradiation, the cells were collected immediately with 0.25% trypsin-EDTA, counted and inoculated in a dish 60 mm in diameter by adding 4 ml RPMI-1640 medium containing 10% fetal calf serum and cultured for 14 days. The medium was discarded and the cells were fixed with 95% ethanol for 20 min, stained with crystal violet (0.5%) for 15 min, and then the clone numbers in each dose group were counted (for colonies with more than 50 cells) and the cell survival fraction (SF) was calculated. The experiment was repeated three times. The singlehit multitarget mathematical model was used to draw a dose-survival curve, D₀, Dₚ and radiosensitization ratios (SER) were obtained. The radiosensitivity of each group was analyzed and compared.

**Cell immunocytochemistry**

Sterile coverslips were put into a 24-well plate, 1 × 10⁵ cells/well, when the confluence reached approximately 70%. Cells were put into a hypoxic condition and treated with IC10 TSA for 24 h, with cells under a normoxic condition as the control. The coverslips were put into 4% paraformaldehyde for 30 min, then fixed on the slides with glue and blocked for endogenous peroxidase and avidin/biotin. The cells were permeabilized in PBS, incubated in mouse anti-human monoclonal VEGF antibody and rabbit anti-human monoclonal HIF-1α antibody overnight at 4°C. After washing with PBS, cells were stained with secondary antibody for 10 min at room temperature, washed with PBS, and added into horseradish peroxidase-conjugated avidin. After washing with PBS, DAB was used as the chromogen for active HIF-1α and VEGF cytochemistry. Cell slides were stained with hematoxylin, dewaxed conventionally, treated with transferase and sealed. Cell slides were observed and photographed were taken. The location expression of HIF-1α was cytoplasm and (or) nucleus, and VEGF was cytoplasm. There were three cell slides in each group, and immunocytochemistry studies were performed in triplicate.

**Immunocytochemical evaluation**

The immunocytochemical evaluation was calculated by combining an estimated percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score), as follows [20]: no staining was scored as 0, 1-10% of cells stained were scored as 1, 11-50% as 2, 51-80% as 3, and 81-100% as 4. Staining intensity was rated on a scale of 0 to 3, with 0-5 negative; 1-5 weak; 2-5 moderate, and 3-5 strong. The raw data were converted to the immunocytochemical evaluation by multiplying the quantity and staining intensity of scores. Theoretically, the scores could range from 0 to 12. An immunocytochemical evaluation score of 9-12 was considered strong immunoreactivity, 5-8 was considered moderate, 1-4 was considered weak, and 0 was scored as negative.

**Statistical analysis**

Experimental data are expressed as mean ± SD and analyzed by the t-test. Analysis of variance was used to compare the two groups. SPSS 13.0 statistical software was used and the test level was 0.05.

**Results**

**Effects of TSA on cell growth**

TSA significantly inhibited the proliferation of Hela cells in a dose-and time-dependent manner under normoxic and hypoxic conditions as shown in Figure 1. The 50% inhibition concentration and 10% inhibition concentration under normoxic conditions were 0.031 µmol/l, 0.587 µmol/l; the 50% inhibition concentration (IC50) and 10% inhibition concentration (IC10) under hypoxic conditions were 0.076 µmol/l and 0.947 µmol/l. Multivariate analysis of variance of cells under normoxic conditions showed that for different concentrations of the experimental group inhibition rate there was a significant difference (F = 37.930, p = 0.000), and the different times of experimental groups had a significantly statistical difference (F = 48.420, p = 0.000); multivariate analysis of variance of cells under hypoxic conditions showed that for different concentrations of the experimental group inhibition rate there was a significant difference (F = 54.429, p = 0.000), and the different times of the experimental groups had significant statistical difference (F = 59.398, p = 0.000). According to the experimental requirements, selection of the IC10 of TSA under hypoxic conditions was used as the drug concentration in the follow-up experiments. Furthermore, IC50 of Hela cells under hypoxic conditions were higher than under normoxic conditions, and resulting in chemo-resistance.
Effect of pretreatment with TSA on radiation sensitivity

The Hela cell line had a $D_0$ value of 1.16 Gy and a $D_{q}$ value of 1.57 Gy in the normoxic irradiation group, a $D_0$ value of 3.24 Gy and a $D_{q}$ value of 2.95 Gy in the hypoxic irradiation group, and a $D_0$ value of 2.19 Gy and a $D_{q}$ value of 1.64 Gy in the hypoxic drug-added irradiation group. Figure 2 shows the radiation survival curves and parameters of Hela in the normoxic group, hypoxic group and hypoxic drug-added group, respectively. Hela cells had a significantly lower radiosensitivity after treat-
Figure 3. — Expression of HIF-1α and VEGF by an immunocytochemical method (DAB × 400). Cells of the normoxic condition group were collected in the cell exponential growth phase and inoculated into sterile coverslips; when the confluence reached approximately 70%, the expressions of HIF-1 and VEGF proteins were detected by immunocytochemistry (A, B). Cells of the hypoxic condition group were collected in the cell exponential growth phase and inoculated into sterile coverslips; when the confluence reached approximately 70%, the expressions of HIF-1 and VEGF proteins were detected by immunocytochemistry (C, D). Cells of TSA combined with the hypoxic condition group were collected in the cell exponential growth phase and inoculated into sterile coverslips; when the confluence reached approximately 70%, cells were put into a hypoxic condition and treated with IC10 TSA for 24 hours. The expressions of HIF-1 and VEGF proteins were detected by immunocytochemistry (E, F). The location expression of HIF-1 was cytoplasm and (or) nucleus, and VEGF was cytoplasm.
ment by hypoxia ($p < 0.05$), while the radiosensitivity significantly increased after TSA treatment ($p < 0.05$). Compared with the hypoxic irradiation group, the hypoxic drug-added irradiation group $\text{SER}_{250}$, $\text{SER}_{100}$, and $\text{SER}_{0}$ were 1.48, 1.80 and 1.3, respectively.

**Expression of VEGF and HIF-1α proteins**

As shown in Figure 3, in the cervical carcinoma cell line Hela under normoxic conditions, expression of HIF-1α in the nucleus was negative (−), in the cytoplasm it was weakly positive (+) (A), and the expression of VEGF in the cytoplasm was weakly positive (+) (B); after preconditioning in the hypoxic condition for two hours, the expression of HIF-1 was strongly positive in the nucleus and cytoplasm (+++) (C), the expression of VEGF was strongly positive in the cytoplasm (+++) (D), the cells were deformed into polygonal shapes and the expression of nuclear particles was increased. As for the role of the hypoxic drug-added group, the cells further deformed, emerged into a membrane dissolution phenomenon, the expression of HIF-1α in the cytoplasm and nucleus was weakly positive (+) (E), and the expression of VEGF in the cytoplasm was weakly positive (+) (F), similar to the normoxic group.

**Discussion**

HDAC inhibitors including TSA have shown potential as antineoplastic agents for the treatment of many solid and hematological malignancies, and currently there is an intense research focus aimed at developing this new class of targeted anticancer agents [21, 22]. They predominantly act by inducing differentiation, apoptosis, cell-cycle arrest, anti-angiogenic, anti-invasive and immunomodulatory activities related to transcriptional changes with a preferential cytotoxicity for tumor cells.

In this study the results showed that under normoxic or hypoxic conditions the concentration of TSA from 0.05 µmol/l to 0.8 µmol/l induced a dose-and time-dependent inhibition on the human cervical carcinoma cell line Hela. Hela cells under hypoxic conditions performed drug resistance to TSA, while the IC50 of Hela cells under normoxic conditions and hypoxic conditions rose from 0.587 µmol/l to 0.947 µmol/l. Many experiments have produced similar results [23-25]. Hypoxia enhances chemoresistance of cancer cells. First, the delivery of drugs in a hypoxic area and cellular uptake are affected by hypoxia or associated acidity. Second, some chemotherapeutic drugs require oxygen to generate free radicals that contribute to cytotoxicity. Last, hypoxia induces cellular adaptations that compromise the effectiveness of chemotherapy. In response to nutrient deprivation due to hypoxia, the rate of proliferation of cancer cells decreases and chemotherapeutic drugs are more effective against proliferating cells. On the other hand, hypoxia induces adaptation by post-translational and transcriptional changes that promote cell survival and resistance to chemotherapy.

In addition, there is emerging interest in the use of HDAC inhibitors to modulate the effects of irradiation [26-29]. Preclinical studies have also shown that HDAC inhibitors exhibit radiosensitizing effects in a variety of malignancies, such as glioblastoma, non-small cell lung cancer, colorectal cancer, prostate cancer, and metastatic breast cancer. While the mechanism of radiosensitization is not well understood, accumulating evidence suggests that it is in part due to inhibition of DNA DSB repair as evidenced by prolonged expression of $\gamma$H2AX [30]. Besides influencing repair pathways, HDAC inhibitors can also change the acetylation status of other proteins such as Hsp90. Some authors have attributed the observed radiosensitization to dissociation of EGFR from the Hsp90 complex, resulting in receptor degradation [31]. Furthermore, in preclinical studies HDAC inhibitors appeared to radiosensitize tumor cells without an increase in radiosensitization of normal cells, potentially improving the therapeutic effect. Interestingly, HDAC inhibitors not only act as tumor-selective radiosensitzers, but also as protectors of radiation-induced normal tissue damage. Topical applications of TSA, VPA and NaB were shown to enhance protection from radiation-induced injuries in an animal model of skin radiation syndrome. Although the mechanisms behind this radioprotection remain to be clarified, HDAC inhibitors mediated decrease in the inflammatory cytokine tumor necrosis factor alpha (TNF-β) and transforming growth factors (TGF)-β1 and TGF-β2 could be partly responsible [32].

In this study, we have found that TSA is a potent radiation sensitizer in Hela cells under hypoxic conditions. Compared to the normoxic irradiation group, the D0 value of the hypoxic irradiation group rose from 1.16 to 3.24, and the $D_{0}$ value rose from 1.57 to 2.95. Compared to the hypoxic irradiation group, the $D_{0}$ value of the hypoxic drug-added irradiation group decreased from 3.24 to 2.19, and the $D_{0}$ value decreased from 2.95 to 1.64. In short, data from the clonogenic survival assays indicated that under hypoxic conditions the radiosensitivity of Hela cells was reduced, and exposure of Hela cells under hypoxic conditions to TSA prior to irradiation results in a radiosensitization effect. Previous studies have demonstrated that low doses of TSA as a result of increasing human leukemia cell levels of histone acetylation, enhanced the radiosensitivity to the γ-ray [33].

In an attempt to elucidate the mechanisms by which TSA potentiates the biological effects of irradiation, experiments were performed with immunocytochemistry. Furthermore, the results demonstrated that Hela cells under hypoxic conditions resulted in increased levels of the protein expressions of HIF-1α and VEGF, and pre-treatment of Hela cells under hypoxic conditions with TSA resulted in decreased levels of protein expressions of HIF-1α and VEGF. An experiment done on liver cancer cells showed that [34] TSA could activate the expressions of tumor suppressor gene p53 and von Hippel Lindau (VHL) and via the ubiquitin-proteasome pathway reduced hypoxia-induced over-expression of HIF-1α. The low expression of HIF-1α decreased the DNA binding activity of the hypoxia response element (HRE). VEGF as a downstream signaling regulator of HIF-1α reduces expression of HIF-1α affecting expression of VEGF, and thus has targeted anti-tumor activity.
and a radiosensitizing effect. Some other studies have demonstrated TSA to be a potential anti-angiogenic agents [35]. TSA reduced the VEGF stimulated human vein endothelial cell invasion of collagen type I-like structures and capillary formation by decreasing the expression of human endothelial growth factor receptor (VEGFR) VEGFR1, VEGFR2, and neuropilin-1.

In summary, we demonstrated that HDAC inhibitor TSA enhanced the radiosensitization of Hela cells under hypoxic conditions via down-regulated expression of HIF-1 and VEGF. In conclusion, the current data provide new evidence for the potential application of combining TSA and X-ray irradiation as a valuable anticancer strategy for cervical carcinoma treatment.

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Toll-like receptor 4 signaling promotes the immunosuppressive cytokine production of human cervical cancer

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Summary

Objectives: To investigate the expression of TLR-4 (toll-like receptor) on human cervical cancer and find the biological function of the TLR-4 signal system. Methods: The immunohistochemistry method was performed to study the protein expression and distribution of TLR-4. The viability of HeLa cells was determined by cell viability assay. Cell proliferation was detected by FCM, ELISA and Western blot were used to observe the gene and protein expression of IL-6 and TGF-β1 in Hela cell lines. Results: TLR-4 was over-expressed in cervix cancer, and its activation by LPS promotes proliferation and anti-apoptosis in Hela cells in vitro. Moreover the cell line proliferation increased in a dose- and time-dependent manner. The production of IL-6 and TGF-β1 were promoted through the activation of the NF-κB signaling pathway.

Key words: Cervical cancer; TLR-4; IL-6; TGF-β1.

Introduction

Cervical cancer is one of the most frequent female malignant tumors worldwide, accounting for approximately 500,000 new cases and the deaths of 270,000 women every year. Despite great improvement in the treatment of cervical cancer, the 5-year survival rate is still lower for cervical cancer at IIb-IV Stage. Therefore, in sight into the molecular biological mechanisms underlying cervical cancer is important for diagnosis, prevention and treatment of cervical cancer.

Toll-like receptors (TLRs) are a family of pattern recognition receptors that are composed of 13 types which play a key role in innate immune responses and participate in the regulation of adaptive immune responses. TLRs are mainly expressed in immune cells such as dendritic cells (DC), macrophages and B cells. Recently, TLRs have also been detected in many cancers, including stomach cancer [1], ovarian cancer [2], lung cancer [3], prostate cancer [4] and breast cancer [5]. There were some studies suggesting that the activation of the TLR signaling pathways may fuel the proliferation, anti-apoptosis, invasion and immune escape of tumors [6]. However, the role of TLR-4 in cervical cancer is poorly understood.

Many epidemiological studies have demonstrated that chronic infection and inflammation are important epigenetic and environmental factors promoting tumorigenesis. Progress has been made in the understanding of tumor immune evasion, which is facilitated by the production of inflammatory profile of cytokines and chemokines, including IL-6 and TGF-β1, the sources of which are to a certain extent tumor cells stimulated by ligand lipopolysacharide (LPS) (stimulating TLR-4) potentially through nuclear factor kB (NF-κB) pathway activation.

Recently, TLR-4 has been found to be expressed in tumor cells, and whether human cervical cancer cells can express TLR-4 remains poorly understood. In this study, we attempted to detect the expression of TLR-4 on human cervical cancer tissue and to find the biological function of the TLR-4 signaling system which was activated by LPS.

Materials and Methods

Reagents

LPS, carboplatin, HTA125, PDTC and all other chemicals mentioned in the methods were purchased from Sigma Chemical Co. (St Louis, MO, USA). The mouse anti-TLR-4, mouse anti-NF-κB, mouse anti-IL-6, mouse anti-TGF-β1 and rabbit anti-β-actin were purchased from Abcam plc. (Cambridge, UK). FITC-conjugated anti-TLR-4 mAb and FITC-conjugated isotype IgG1 mAbs were from eBioscience (San Diego, CA, USA) and Serotec (Oxford, UK), respectively.

Study samples

A total of 68 patients were recruited from women who were scheduled to undergo cervical cancer radical surgery in our hospital between January 2008 and May 2011, while the control group comprised 39 women who underwent surgery for other diseases and had a normal cervix. The mean age of the patients was 43 ± 12 (range 25 to 68 years).

Cell line and culture

HeLa cells, a human cervical cancer cell line, were obtained from Wuhan University, and grown in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and penicillin/streptomycin (100 units ml⁻¹) in a 5% CO₂ incubator that was maintained at 37°C.

Immunohistochemistry

All tissues were formalin fixed, paraffin embedded, and then cut into 5 μm sections on glass slides. Following deparaffinization in xylene and rehydration in alcohol, sections were treated...
of cell suspension (1 × 10^6 cells per ml) and incubated at 4°C for 30 min. FITC-conjugated isotype IgG 1 mAbs were used as negative controls. Cell-surface expression of TLR-4 was analyzed on a FACS scan flow cytometer to detect the log of the mean channel fluorescence intensity with an acquisition of FL1. A minimum of 10,000 events were collected and analyzed on Cell Quest software.

**ELISA analysis**

Concentrations of the IL-6 and TGF-β1 were evaluated by ELISA analysis by collecting different treatment group supernatants according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN).

**Western blot analysis**

Protein (20 µg) was denatured in sample buffer [2.5% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.15 mol/l Tris (pH6.8), and 0.01% bromophenol blue] and subjected to 12% SDS-PAGE as previously described [17]. Membranes were probed with the following primary antibodies specific for: mouse anti-NF-κB (Abcam; 1:1,000), mouse anti-IL-6 (Abcam; 1:1,000), mouse anti-TGF-β1 (Abcam; 1:1,000) and mouse anti-β-actin (Abcam; 1:10,000). Proteins were visualized using enhanced chemiluminescence (Pierce Biotechnology).

**Results**

**TLR-4 in cervix cancer**

To determine whether TLR-4 is expressed in cervix cancer, we evaluated TLR-4 expression in cervix cancer tissues by immunohistochemistry. As shown in Figure 1, positive staining for TLR-4 was easily detected in the cytoplasm and membrane of tumor cells and its expression was 68.7% in tumor tissue, which was significantly higher than that in the neighboring nondysplastic tissue.

**ELISA analysis**

Concentrations of the IL-6 and TGF-β1 were evaluated by ELISA analysis by collecting different treatment group supernatants according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN).

**Cell viability assay**

Cell viability was evaluated using the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay according to the manufacturer’s instructions. The values from the treated cells were compared with the values generated from the untreated cells and reported as percent viability. Each experiment was done at least thrice. In order to assess the role of LPS, cells cultured in six-well plates (1 × 10^5 cells per well) were exposed to various concentrations of LPS (0, 1, 5 and 10 µg/ml) for different time periods (12, 24 and 48 h) at 37°C in humidified 5% CO2 conditions. Cell-free supernatants were collected by centrifugation at 400 g for 10 min and frozen at -70°C or analyzed immediately.

**Flow cytometry (FCM) assay**

Cells were cultured in 6-well plates for 24 hours. Nonadherent cells were removed by gentle washing. Medium was removed and pretreated with or without carboplatin (80 µg/ml) for 12 h and HeLa cells (4 × 10^5/ml) were exposed to the accompaniment of 10 mg/ml LPS for 24 h at 37°C in humidified 5% CO2. The expression of TLR-4 on HeLa cells was determined using direct immunofluorescent staining. Briefly, 20 µl of FITC-conjugated anti-TLR-4 mAbs was added to 100 µl of cell suspension (1 × 10^6 cells per ml) and incubated at 4°C for 30 min. FITC-conjugated isotype IgG1 mAbs were used as negative controls. Cell-surface expression of TLR-4 was analyzed on a FACS scan flow cytometer to detect the log of the mean channel fluorescence intensity with an acquisition of FL1. A minimum of 10,000 events were collected and analyzed on Cell Quest software.

**Figure 1. — Expression of TLR-4 in cervical cancer samples. Brown color displays positive expression. A: Negative control (isotype-matched control) B: Expression of TLR-4 protein in carcinoma tissue (SP × 200).**

Legend 1. — Differential response of Hela cells to ligation of TLR-4 with LPS. Monolayers of Hela cells, exposed to different periods in the presence of increasing amounts of LPS. Cell viability was determined using CellTiter 96 AQueous One Solution Cell Proliferation Assay.
Functional Grade Purified anti-human Toll-like receptor 4) is known to function as an inhibitor of TLR-4. HeLa cells were pretreated with HTA125 (10 µg/ml−1) before incubation with 10 µg/ml−1 LPS for 24 h. Here we demonstrate that the inhibition of TLR-4 reduced LPS-mediated proliferation of HeLa cell to normal levels (Figure 4). Furthermore, on the basis of the carboplatin mentioned above, we added HTA125 (10 µg/ml−1) before incubation with LPS for 24 h. The inhibition of TLR-4 resumed apoptosis of HeLa cell induced by carboplatin (Figure 3).

TLR-4 upon recognition of its LPS can stimulate the activation of the NF-κB signaling pathway and promote the production of IL-6 and TGF-β1 in human cervix carcinoma cells.

Some studies have confirmed that the TLR-4 upon recognition of its LPS can induce the production of inhibitory cytokines, inflammatory factors, proteinases, and other small molecules in the immune system [7]. Thus, our next objective was to evaluate whether TLR-4 ligation by LPS would have a similar effect in human cervix carcinoma cells.

As IL-6 and TGF-β1 have been reported to be involved in proliferation and anti-apoptosis of tumor cells, we incubated HeLa cells in the presence or absence of 10 µg/ml−1 LPS for 24 h, and cytokine secretion was evaluated by ELISA analysis. As expected, the secreted levels of IL-6 and TGF-β1 were demonstrated to be significantly increased by LPS treatment (Figure 5). A similar result turned up with the inhibition of TLR-4 (10 µg/ml−1 HTA125); the levels of IL-6 and TGF-β1 were all downregulated (Figure 5).

TLR-4 ligation to LPS activates the NF-κB signaling pathway in immune cells, which results in cytokine secretion, proliferation and anti-apoptosis of immune cells. Thus, we observed the activation status of NF-κB in human cervix carcinoma. HeLa cells were stimulated with 10 µg/ml−1 LPS for 0.5, 1, 2, and 4 h. The nuclear localization sequence of NF-κB p65 was assessed by Western blot analysis. Stimulation of HeLa cells with 10 µg/ml−1 LPS for 0.5 h increased NF-κB activity (Figure 6).

**Figure 2.** — LPS affects carboplatin induced Hela cell line apoptosis. Apoptosis detected by Annexin V/FITC staining.

**Legend 2.** — HeLa cells pretreated with HTA125 (10 µg/ml−1) before incubation with 10 µg/ml−1 LPS for 24 h, were partially rescued from proliferation promotion induced by LPS. Cell viability was determined using CellTiter 96 AQueous One Solution Cell Proliferation Assay.

**Figure 3.** — TLR-4 ligation to LPS activates the NF-κB signaling pathway. HeLa cells stimulated with 110 µg/ml−1 LPS for for 0.5, 1, 2 and 4 h. The nuclear localization sequence of NF-κB p65 was assessed by Western blot.

**Legend 3.** — Enhancement of IL-6 and TGF-β1 secretion in Hela cells by LPS samulation which was mediated by NF-κB signaling pathway. The cytokine secretion was evaluated by ELISA analysis.
Finally, to confirm whether IL-6 and TGF-β1 secretion induced by LPS was mediated by the NF-κB signaling pathway. Hela cells were incubated with the NF-κB specific inhibitor (PDTC) (10 µg/ml) for 1 h with LPS of 10 µg/ml for 24 h. PDTC significantly inhibited the secreted levels of IL-6 and TGF-β1 induced by LPS (Figure 5). These results indicated that TLR-4 ligation by LPS activated the NF-κB signaling pathway leading to proliferation and anti-apoptosis of HeLa cells.

Discussion

It has been well established that a variety of microbe-induced inflammatory processes promote tumorigenesis but the mechanisms of the phenomenon are poorly understood. Substantial evidence has identified TLR-4 as a crucial component of the immune system that plays an important role in regulating tumor cell proliferation, anti-apoptosis, angiogenesis, invasion, metastasis and immune escape. Kelly et al. [2] confirmed that the activation of the TLR-4 signaling pathway promoted an inflamed environment, improved tumor cell survival ability and chemotherapy resistance. However, whether human cervical carcinoma cells can express TLR-4 and what the biological function of TLR-4 on cervical carcinoma is remains to be fully understood.

Up to date, there have been increasing studies about the effect and corresponding mechanism of the TLR-4 signal pathway on tumorigenesis. Chronic inflammation is a high risk factor for the development of cancer. Progression of tumor is often associated with a generalized immunosuppression of the host. Tumors evade immune surveillance by multiple mechanisms, including the production of factors such as transforming growth factors (TGF-β1) and interleukin-6 (IL-6), which inhibit DC activation and impair tumor-specific T cell immunity. The TLR-4 signaling pathway involves the activation of NF-κB, which leads to the production of inflammatory cytokines. LPS-ligand for TLR-4, which has been shown to induce NF-B activation, cytokines/chemokine production, and inflammation [8], is expressed by the cells of the innate immune system. He et al. [9] has found that TLR-4 ligation promoted production of immunosuppressive cytokine TGF-β1, vascular endothelial growth factor (VEGF), proangiogenic chemokine IL-8, and NF-κB was activated and contributed to apoptosis resistance of human lung cancer cells induced by LPS in human lung cancer cells.

In this study, we have described a specific inflammatory and innate host defense mechanism, that could regulate the release of proinflammatory cytokines, leading to tumorigenesis. We found, for the first time, that TLR-4 expressed in cervical cancer tissue is significantly higher than in surrounding normal cervical tissue. At the same time, we ascertain that the activation of TLR-4 by LPS can accelerate the growth of cervical cancer cells by promoting proliferation and anti-apoptosis, mainly associated with the process where NF-κB mediates IL-6 and TGF-β1 secretion.

In conclusion, our study revealed that TLR-4 was over-expressed in cervical cancer. On the other hand, TLR-4 activation by LPS could promote proliferation and anti-apoptosis in cervix cancer. In addition, functional activity of TLR-4 was demonstrated by stimulation of LPS and subsequent activation of NF-κB, and release of the proinflammatory IL-6 and TGF-β1. Thus, TLR-4 might be a new marker for cervical cancer.

Conclusion

We investigated the expression of TLR-4 on human cervical cancer cells and found the biological function of the TLR-4 signal system. We have reported for the first time that TLR-4 expressed in cervical cancer tissue is significantly higher than in surrounding normal cervical tissue. At the same time, we ascertain that the activation of TLR-4 by LPS can accelerate the growth of cervical cancer cells by promoting proliferation and anti-apoptosis – mainly associated with the process where NF-κB mediates IL-6 and TGF-β1 secretion. Therefore, we conclude that TLR-4 might be a new biological marker for cervical cancer.

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Evaluation of endometrium by transvaginal ultrasonography and Doppler in tamoxifen-treated women with breast cancer

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Summary

Purpose: The purpose of this study was to investigate the discriminative role of transvaginal ultrasonography and Doppler measurements on the detection of endometrial pathologies in tamoxifen-treated breast cancer patients. Methods: Tamoxifen-treated breast cancer patients were included in this prospective study between February 2009 and June 2010. The subjects were assessed by gynecologic examination and transvaginal gray-scale and Doppler sonography. The patients whose endometrial thicknesses were more than 6 mm underwent endocervical/endometrial curettage for histopathological examination. Results: There were 98 tamoxifen-treated patients with breast cancer enrolled in the study, providing 141 ultrasound evaluations. Uterine artery pulsatility index was significantly lower in postmenopausal than premenopausal patients (p: 0.013). Endocervical and endometrial curettage was performed in 52 patients. It was more prevalent that the endometrial strip was ≥ 6 mm in women with abnormal endometrial histopathology (p: 0.020). However the women with abnormal endometrial histopathology presented lower vascular indices; the only significant difference was in myometrial pulsatility index (p: 0.036). Conclusion: The most evident tool for evaluating the endometrium in tamoxifen-treated breast cancer patients is still the transvaginal measurement of its thickness. It exists that Doppler ultrasonicographic assessment of uterine, radial and spiral vasculature has no additional benefit for detection of endometrial pathology.

Key words: Doppler ultrasonography; Endometrial thickness; Tamoxifen; Transvaginal ultrasonography.

Introduction

Tamoxifen is the anti-hormonal treatment of choice for breast cancer patients with positive estrogen receptors. One of the most significant and deleterious effects is proliferation of the endometrium. Primarily, tamoxifen is an estrogen receptor agonist of endometrium. Tamoxifen develops cystic hyperplasia on the glandular epithelium within the stroma, whereas atrophy on the luminal epithelium. Histological changes on endometrium are seen in one-third of tamoxifen treated patients [1].

In an estrogen-rich environment, tamoxifen acts primarily as an anti-estrogen, whereas with low endogenous estrogens the agonistic effects may prevail. Relative risk of endometrium cancer does not increase in premenopausal women treated with tamoxifen, but 2-3 fold increases are seen in postmenopausal women [2, 3]. Increased risk is related to the longer duration and higher cumulative tamoxifen doses [1]. Breast cancer patients also have a higher risk of endometrial cancer. Many of estrogen-related risk factors are shared in both endometrial and breast cancer promotion [4].

Endometrial pathologies including polyps, hyperplasia, carcinoma, and malignant mixed mesodermal tumors have been identified in up to 35.5% of postmenopausal women who used tamoxifen [5].

Measuring endometrial thickness by transvaginal ultrasonography (TVS) stands out with its easy of the use and high sensitivity. When used in particular, with lower cut-offs of 4-5 mm, sensitivity reaches 95-100% for all endometrial lesions of postmenopausal women [6]. However, this cutoff value is not suitable for tamoxifen users. It is well known that women treated with tamoxifen frequently have a remarkable increase in endometrial thickness. Endometrial thickness from 5-10 mm has been proposed as the cutoff in several studies for tamoxifen users [2, 3, 5]. While a lower cutoff raises false-positive results, a higher cutoff raises specificity and reduces sensitivity. Dilated endometrial glands, dense edematous stroma and adenomyosis-like alterations in the proximal myometrium cause an increase in the endometrium measurement. Benign endometrial lesions as polyps and hyperplasia are more frequent than endometrial carcinoma in cases with thick endometrial measurements [2, 3]. To avoid unnecessary invasive procedures, new methods are being investigated to reduce false positivity.

In endometrial pathologies, marked changes in blood flow due to the lower impedance arising from the newly formed tumoral vessels could be defined by Doppler ultrasonography and the sensitivity of TVS could be increased. Doppler analysis of the uterine, myometrial and endometrial vasculature could be utilized to distinguish benign and malignant endometrial pathologies. Color Doppler studies in tamoxifen users demonstrated a
higher percentage of cases with subendometrial blood flow and lower impedance of blood flow in the uterine arteries. This last parameter seemed to correlate with the presence of benign pathologies in biopsies [3, 5].

The purpose of the study was to investigate the discriminated role of TVS and Doppler measurements on the detection of endometrial pathologies in tamoxifen-treated patients with breast cancer.

Material and Methods

Between February 1, 2009 and July 1, 2010 overall 98 breast cancer cases having positive estrogen receptors and tamoxifen-treated patients who were under medical observation at the Gynecology and Obstetrics Clinic of Izmir Ataturk Training and Research Hospital were followed using an investigative protocol.

All patients were treated by primary breast surgery. Adjuvant radiotherapy and/or chemotherapy could be included in the therapeutic plan, according to current guidelines which were accomplished before the start of endocrine treatment. Physiological menopausal status was established when at least 12 months had elapsed from the last menstrual period. In all premenopausal breast cancer patients, recording amenorrhea occurred after chemotherapy or chemo-induced menopause was established by rising serum levels of FSH. All patients were treated with 20 mg daily of tamoxifen (Novadex, AstraZeneca Pharmaceuticals LTD, Seoul, KOREA) with a range 3-7 convex array transvaginal sound system equipped with SonoaceX8 (MEDISON, CO, LTD, Seoul, KOREA) with a range 3-7 convex array transvaginal probe C3-7EP.

The endometrium was measured sonographically in double layers in the sagittal plane from the anterior endometrial-myometrial interface to the posterior. The vascularization of the uterine arteries was visualized with the color Doppler technique and blood flow velocity waveforms were obtained by placing the Doppler gate over the colored areas and activating the pulse Doppler function. The examination of the uterine arteries was made lateral to the cervix at the level of the internal os. The radial arteries were visualized and measured on the middle or inner layer of the myometrium to the anterior uterine corpus. The basilar and spiral arteries could be visualized within the endometrial strip. Power Doppler examination was carried out using a predetermined standardized setting (frequency 6.5 MHz, power Doppler gain 50, dynamic range 100 dB, edge 1, persistence 2, color map 1, gate 2, filter 3). The measurements such as uterine size, endometrial thickness, pulsatility index (PI), and resistance index (RI) of uterine, myometrial and endometrial vasculature were also recorded. Patients were assessed using TWS and color Doppler flow imaging every six months for the duration of the study. The endometrium was sampled by dilatation and curettage when the endometrial thickness was more than 6 mm or presented uterine bleeding. Diagnosis was based on histologic examination of the biopsy specimen.

The results obtained by transvaginal Gray-scale and Doppler ultrasound were compared with pathological diagnosis results, and then diagnostic value of Gray-scale and Doppler ultrasound was calculated as sensitivity and specificity.

Statistical analysis was carried out using SPSS 16.0 for Windows (SPSS Inc, Chicago, IL, USA) statistical software. Categorical variables were described using frequency distribution. For continuous variables, descriptive statistics were calculated and reported as median and inter quartile ranges. The Mann-Whitney U test was used to compare age, parity, age at first birth, duration of breastfeeding, exposure to tamoxifen, endometrial thickness, RI and PI of normal and abnormal endometrium. Spearman’s coefficient for correlation was carried out; \( p < 0.05 \) was accepted as the level of significance.

Results

Ninety-eight tamoxifen-treated patients with breast cancer were enrolled in this study, providing 141 ultrasound evaluations. The ultrasound examination was performed three times in 13 patients and two times in 30 patients. All of the patients were in an amenorrheic state. When the breast cancer was diagnosed, 25 of 98 patients were postmenopausal and 73 premenopausal. In 50 of 73 premenopausal patients, amenorrhea occurred after chemotherapy and in 23 patients by GnRH analogues. Of the patients 58.18% were diagnosed with early-stage breast cancer (Stage I, II). Mastectomy was performed in 64.26% of patients, breast conserving treatment in 35.71% of patients, and axillary staging was performed in all patients. Adjuvant chemotherapy and irradiation were applied in 87.75% and 80.61%, respectively. The mean and median exposure to tamoxifen was 21.9 and 17 months, respectively (95% CI 18.99-24.95).

Seventy-four endometrial curettings were performed in patients with more than 6 mm endometrial thickness or vaginal bleeding. The results were classified histopathologically as atrophic in 46 patients, as functional in 13 patients, as benign lesions (endometrial hyperplasia and polyps) in 12 patients, and as malignant in three patients.

All measurements of continued variables were compared between the subjects with normal and abnormal endometrial histopathology and are shown in Table 1. The ages of women with abnormal histopathology were higher and endometrium was thicker than that of women with normal histopathology. Radial artery and spiral artery vascular indices of women with abnormal histopathology were lower than for women with normal histopathology.

Continued variables which were obtained from the measurements of premenopausal patients were compared among women with normal and abnormal endometrial histopathology. Statistically significant differences of endometrial thickness and radial artery vascular indices were recorded for women with normal and abnormal endometrial histopathology (Table 2). Then continued variables of postmenopausal patient measurements were compared between the women with normal and abnormal
endometrial histopathology. The duration of breastfeeding and tamoxifen exposure was longer in postmenopausal patients with abnormal histopathology than that of women with normal histopathology. The statistically significant thickness of endometrium was also observed in postmenopausal patients with abnormal histopathology (Table 3).

The prevalence of an endometrium ≥ 6 mm was found higher in women with abnormal than normal histopathology (p: 0.008). When the cutoff was accepted as ≥ 6 mm, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of TVS were found as 100%, 55.8%, 46.8%, 100%, respectively. Endometrial blood flow could not be visualized in 18 of 77 measurements with endometrium thickness less than 6 mm and three of 64 measurements with endometrial thickness more than ≥ 6 mm (p: 0.002). We chose an endometrial thickness of 9.5 mm, radial artery RI of 0.66, and spiral artery PI of 0.52 as the cutoff points according to the area under ROC curve. Other AUC values were worthless. The results of ROC curve analysis are summarized in Table 4. No correlation was found between endometrial thickness with Doppler indices and duration of tamoxifen use.

Discussion

The role of TVS and Doppler measurements on the detection of endometrial pathologies was investigated in tamoxifen-treated women with breast cancer. Because of the increased risk of uterine malignancies in tamoxifen users, the patients are subjected to several diagnostic endometrial biopsies. Vaginal bleeding and thickening of the endometrial strip on TVS are the most common indications for this procedure. Usage of tamoxifen causes the endometrium to be measured as thickened on ultrasonography. The mean endometrial thickness is up to 10 mm and the rate of an abnormally thickened endometrial strip ranges from 75% to 98% in tamoxifen-treated patients with breast cancer. Of the cases 46-72% have histologically normal endometrium [2].

When the measurement of the endometrial thickness is used for the detection of endometrial pathologies, it may lead to a high false-positive rate and unnecessary invasive procedures. When the endometrial thickness cutoff is increased, it does not improve specificity. In our series, more than 10 mm and 6 mm of endometrial thickness prevalence was 32.6% and 45.4%, respectively. Of patients who underwent endometrial sampling 66.2% showed normal endometrial histopathology.

Develioğlu et al. investigated 60 patients with breast cancer and proposed a 9.5 mm endometrial thickness as the cutoff for all tamoxifen-treated patients regardless of menopausal status; the sensitivity and specificity of TVS were 89% and 78%, respectively [7]. In another study, Funk et al. followed 304 patients with breast cancer studied prospectively and obtained 1,061 measurements. When the cutoff of 9 mm for endometrial thickness was accepted, the sensitivity, specificity, PPV, and NPV were as 63%, 60%, 43% and 77%, respectively [8]. In a

Table 1. — Comparison of continued variables in patients with sampling results showing normal and abnormal endometrial histopathology.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal histopathology</th>
<th>Abnormal histopathology</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>median IQR</td>
<td>median IQR</td>
<td></td>
</tr>
<tr>
<td>Premenopausal patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44 (7)</td>
<td>50 (12)</td>
<td>0.106</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.6 (7.9)</td>
<td>28.8 (16.3)</td>
<td>0.415</td>
</tr>
<tr>
<td>Parity</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>0.717</td>
</tr>
<tr>
<td>Age at first birth (years)</td>
<td>22 (5)</td>
<td>19 (5)</td>
<td>0.188</td>
</tr>
<tr>
<td>Duration of breastfeeding (months)</td>
<td>11 (24)</td>
<td>12 (21)</td>
<td>0.711</td>
</tr>
<tr>
<td>Exposure to tamoxifen (months)</td>
<td>15.5 (23.2)</td>
<td>11.0 (22.5)</td>
<td>0.344</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>6 (8)</td>
<td>13 (7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Uterine artery pulsatility index</td>
<td>2.12 (0.88)</td>
<td>2.25 (1.54)</td>
<td>0.872</td>
</tr>
<tr>
<td>Uterine artery resistance index</td>
<td>0.84 (0.12)</td>
<td>0.88 (0.22)</td>
<td>0.592</td>
</tr>
<tr>
<td>Radial artery pulsatility index</td>
<td>1.92 (1.22)</td>
<td>1.24 (0.72)</td>
<td>0.033</td>
</tr>
<tr>
<td>Radial artery resistance index</td>
<td>0.80 (0.19)</td>
<td>0.68 (0.16)</td>
<td>0.027</td>
</tr>
<tr>
<td>Spiral artery pulsatility index</td>
<td>0.80 (0.40)</td>
<td>0.72 (0.58)</td>
<td>0.216</td>
</tr>
<tr>
<td>Spiral artery resistance index</td>
<td>0.53 (0.14)</td>
<td>0.49 (0.24)</td>
<td>0.215</td>
</tr>
</tbody>
</table>

Table 2. — Comparison of continued variables in premenopausal patients with normal and abnormal endometrial histopathology.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal histopathology</th>
<th>Abnormal histopathology</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>median IQR</td>
<td>median IQR</td>
<td></td>
</tr>
<tr>
<td>Premenopausal patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>22 (5)</td>
<td>22 (7)</td>
<td>0.188</td>
</tr>
<tr>
<td>Duration of breastfeeding (months)</td>
<td>11 (24)</td>
<td>12 (21)</td>
<td>0.711</td>
</tr>
<tr>
<td>Exposure to tamoxifen (months)</td>
<td>15.5 (23.2)</td>
<td>11.0 (22.5)</td>
<td>0.344</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>6 (8)</td>
<td>13 (7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Uterine artery pulsatility index</td>
<td>2.12 (0.88)</td>
<td>2.25 (1.54)</td>
<td>0.872</td>
</tr>
<tr>
<td>Uterine artery resistance index</td>
<td>0.84 (0.12)</td>
<td>0.88 (0.22)</td>
<td>0.592</td>
</tr>
<tr>
<td>Radial artery pulsatility index</td>
<td>1.92 (1.22)</td>
<td>1.24 (0.72)</td>
<td>0.033</td>
</tr>
<tr>
<td>Radial artery resistance index</td>
<td>0.80 (0.19)</td>
<td>0.68 (0.16)</td>
<td>0.027</td>
</tr>
<tr>
<td>Spiral artery pulsatility index</td>
<td>0.80 (0.40)</td>
<td>0.72 (0.58)</td>
<td>0.216</td>
</tr>
<tr>
<td>Spiral artery resistance index</td>
<td>0.53 (0.14)</td>
<td>0.49 (0.24)</td>
<td>0.215</td>
</tr>
</tbody>
</table>
prospective study with 279 patients, Markovitch et al. found that receiver curve analysis revealed 15 mm as the most accurate endometrial cutoff value for the diagnosis of endometrial pathologies and the sensitivity and specificity were reported as 38% and 63%, respectively [5].

In our study, endometrial sampling was not performed when endometrial thickness was less than 6 mm, because the prevalence of endometrial pathology was reported to be negligible in tamoxifen studies. We found a 9.5 mm endometrial thickness as the most advisable endometrial cutoff value for the diagnosis of endometrial pathologies according to receiver curve analysis and the sensitivity and specificity were as 73.3%, 50.8%, respectively.

Doppler studies in postmenopausal patients demonstrated that an increase in the endometrial thickness leads to an easier displaying of Doppler signals [9, 10]. The color Doppler studies in tamoxifen users demonstrated that a thicker endometrium shows a higher percentage of cases with subendometrial blood flow [2]. We could measure the endometrial blood flow in 81.5% of patients and established a higher percentage of endometrial blood flow in patients with an endometrial thickness of more than 6 mm.

The most prevalent pathology in women receiving tamoxifen is endometrial polyps. In cases with endometrial polyps, the existence of the pedicle artery makes blood flow measurement easier at the endometrial level.

### Table 3. — Comparison of continued variables in postmenopausal patients with normal and abnormal endometrial histopathology.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal histopathology</th>
<th>Abnormal histopathology</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmenopausal patients</td>
<td>median</td>
<td>median</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>64</td>
<td>43</td>
<td>0.011</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.8</td>
<td>23.1</td>
<td>0.064</td>
</tr>
<tr>
<td>Parity</td>
<td>2</td>
<td>9</td>
<td>0.371</td>
</tr>
<tr>
<td>Duration of breastfeeding</td>
<td>21</td>
<td>18</td>
<td>0.018</td>
</tr>
<tr>
<td>Exposure to tamoxifen</td>
<td>28</td>
<td>16</td>
<td>0.020</td>
</tr>
<tr>
<td>Endometrial thickness</td>
<td>5</td>
<td>9</td>
<td>0.010</td>
</tr>
<tr>
<td>Uterine artery pulsatility index</td>
<td>1.88</td>
<td>1.22</td>
<td>0.53</td>
</tr>
<tr>
<td>Resistance index</td>
<td>0.79</td>
<td>0.19</td>
<td>0.12</td>
</tr>
<tr>
<td>Radial artery pulsatility index</td>
<td>1.08</td>
<td>0.75</td>
<td>0.68</td>
</tr>
<tr>
<td>Resistance index</td>
<td>0.63</td>
<td>0.24</td>
<td>0.18</td>
</tr>
<tr>
<td>Spiral artery pulsatility index</td>
<td>0.73</td>
<td>0.40</td>
<td>0.19</td>
</tr>
<tr>
<td>Resistance index</td>
<td>0.50</td>
<td>0.17</td>
<td>0.10</td>
</tr>
</tbody>
</table>

The existence of the pedicle artery has a 94% PPV for endometrial lesions [11]. In our series, endometrial blood flow was measured in all patients with endometrial polyps.

A study which investigated the effect of tamoxifen on the uterus of postmenopausal healthy women in a randomized breast cancer prevention trial demonstrated that the women using tamoxifen had a lower impedance to blood flow in the uterine arteries [12]. Mean pulsatility and resistance indexes of uterine and spiral arteries in tamoxifen-treated breast cancer patients were significantly lower compared with normal postmenopausal women [13].

Fung et al. stated that the addition of color flow Doppler imaging to regular 2D ultrasound examination enhances the sensitivity of the ultrasound examination. The results of the study in which 304 patients were followed prospectively showed endometrial thickness greater than 9 mm and spiral artery pulsatility index measurement to be associated with significant uterine abnormalities [8]. However on the contrary, there were studies which were unable to define a significant predictive role for uterine artery Doppler indices in predicting endometrial pathologies in patients treated with tamoxifen [7, 14].

In this study lower vascular indices were found in patients with abnormal endometrial histopathology. However, the difference did not reach statistically significant levels for most measurements. Doppler indices were obtained by measurements not only for uterine, but also myometrial and endometrial blood flows. There was no difference between patients with normal and abnormal histopathology in terms of Doppler indices of the uterine artery. Radial artery pulsatility and resistance index were
found significantly lower in all patients with abnormal histopathology. These lower values of radial artery pulsatility indices were found not only in the average of all patients but also in premenopausal patients with abnormal histopathology.

Endometrial pathologies have been suggested to be associated with long-term and high cumulative doses of tamoxifen administration to breast cancer patients [1,7]. In patients receiving tamoxifen, particularly in those who start therapy many years after the onset of menopause, the risk of developing endometrial pathology increases. It should be closely monitored by TVS and color Doppler imaging to detect endometrial lesions [15]. We determined that the association with pathologic endometrium and tamoxifen exposure was only in postmenopausal patients.

In this study, using TVS scans with color Doppler imaging, we detected only three endometrial cancers exposed to tamoxifen from 141 ultrasound examinations. Because of the scarcity of malignant cases, the measurement of Gray-scale and Doppler ultrasound revealed no statistically significant results.

In conclusion the most evident tool for evaluating the endometrium in tamoxifen-treated breast cancer patients is still transvaginal measurement of its thickness. Doppler ultrasonographic assessment of uterine, radial and spiral vasculature has no additional benefit for detection of endometrial pathology. The duration of exposure is the most important prognostic factor for endometrial disturbances of tamoxifen-treated breast cancer patients. In our study, radial artery Doppler indices showed little evidence for discovering the endometrial pathologies in tamoxifen-treated breast cancer patients but it was not found to be superior to TVS measurement of the endometrium.

References


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E-mail: drinimbezircioglu@yahoo.com
Status quo and prevention of overtreatment in cervical diseases

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Summary

The objective of this paper was to review the diagnosis and treatment of cervical diseases. Often, due to improper judgment of interventional indications for cervical lesions, overtreatment to various degrees takes place, influencing patients' health and lives. This review analyzes the expression, causes and negative aspects of overtreatment of cervical lesions, and discusses the available therapeutic methods for cervical lesions, to remind gynecologists to master the interventional indications for proper treatment and avoid overtreatment, so as to achieve normalization and individualization in treating gynecologic diseases.

Key words: Cervical Diseases; Overtreatment; Prevention.

Introduction

Cervical lesions refer to various pathological changes happening in the cervix, including benign cervical lesions, cervical intraepithelial neoplasia (CIN), cervical cancer, etc. [1]. In recent years, medical knowledge has spread, diagnostic techniques have developed, and women's healthcare sense has increased, and accordingly the majority of cervical diseases can be diagnosed in early stages and treated in time. However, even though most patients are cured, because of insufficient control of the interventional indications for cervical lesions, overtreatment can occur, such as unnecessary therapy, complications of simple treatment, etc., affecting patients' health to various degrees. The paper discusses various issues, for example, whether it is necessary to treat or not, what therapy should be adopted, what the interventional indications are for various therapeutic methods, etc., with a view of making the treatment of cervical lesions more normalized and accurate.

Status quo of overtreatment of cervical diseases

Overtreatment of cervical lesions

a) Treat patients unnecessarily: carry out treatment of patients with benign lesions and physiological phenomena which do not require treatment, such as cervical hypertrophy, cervical Nabothian cyst, cervical columnar ectopy, etc., which do not belong to infectious cervical diseases, are free of clinical symptoms, and are not combined with other cervical lesions, as well as some patients with follow-up CIN 1 (without clinical symptoms, without apparent abnormalities under colposcope, human papilloma virus (HPV)-negative).

b) Improper treatment method selected for cervical lesions requiring treatment: for example, patients with a cervical polyp, true erosion or CIN 1, who could be treated with therapy such as laser, electrosurgery, cryotherapy, microwave, focused ultrasound, etc., use of loop electrosurgical excision (LEEP) instead; young patients with CIN 3 who can undergo cervical conization, as well as patients who desire children who have early cervical cancer and can undergo radical trachelectomy, undergo hysterectomy instead; while carrying out hysterectomy on young patients with cervical cancer, both ovaries are removed simultaneously. Cervical conization performed with the purpose of diagnostic clarification belongs to the diagnosis, rather than over-treatment [2-4].

Causes for overtreatment

Patient factors: due to lack of medical knowledge, many patients think that once they see a doctor, the doctor has to find a disease, and if a disease exists, therapy must be undertaken; only the doctor is “responsible” and “has expertise”. Additionally, due to fear of diseases, the patients often ask doctors to choose a therapeutic method stronger than necessary, even at the cost of losing the uterus, in order to obtain some so-called “peace”.

Physician factors: lack of normalization of the diagnostic and therapeutic process: for example, the current screening of cervical cancer and precancerous lesions mainly follows a three-step procedure: ThinPrep cytology (TCT) and/or HPV, colposcopy, and histology. TCT with HPV detection is the main method for examination of cervical lesions. When TCT and/or HPV detect abnormalities, then a colposcopic examination is carried out and a biopsy is taken at the suspected locations. The issue is that without carrying out a basic TCT examination and necessary HPV detection, if the visual examination finds abnormalities, the doctor will determine the treatment; or otherwise, without TCT and HPV examinations, colposcopy is chosen directly, and the diagnosis is made according to colposcopic images; and even without taking living tissue, a CIN diagnosis is made. Moreover

Summary

The objective of this paper was to review the diagnosis and treatment of cervical diseases. Often, due to improper judgment of interventional indications for cervical lesions, overtreatment to various degrees takes place, influencing patients' health and lives. This review analyzes the expression, causes and negative aspects of overtreatment of cervical lesions, and discusses the available therapeutic methods for cervical lesions, to remind gynecologists to master the interventional indications for proper treatment and avoid overtreatment, so as to achieve normalization and individualization in treating gynecologic diseases.

Key words: Cervical Diseases; Overtreatment; Prevention.

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the phenomenon that treatment is carried out directly based on TCT and HPV results without pathological examination also exists. Confronted with patient preoccupation about whether the existing disease will progress or turn into malignity, to reduce medical disputes possibly caused by inestimable disease progress, without considering the possible complicating diseases and negative results from treatment, carry out the interventional therapy too early on during the follow-up of lesions [5].

**Overtreatment**

While bearing the economic burden, the patients suffering from overtreatment also undertake the possible disadvantages from the treatment itself to various degrees. Under normal conditions, the cervical columnar epithelium can secrete mucilage, forming a mucous plug, which not only wets the vagina but also plays the role of barrier against infection. Additionally, a sufficient amount of mucilage facilitates the passing of sperm. Treatment can influence the function of columnar epithelium to various degrees, reducing the mucous and adversely affecting passing of sperm. Especially after carrying out LEEP surgery, possible cervical stenosis, even atresia, may affect conception. Moreover, cervical incompetence caused by too deep cervical conization to the internal cervical orifice may cause complications such as miscarriage, etc. After treatment, patients must also face problems such as short-term secretion increase, exuvial hemorrhage, secondary infection, short-term abstinence of sexual activity, etc.

**Prevention of overtreatment of cervical diseases**

Effective preventive measures for treating cervical cancer are: master the intervention indications for treatment of cervical lesions, select proper therapeutic methods, and comply with the principles of normalization, individualization and humanization. Depend on the type of lesion, choose local administration, therapy (including laser, cryotherapy, infrared, microwave, electrosurgery, focused ultrasound), cervical conization, radical trachelectomy, hysterectomy, etc. [6].

**Benign cervical lesions**

Overtreatment of benign lesions, mainly cervicitis and cervical dysplasia (cervical polyp), mainly originate from misunderstandings generated about infective cervical diseases. Previously, the description of cervicitis was taken from the concepts of chronic cervicitis and acute cervicitis, thinking that chronic cervicitis comes from the incomplete treatment of acute cervicitis, including cervical erosion, cervical Nabothian cyst, cervical hypertrophy, cervical polyp, endocervicitis, etc. However, the concept of chronic cervicitis has been discarded at present, and only the term cervicitis has been adopted, i.e., limited to inflammation of the uterine cervix and vagina, as well as mucous membranes of the canal of uterine cervix [7]; moreover, the diagnosis can only be made when one of two characteristic signs is available (when rubbing the canal of the uterine cervix with a cotton swab and purulent secretion is visible or bleeding is induced) and leukocyte examination of secretion and the determined pathogenic examination results [8].

How should the various expressions of chronic cervicitis in the old days be regarded? In the past, so-called cervical erosion consisted of two cases, one was under physiological conditions, i.e., exfoliation of cervical squamous metaplasia caused by change of the vaginal acid environment following fluctuation of estrogenic levels, is covered by the columnar epithelium and immature squamous metaplasia, thus it seems like erosion (also called fake erosion); in fact, it is the columnar epithelium in the wide transformation zone and results from ectopy of the squamous columnar junction, normal physiological phenomena, known as “cervical columnar epithelium ectopy”. Cervical erosion belonging to pathologic phenomena refers to epithelial exfoliation caused by pathogenic infection (herpes simplex virus, syphilis, Chlamydia trachomatis, Neisseria gonorrhoeae, etc.), i.e., the commonly called true erosion [9]. Therefore, cervical erosion seen colposcopically is differentiated as physiological or pathologic according to whether there are clinical symptoms or not, as well as the examination results of secretions and pathogens. Pathologic erosion should be treated actively, and the treatment should be mainly focused on the causes of erosion; for columnar epithelium ectopy, if the cervical cytological (or HPV) examination is normal, and there are no symptoms such as increased secretions, contact bleeding, etc., there is no need to treat; if the cervical columnar epithelium ectopy shows symptoms or co-infection, carry out the corresponding local treatment (medication or physical therapy), but LEEP is not advisable. However, the current treatment of cervical columnar epithelium ectopy should attend to two phenomena – one is overtreatment, neglecting the physiological and pathologic examination, and the other is negligence of its similarity to cervical precancerous lesions due to the wrong opinion that cervical erosion belongs to chronic inflammation, so that the diagnosis and treatment are delayed because of no cytological screening [10, 11].

Today, there is still no determinant standard for the diagnosis of cervical hypertrophy; if patients do not show clinical symptoms or any other pathologic changes, there is no need to perform therapy generally.

Cervical Nabothian cysts are formed from gland secretions retained inside the gland duct, which is narrowed or blocked because the hyperplasia of connective tissue around the gland duct or the scar presses the gland duct. Afterwards new squamous metaplasia covers the cervical gland duct or enters into the duct and blocks its mouth during formation of the transformation zone, without special clinical meaning. It can be followed up regularly, and no treatment is needed. If the cyst is too large, showing clinical symptoms or co-infection, then it should be treated with laser [12].

Since it may cause symptoms such as contact bleeding or increased secretion, cervical polyps should be extirpated preferably with surgery. If the polyp is located inside the cervical tube, hysteroscopic polyp electri-
Cervical intraepithelial neoplasia (CIN)

CIN was reported by Richart for the first time in 1967. The risks of CIN developing into carcinoma in situ and invasive cancer are 20 times and 7 times greater in normal people, respectively [15]. The risks of CIN I, CIN II and CIN III developing into cancers are 15%, 30% and 45%, respectively. CIN develops slowly, showing certain progress, but it may regress or reverse. The development from CIN I, CIN II and CIN III into cervical cancer takes several years, even more than ten years [16]. The selection of a therapeutic method for patients should comply with the individualization principle, i.e., considering the treatment is integratedly according to the patient’s age, procreation, lesion range and level, and follow-up condition. Among CIN I patients, 15% may further develop, 20% will remain constantly, and 65% will regress naturally. If these patients do not also have highly dangerous HPV infection, carry out the regular inspection and monitor them closely. For patients with cytological ASC-US, who are HPV positive, or showing positive after converting negative, local physical treatment should be carried out [17]. If patients also suffer from middle- or high-grade cervical columnar ectopy and are HPV positive requiring prompt treatment; local physical treatment and/or medication should be carried out. CIN II patients can select the therapy according to specific conditions: if CIN III patients have severe pathologic atypical hyperplasia, LEEP or cold knife conization (CKC); at the same time, excised samples need to be labeled. If it is carcinoma in situ CKC or hysterectomy should be performed. If the edge of the conization sample still has a CIN II focus, it should be reexamined after three months, and it is inadvisable to carry out surgical treatment again immediately. If the edge of the conization sample still has a CIN III focus, colposcopy should be done after one month, and then handled properly. If CIN III patients are older without child-bearing desire and requiring hystrectomy, surgery should be carried out [18-20].

The general principles for CIN treatment during pregnancy are as follows: watch conservatively and delay the therapy. During pregnancy, under the influence of estrogen, cervical squamous metaplasia thickens, basal cell hyperplasia is active, and the gland hypertrophies, which may make the exfoliated cells show phenomena such as nucleus augmentation, stronger staining, etc., so that the cytological examination is likely to misdiagnose it as cervical CIN. Cytological changes of the uterine cervix during pregnancy can recover six weeks after child-birth generally [21]. Therefore, it is advisable to carry out the examination again after giving birth, diagnose again, and treat the patient at that time. For pregnant women with CIN I or II, the treatment can be suspended, with regular cytological examination and colposcopy follow-up. The patient should be reexamined six weeks after giving birth; if the result is still CIN I or II she should be treated based on the non-pregnancy period. CIN III treatment should be determined according to gestational weeks. In principle follow-up should be carried out closely, and it is unnecessary to terminate the pregnancy. At the 6th–8th week after giving birth, if the cytological examination and biopsy are still CIN III, the patient should be treated according to CIN III during non-pregnancy or according to the patient’s wishes, the pregnancy should be terminated first and then cervical conization should be carried out. Cervical conization during pregnancy will increase the risk of bleeding, miscarriage and premature delivery [22-24].

For patients with early cervical cancer who want children, it is advisable to carry out radical tracheectomy plus pelvic lymphadenectomy or biopsy. However, the following conditions must be satisfied: the patients possess no sterile factors, FIGO staging is IA2-IB1, colposcopy shows that there is no invasion above the internal cervical orifice, no regional lymph node metastasis, no parametrial or uterine implication, and no blood vessel or lymphatic vessel invasion [25].

Know the function of cervical conization during the diagnosis and treatment of cervical lesions: cervical conization includes LEEP and CKC [26]. These are used for the diagnosis and treatment of cervical diseases; not only do they cut off the focus, but they also provide the sample for pathologic examination. However, the abuse of LEEP or CKC currently exists. Some doctors regard LEEP as the gold standard treatment of cervical diseases whether the disease is a pathologic phenomenon or not, and whatever the type of cervical disease is, LEEP is resorted to. For some patients with cervical columnar ectopy of a larger cervical area or CIN I, LEEP is also adopted. Diagnostic conization is mainly suitable for cases where the colposcope cannot see the border of cervical lesions, or the main focus is located inside the canal of the uterine cervix [27], exceeding the colposcopic range; cytological or histological evidence proves that cervical gland epithelium has a precancerous lesion or carcinomatous change. Pathologic results of samples from endocervical curettage report, abnormal or unsure; cytological results, colposcopic results and living biopsy results are inconsistent; the cytological, colposcopic and living biopsy results are suspicious cervical invasive cancer; the cervical biopsy pathologic diagnosis is CIN; however, cervical micro-invasive cancer or invasive cancer can not be excluded definitely. In comparison with diagnostic conization, therapeutic conization is widely used in the treatment of cervical diseases, with advantages such as short surgical duration, rapid recovery, little pain, low expense, and readily accepted by patients. Today, the comparative research on cervical conization and hysterectomy for CIN III finds that there is no statistical difference in prognosis between these two surgical methods [28, 29]. Cervical conization is a reasonable selection for young patients with cervical CIN III, cervical squamous cell carcinoma in situ and Stage IA cervical invasive cancer especially if they want children.

For older patients, those who have finished child
bearing and patients with incomplete incisional margins in cervical conization samples with CIN III together with benign diseases such as uterine myoma or uterine pro lapse, cervical adenocarcinoma in situ, early micro-invasive carcinoma, conization can be performed with the hysterectomy [30].

In summary, overtreatment of cervical lesions can be eliminated or at least reduced by mastering the interventional indications for cervical lesions, and by selecting the therapeutic measures for cervical lesions, which are more compliant with the principles of normalization, individualization, humanization and minimal invasion advocated in the current treatment of gynecological diseases.

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References


Sentinel lymph node detection by intranipple injection of patent blue dye in breast cancer: a preliminary report of a feasibility study

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Summary
Sentinel lymph node (SLN) biopsy is a well established option for assessing axillary lymph node status in breast cancer. Several techniques have been applied so far (superficial or deeper ones). Based on anatomical features of the lymphatic drainage in the breast, we assessed the feasibility of an intranipple approach for SLN mapping. Our data support the feasibility of SLN detection by our technique, with a high rate of SLN identification, which could be used in clinical practice as an alternative to the peri-areolar approach.

Key words: Sentinel lymph node detection; Breast cancer; Patent blue; Intranipple injection; Techniques for SNL identification.

Introduction
Sentinel lymph node (SLN) evaluation can accurately predict the regional nodal status [1], and hence, there is a growing trend for SLN biopsy to replace axillary dissection in clinically node-negative cases. Although considered routine, several techniques are applied regarding the site of injection of the tracer. Nearly all anatomic boundaries of the external superficial anatomy have been used as sites of injection (dermis, dermo-areolar boundary, areola). However, no report has identified the intranipple route as an alternative so far. Herein, we present a multicentric pilot study regarding intranipple administration of a blue tracer as an alternative approach of SLN detection in an effort to investigate if such a technique is feasible and simple to perform.

Material and Methods
The study was approved by the Ethics Committees of the four participating institutes: Lito Maternity Hospital of Athens (Greece), Rea Maternity Hospital of Athens (Greece), University Hospital of Ioannina (Greece), and Saint Joseph University of Beirut (Lebanon).

Sixty-seven patients with breast cancer and non-palpable axillary nodes (N0) were enrolled in the study. All patients were informed about treatment options and opted for axillary lymph node dissection. After the management plan had been established, the patients were informed about the current research protocol and gave their written informed consent. The mean age of the participants was 50.7 (SD = 10.19) years, ranging from 26 to 80 years. SLN detection was performed intraoperatively. An intranipple injection (via major mammary ducts) of 2 ml of patent blue solution was administered, followed by a gentle massage of the breast for 3-5 min (Figure 1). Fifteen minutes post-administration, a standard axillary lymph node dissection was performed. Any node marked as blue was removed separately and sent for pathology evaluation. The rest of the lymph nodes as well as the breast specimen followed.

Results
At least one SLN was detected in 62 out of the 67 patients enrolled (SLN detection rate: 92.5%). The overall mean number of SLNs colored was 1.86 (SD = 1.2). By omitting the five cases where no SLN was detected, the mean number of SLNs colored was 2.0 (SD = 1.1) which did not differ significantly (p = 0.5) from the mean number of SLNs in the total number of patients.

Taking into account that the lymphatic system of the breast is vast (comprising a network over the entire surface of the chest, neck, and abdomen with increased density under the axilla), it could be suggested that the proportion of patients in whom lymph nodes were colored (92.5%) is the sensitivity of the method in terms of its capability to color lymph nodes [1]. In cases of no SLN coloration, we proceeded to axillary lymphadenectomy (with negative nodes in all cases). Taking into account the negative results for cancer cases after axillary lymph node dissection (56 cases) and negative results for cancer SLN cases (50), the negative predictive value (NPV) of the method was 89.3%.

In three patients, although our method showed a colored negative SLN for cancer, after axillary lymphadenectomy, it was proven that the axillary nodes were positive for cancer. This could be explained as a “skip” phenomenon or as a false-negative result of our method.

Even if we assume that all of these three cases (from a total of 62 colored cases) were false-negatives, the sensitivity of the method is high (95%) in terms of its capability to correctly identify the “true” sentinel nodes.
Figure 1.—Intrinipple injection before batwing procedure.

No local infection or wound complications from the nipple were observed, underlying the safety of the procedure. Similarly, patient satisfaction was very good and not even minimal morbidity was related to the method.

Discussion

In the past, lymphatic mapping in breast cancer, performed solely by intraparenchymal injections of blue dye, remained an accepted method of identifying sentinel nodes, largely because of its simplicity. However, the technique was associated with a marked learning curve, variable identification rates of sentinel nodes, and high false-negative rates. Later on, dye injections into the subareolar plexus demonstrated a high sentinel node identification rate, low false-negative rate, and rapid learning curve.

The concept of the intranipple administration of a tracer is based on certain anatomic characteristics of the breast: following the galactophore ducts, each periductal lymphatic plexus converges into Sappey’s subareolar plexus which is the major anatomic site where lymph from different breast sites is mostly drained [2]. The lymph from the nipple also drains into Sappey’s subareolar plexus [3], and then to axillary nodes, whereas a small lymph portion is directed to the internal mammary glands and other adjacent lymph node groups [2, 4]. It was also shown that breast dermis lymphatics are concentrated in the nipple-areolar area [5]. By combining these together, it can be hypothesized that the tracer can mostly be accumulated in an axillary lymph group that should be the sentinel node.

The major advantages of this technique are:

a) easiness to perform,

b) short learning curve and

c) standardization of injection site.

Finally, as mentioned above, wound complications were not observed with our technique, in contrast with peritumoral injection (discoloration of the skin for many months) [6, 7].

The blue dye used has been considered as biologically inert and, as such, it has been allowed to be injected for SLN detection. However, several objections could rise regarding potential harm in the breast parenchyma, although no serious (or mild) adverse effects have been observed in our patients.

Pressure-induced damage due to the dye could also be hypothesized. However, both galactography and ductoscopy so far (both increasing parenchyma pressure) have not been reported as harmful procedures. Finally, possible iatrogenic infections due to a major mammary duct approach are unlikely by applying a good antiseptic technique.

A previous study by Kern et al. with smaller samples concluded definitely that: “on the basis of these findings, we propose that injections into the subareolar lymphatic plexus are the optimal way to perform dye-only lymphatic mapping of the breast” [8].

Taking into account that in the cases of no SLN colorization we proceeded to axillary lymphadenectomy (with negative nodes in all cases), our method “retains” its safety. This approach (if no blue nodes are detected; a standard axillary dissection is performed) is the usual practice published in previous studies [9]. However, in the previous studies, although a greater number of patients were included, the percentage of no colored nodes with blue dye - 18% (47 out of 260) was much higher compared to our data.

Conclusion

Although we believe that our results almost prove that the “intranipple injection” approach is feasible in detecting the SLN of the breast and that this technique could be used in clinical practice as an alternative to the periareolar or subcutaneous approach, the technique needs to be validated in larger studies.

References


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Leiomyosarcoma of the vulva

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Introduction

Primary sarcomas of the vulva are very rare gynecologic malignancies, accounting for 1-3% of all vulvar malignancies [1-3]. The tumor can be mistaken as a benign lesion as is described in the literature when it occurs in the Bartholin’s gland area [4, 5].

Leiomyosarcoma is the most common histologic type of vulvar sarcoma [6], although also reported liposarcomas, neurofibrosarcomas, angiosarcomas and epithelioid sarcomas have been reported [3].

The location of the leiomyosarcoma is in the labia majora, followed by, in decreasing order, the Bartholin gland area, clitoris and labia minora [7].

The age of the patients varies between 31 and 69 years and the size of the tumor varies from 2-10 cm [8].

The case of a patient who had a leiomyosarcoma growth in the left labia majora is reported.

Case Report

A 71-year-old, gravida 3, para 2, postmenopausal German woman presented with a vulvar nodule. The patient consulted a gynecologist for a preventive medical checkup. She had an unremarkable gynecology history and she had never used any kind of hormonal therapy. The family history was lung cancer of the patient’s mother. The gynecology history of the family was unremarkable.

Clinically there was a painless vulvar mass, 2 cm in diameter, which was initially thought to be a myoma. A biopsy had already been done when the woman came to our hospital. Histology showed an infiltrate margin of a tumor composed of interwoven fascicles of spindle cells. The mitotic count was 10 per high-power field and the nuclear atypia was grade 2. A leiomyosarcoma was diagnosed and surgery was planned.

On palpation, there was still a solid mass and a hematoma which was not attached to skin or bone. There were no palpable groin inguinal lymph nodes. The uterus was hardly circumscribable and in ultrasound it appeared enhanced. Computed tomography (CT) of the abdomen followed. The uterus was manifold lobated and the absorption of the barium meal was irregular. The uterus seemed to be necrotic. Malignancy of the uterine tissue could not be ruled out. X-ray of the chest, CT of the liver, coloscopy and urethroscopy were without any pathology.

Preoperatively we planned a wide local excision of the leiomyosarcoma with adequate margins done as a hemivulvectomy of the left side. In addition to this hysterectomy and salpingo-oophorectomy were carried out as well as inguinal lymphadenectomy.

Pathology examination of the entire specimen demonstrated an uterus with multiple myomas encompassing sizes up to 6 cm diameter with no malignant aspects. The ovaries were inconspicuous.

In the preparation of the vulva the intermediate differentiated leiomyosarcoma was seen with free surgical margins. Inguinal and pelvic lymph nodes were negative for leiomyosarcoma. Tumor cells were immunoreactive for smooth muscle actin, desmin and ki-67 antigen.

Because of the direct location to the anus – there were 7 mm between the tumor and anus – radiotherapy was not possible.

Fifteen days after the operation the patient left the hospital in good condition. Close-meshed examinations were suggested.

Discussion

As already mentioned, sarcomas of the vulvar are rare and they appear painless. Symptoms are often only enlarging nodularity and vulvar discomfort. The vast majority of smooth muscle tumors of the female genital tract occur in the uterus, and for this area criteria for distinction between leiomyomas, leiomyosarcomas and smooth muscle tumors of uncertain malignant potential have been formulated.

Clinically leiomyosarcomas can be mistaken as benign processes, such as a Bartholin cyst, infectious granuloma, fibroma, lipoma, or as in our case as myomas [9].

If misdiagnosed, the management is inadequate and the malign tissue expands.

Our knowledge of this disease is limited because of the rarity of these tumors. In 1996 Nielsen et al., delineated 36 cases of leiomyosarcomas of the vulva [10]. At least

Summary

Malignant tumors of the vulva soft tissue are uncommon. About 1-3% are sarcomas. They can be mistaken as benign lesions, leading to misdiagnosis and mistreatment. A case of a 71-year-old woman with a leiomyosarcoma of the vulva is presented. The surgical excision of the lesion is described and there were no additional malignancies or lesions found. There was no need for adjuvant therapy.

Key words: Vulvar carcinoma; Leiomyosarcoma.
Leiomyosarcoma of the vulva

where radiation is required; these are cases with high-grade tumors, margin involvement or tumor size > 5 cm [13].

In conclusion we would like to mention that this disease is extremely rare and because of the small number of cases further studies should be done. Nevertheless any vulvar lesion with unusual characteristics in the labia majora or Bartholin gland area should be carefully and promptly studied. Based on this, the role of hormones of the tumor should be elucidated because of the higher percentage during reproductive years and especially during pregnancy.

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Bilateral juvenile fibroadenosis of the breast: management with subcutaneous mastectomy and silicone implant placement

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Introduction

Fibroadenomas are the most common fibroepithelial benign lesions of the breast affecting adolescent girls and young women, with an incidence of 7-13% in this age group [1-3]. Generally, the tumor presents as a round, elastic, nodular, nontender palpable mass, measuring up to 3 cm in diameter [2]. Fibroadenomas can be solitary or multiplex, sub-classified as simplex (80%) and complex (20%), respectively, or histologically classified as intracanalicular, depending on the relative amount of epithelial and stromal tissue present [2, 3]. The epithelial elements of a fibroadenoma can exhibit a spectrum of proliferative lesions [2, 4]. Usually the diagnosis is made clinically, based on physical and ultrasound examination, and confirmed by core biopsy [3]. The decision to perform a biopsy of the lesion is based on the Breast Imaging-Reporting and Data System (BI-RADS) criteria of the American College of Radiology [3]. The risk of malignant transformation to any fibroadenoma is low (0.0125-0.3%), with approximately 100 cases reported in the literature [2, 5]. Fibroadenomas are believed to be stimulated by estrogen and progesterone, pregnancy or lactation, and then to regress, lose their cellularity and become smaller in menopause. In young patients with histologically proven fibroadenomas, the current management tends to be more conservative, with a recommendation of 6-month sonographic surveillance [4]. The invasive principles are in situ cryoablation (in lesions < 4 cm), percutaneous ultrasound-guided vacuum-assisted eradication and local excision of the lesion through a circumareolar or inframammary incision [1-7].

In 5-10% of all adolescent fibroadenomas, the uncommon juvenile, or giant variant is present, characterized by a diameter larger than 5 cm, rapid enlargement, or a weight greater than 500 g [1, 2, 5]. Up to 25% of these patients have multiple bilateral tumors. The etiology is believed to be tissue hypersensitivity to normal levels of estrogen [4, 6]. The involved breast is enlarged with prominent superficial veins, stretched, and has an enlarged areola. Juvenile fibroadenomas usually harbor one or more complex features, including epithelial calcifications, papillary apocrine metaplasia, sclerosing adenosis, and cysts [2, 3, 5]. Dupont et al. found that the cumulative risk of breast carcinoma in females with complex fibroadenomas was 3.1-3.72 times that of women in the general population [3]. Despite the benign behavior of these lesions, their large size and multiple and bilateral presentation can cause physical deformity, discomfort or emotional distress for the patient and a challenge for the surgeon both in diagnosis and in selection of the best therapy [3, 5]. Treatment is usually surgical and ranges from simple excision to subcutaneous mastectomy with reconstruction [5-7].

Case Report

A 25-year-old nulliparous woman was admitted to our surgical department in February 2005 with multiple, bilateral, firm, well-circumscribed, mobile masses in the breasts. The diameter of the lesions varied between 1-8 cm, occupying the entire breast. Some of the larger lesions caused deforming skin protuberances. There was no palpable regional lymphadenopathy. Ultrasonography of the breasts revealed several bilateral, round, circumscribed, hypoechoic lesions, ranging in size from 0.5 to 7 cm in diameter. Sonography-guided core biopsies of a couple...
of the largest lesions of both sides confirmed benign juvenile fibroadenomas. According to the patient, the first lesion was palpated at the age of 17. The patient also reported being on hormonal contraception for the previous two years, during which she reported rapid tumor progression. Hormonal assays were normal, as was sonography of the lesser pelvis. Magnetic resonance (MR) imaging ultimately identified a total of 34 separate breast lesions, consisting of 19 in the right and 15 in the left breast, with a range of 5 to 77 mm in diameter (Figure 1 a/b).

The patient was highly anxious and concerned about the possibility of diagnostic inaccuracy, progression of the proliferative lesions and probable cosmesis resulting from the surgical removal of the lesions. A multidisciplinary consultation of the institutional breast oncology board, involving plastic surgeons and a psychiatrist, recommended bilateral subcutaneous nipple-sparing mastectomy and reconstruction with implants. The decision for this major procedure, in accordance with the expectations of the patient, was confirmed by the following: (i) breast tissue might be replaced by the lesions, (ii) optimal oncological solution and accurate histopathological investigation of each lesion can be provided with the minimalization of recurrence, (iii) the disease is managed both oncologically and cosmetically within a short time without multiple subsequent admissions, and (iv) optimal cosmesis can be achieved. In February 2006, bilateral subcutaneous mastectomy was performed. The pathological investigation revealed simple and complex juvenile fibroadenomas in all 37 lesions, without any malignancies. Two months later, cohesive textured-surface silicone implants (McGhan Style 410ML 220 g) were placed submuscularly with superior pedicle vertical scar mammoplasty, resulting in excel-

Figure 1. — a/b: Preoperative MR images of the breasts: the morphologic features of most of the fibroadenomas were generally similar, consisting of smooth margins and round or lobulated shape. Inner septations with heterogeneous structures have been found in a marked part of the tumors, making the accurate differentiation of phyllodes tumors or even of malignant neoplasms impossible.

Figure 2. — a/b: Excellent cosmetic result after reconstruction.

Figure 3. — A control MR image made during the 30th month of follow-up, after performing bilateral subcutaneous mastectomy and submuscular placement of silicone implants. A recurrent fibroadenoma (20 mm in diameter) in the minimal remnant of the retroareolar gland in the right breast was confirmed by FNA.
Bilateral juvenile fibroadenosis of the breast: management with subcutaneous mastectomy and silicone implant placement

Discussion

Bilateral breast fibroadenosis has been related to familial (Carney complex) and hormonal factors (the effect of oral contraception is still a matter of debate), as well as an association with the use of cyclosporin A in female renal graft recipients [1, 5]. Since there are no definite clinical or radiological criteria to differentiate fibroadenomas from phyllodes tumors or carcinoma (especially mucinous carcinoma) developing in a fibroadenoma, histopathological examination of all fibroadenomas should be routinely performed [2]. The goals of treating fibroadenosis are the complete resection of the lesions and a symmetrical cosmetic result [1, 2, 5]. Different approaches to tumor excision and subsequent breast reconstruction have been described [1-7]. The successful use of ultrasound-guided, vacuum-assisted breast biopsy technology (Mammotome system, Ethicon Endo-Surgery, Inc., Cincinnati, OH) for the eradication of small-volume bilateral fibroadenomas was reported [7]. Beyond a certain total volume of a solitary giant or multiple fibroadenomas, enucleation alone is inadequate, and additional reconstructive techniques are necessary for a symmetrical result [2]. According to this principle, a reduction mammoplasty would appear to be the ideal solution if there is sufficient uninvolved parenchyma and the tumors can be completely excised [1, 5]. Successfully treated cases of bilateral fibroadenosis were reported using reduction mammoplasty with the McKissock, Wise or Rezai modified Ribeiro technique [1, 5]. A disadvantage of these techniques is the possible novel formation of fibroadenomas in the remaining breast tissue [2, 3, 5, 7]. For selected patients with bilateral fibroadenosis, the major procedure of subcutaneous nipple-sparing mastectomy with expander-to-implant reconstruction may be the procedure of choice, minimalizing the risk of recurrence, reducing scars and diminishing the need for multiple subsequent admissions that would be a burden, both physically and psychologically, to the young patient [1, 5]. The loss of the potential to breastfeed in these women of childbearing age should be assessed by the surgeon for careful consideration of therapeutic options [2, 3, 6].

Conclusions

Although juvenile bilateral fibroadenosis is a benign disorder, choosing the most optimal therapy, which treats both the physical and psychological aspects of the disease at a young age, is a challenge. To achieve an optimal solution, a multidisciplinary consultation, strict clinical surveillance and the education of the patient and family are mandatory.

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Primary ovarian small cell carcinoma of pulmonary type with enlarged paraaortic lymph node masses: a case report and review of the literature

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Summary

Introduction: Small cell carcinoma of the ovary of pulmonary type, is a rare, aggressive tumour with poor prognosis and its optimal management is unclear. Case presentation: A 55-year-old Caucasian woman presented with abdominal discomfort and left lumbar pain within a three-week period. At exploratory laparotomy, a 8 cm solid cystic mass of the left ovary was found infiltrating the sigmoid colon, and a bulky mass (11 x 7 x 4 cm) in the left paraaortic infrarenal region. Histopathological features of Ovarian Small Cell Carcinoma of the lungs and positive immunohistochemical stains provided a definite diagnosis of IIIC ovarian small cell carcinoma of pulmonary type. After six cycles chemotherapy with carboplatin and etoposide, the patient is still alive at 21 months from initial diagnosis. Discussion: In this case, the absence of peritoneal involvement and the extensive paraaortic adenopathy is suggestive of a different pattern of spread of this rare tumour. Optimal treatment seems to be radical primary debulking surgery resulting in no residual disease, maximizing the effect of adjuvant chemotherapy for this biological aggressive tumour.

Key words: Ovarian small cell carcinoma pulmonary type; Debulking surgery.

Introduction

Primary ovarian small cell carcinoma (OSCC) is a rare and highly aggressive neoplasms with poor prognosis, constituting about 1% of all ovarian neoplasms. Up to 5% of all small cell carcinomas (SCCs) arise in extrapulmonary sites and SCCs of female genital tract represent less than 2% of all gynaecological malignancies [1, 2]. The histogenesis of these tumours is unclear. According to the World Health Organization (WHO) classification, OSCC is categorised in the group of miscellaneous tumours that is further divided in the hypercalcaemic type and in the extremely rare pulmonary type [3]. Up to date, approximately 20 cases of OSCC have been reported (Table 1) in the literature [4-13]. Due to limited experience on the pattern of spread and the management and outcome of this rare tumour, there is no consensus on optimal treatment. We present a case of OSCC from our hospital to pool our experience with previous reports in order to improve the management of patients with OSCC.

Case Report

A 55-year-old Caucasian woman presented to our department with abdominal discomfort and left lumbar pain. On physical examination, a left firm adnexal mass was found. Transvaginal ultrasound revealed a multiloculated predominantly solid mass with smaller cystic components at the periphery of the left ovary, measuring 8 x 7 x 4 cm suggestive of an ovarian neoplasm. Magnetic resonance imaging (MRI) of the abdomen showed dense adherence of the left ovarian tumour to the sigmoid colon, as well as bulky left paraaortic adenopathy, 11 x 7 x 4 cm in size, adjacent to the infrarenal region. All blood tests, serum calcium level and tumour markers were in the normal range except CA-125 which was slightly raised to 82.19 U/ml.

During exploratory laparotomy, a solid pale-gray mass 8 x 5 x 3 cm of the left adnexa was found firmly adjacent to the sigmoid colon with no other obvious metastatic peritoneal implants. Exposure of the retroperitoneum was carried out to identify the paraaortic adenopathy. Frozen sections of the left ovarian mass and the paraaortic adenopathy were inconclusive but suggestive of an undifferentiated ovarian carcinoma. Subsequently, the patient underwent debulking surgery including hysterectomy, salpingo-oophorectomy, infracolic omentectomy, sigmoidectomy and extensive pelvic plus paraaortic lymphadenectomy. The assistance of a vascular surgeon was required because the lymph node mass was densely adherent to the undersurface of the left renal artery and vein. The operation resulted in complete removal of the adenopathy with no residual disease.

Pathology examination (Figure 1) revealed a tumour with microscopic features similar to small cell carcinoma of the lung (A). Immunohistochemical stains showed that the tumour cells were diffusely positive for neuron specific enolase (B), chromogranin (C) and synaptophysin (D). Therefore, an unexpected diagnosis of OSCC was made.

The postoperative course was uneventful and four weeks following debulking surgery, the patient received six cycles of adjuvant chemotherapy with carboplatin (AUC-6) and etoposide (100 mg/m²). She is on regular follow-up and 21 months from the initial diagnosis remains in remission.
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existing ovarian tumours. In our case no surface epithelial tumoral components were identified which might be attributed to the aggressive behaviour of this tumour replacing any other adjacent ovarian tissue.

Accurate preoperative diagnosis of OSCC is difficult. Its diagnosis is usually unexpected even during surgery, as frozen sections are inconclusive. The combination of histopathological features and immunohistochemistry expression of tumour markers on histological material provide the definite diagnosis of primary OSCC. The majority of cases have been diagnosed at advanced stage.

Discussion

Since Eichhorn et al. [4] first reported a small series of 11 patients with primary ovarian small cell carcinoma of pulmonary type, only nine additional sporadic cases have been reported in the literature [5-13]. It usually occurs in peri-post menopausal women with a peak incidence 50-60 years, as in our patient. Its pathogenesis is unclear. In the majority of cases, there was an association with other malignant or benign surface epithelial-stromal tumours, which indicates that these tumours may arise from pre-existing ovarian tumours. In our case no surface epithelial tumoural components were identified which might be attributed to the aggressive behaviour of this tumour replacing any other adjacent ovarian tissue.

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and the tumour size ranges from 4.5 cm to 28 cm. The pattern of tumour spread in advanced stages is comparable to epithelial ovarian tumours. However, our case of advanced OSCC showed a unique pattern of disease dissemination with exclusive spread to the infrarenal para-aortic nodes without peritoneal metastatic implants or omental involvement.

The differential diagnosis mainly includes ovarian tumours composed of small cell with round pattern and scanty cytoplasm. Radiological exclusion of other known primary sites of this type of tumour is mandatory. In our case, chest X-ray and MRI were negative excluding ovarian metastasis from primary lung cancer.

Due to the absence of standard treatment, various chemotherapy regimens have been suggested. However, radical surgery seems to be the cornerstone of treatment for these tumours. Fertility sparing surgery in early stages or suboptimal debulking in advanced stages were associated with early recurrences and poor survival (Table 1).

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Primary ovarian small cell carcinoma of pulmonary type with enlarged paraaortic lymph node masses: a case report and review etc.


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G-CSF induces focal intense bone marrow FDG uptake mimicking multiple bone metastases from uterine cervical cancer: a case report and review of the literature

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Summary

We describe a case of FIGO Stage IB2 uterine cervical cancer which showed focal intense bone marrow FDG uptake mimicking bone metastases after the administration of G-CSF. This case highlights the importance of avoiding the administration of G-CSF prior to PET imaging.

Key words: G-CSF; FDG PET/CT; Cervical cancer; Bone metastasis.

Introduction

It is known that F-18-fluorodeoxyglucose (FDG) accumulates in the bone marrow of healthy subjects [1, 2]. It was also reported that granulocyte colony-stimulating factor (G-CSF) administration induces increased bone marrow FDG uptake [1, 2]. According to previous reports, the increased bone marrow FDG uptake induced by G-CSF treatment is generally homogenous rather than focal [1, 2]. We describe a case of FIGO Stage IB2 uterine cervical cancer which showed focal intense bone marrow FDG uptake mimicking bone metastases after the administration of G-CSF. This case highlights the importance of avoiding the administration of G-CSF prior to positron emission tomography/computed tomography (PET/CT) imaging.

Case Report

A 50-year-old woman presented with postmenopausal vaginal bleeding. Biopsies from the 4.5 cm cervical lesion demonstrated a non-keratinizing type squamous cell carcinoma. A pretreatment work-up including magnetic resonance imaging (MRI) and FDG PET/CT revealed no evidence of adenopathy or metastatic disease. The patient was diagnosed with FIGO Stage IB2 cervical cancer and treated with concurrent chemoradiotherapy.

As serious pancytopenia developed during the course of external beam radiotherapy (EBRT), both concurrent chemotherapy and EBRT were discontinued (day 23) and treatment with blood transfusion (both red blood cells and platelets) and daily subcutaneous injection of 100 µg of G-CSF was initiated. As her pancytopenia persisted, FDG PET/CT was performed to investigate whether systemic metastases including bone marrow were present (day 36). FDG PET/CT showed diffusely intense FDG uptake in the bone marrow with multiple focal lesions in the vertebrae, which were highly suspicious of metastases (Figure 1A). However, a bone marrow biopsy revealed no evidence of malignant cell infiltration (day 39). As none of the other clinical and radiologic findings indicated progressive disease, we concluded that the first PET findings of intense FDG uptake in bone marrow and vertebrae had been caused by a physiological response to the G-CSF administration. On another FDG PET/CT conducted four weeks after the last G-CSF administration, the abnormal focal uptake demonstrated in the first examination completely disappeared (Figure 1B). The patient’s hematological condition gradually recovered, and she completed her radiotherapy without concurrent chemotherapy. She is currently free of disease.

Discussion

FDG PET/CT is reported to be effective for the detection of bone or bone marrow metastases from human malignancies [3]. Some investigators have observed an intense FDG uptake in normal bone marrow after the administration of G-CSF [1, 2]. This increased FDG uptake in normal bone marrow after G-CSF administration could be explained by increased bone marrow metabolism and cellularity due to G-CSF treatment.

Figure 1. — Sagittal positron emission tomography (PET) images. A) obtained just after G-CSF administration. B) obtained 4 weeks after the cessation of G-CSF therapy.
According to previous reports, the increased bone marrow FDG uptake induced by G-CSF treatment is generally homogenous rather than focal [1, 2]. However, two cases showing multiple bone marrow FDG uptake foci after G-CSF administration have been reported [4, 5]. Of these, one was a patient with primary peritoneal carcinoma [4], and the other was a patient with aplastic anemia [5]. In both cases, multiple bone metastases were initially suspected, but the FDG uptake was later found to have been due to the stimulatory effect of G-CSF on the bone marrow [4, 5]. Therefore, in patients receiving G-CSF treatment, the timing of FDG PET/CT study is critical for differentiating between metastatic disease and stimulated bone marrow. In the previous reports, the increased bone marrow FDG uptake was induced immediately after the administration of G-CSF, and sustained for up to four weeks after the cessation of G-CSF therapy [1, 2]. Therefore, it is suggested that FDG PET/CT should be delayed for at least four weeks after completion of G-CSF therapy to avoid the misinterpretation of increased FDG uptake as bone or bone marrow metastases.

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A huge retroperitoneal liposarcoma: case report

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Summary

Background: Soft tissue sarcomas are rare and account for less than 1% of all newly diagnosed malignancies. One-third of malignant tumors arising in the retroperitoneum are sarcomas. Liposarcoma is the most common soft tissue sarcoma and retroperitoneal sarcoma. Liposarcoma accounts for at least 20% of all sarcomas in adults and up to 41% of all retroperitoneal sarcomas. Here we present the case of a huge retroperitoneal liposarcoma and a brief literature review. Case report: A 34-year-old woman was referred to our hospital from a local clinic, because of abdominal distention, pain, and palpable mass. On admission we found that her abdomen was markedly distended. Computed tomography showed a huge left ovarian mass that occupied almost the entire abdominal cavity. The mass consisted mainly of fat, and calcified material. She was operated under the diagnosis of a huge teratoma. The tumor was located in the retroperitoneal cavity and it abutted the left adnexa. The retroperitoneal tumor, including the left adnexa was removed. The tumor measured 22 x 15 x 11 cm, and showed many histological and pathological findings. On the basis of the histopathological finding, the tumor was diagnosed as a dedifferentiated liposarcoma of the retroperitoneum. Presently she is undergoing radiation therapy. Conclusion: In retroperitoneal liposarcoma, histological subtype, incomplete resection, contiguous organ resection, and older age are strongly associated with tumor-related mortality. For liposarcoma, it is necessary to customize the treatment strategy on a case-by-case basis.

Key words: Retroperitoneal liposarcoma; Histological subtype; Customized treatment.

Introduction

Soft tissue sarcomas are rare, accounting for less than 1% of all malignancies, and one-third of all malignant tumors arising in the retroperitoneum are sarcomas. Liposarcoma is the most common soft tissue sarcoma in adults, and is often located in the retroperitoneum. Liposarcomas originate from the mesoderm, and are derived from adipose tissue. The peak age of incidence ranges from 40 to 60 years. Retroperitoneal tumors grow slowly, but they may grow to a very large size [1, 2]. Here we report the case of a huge retroperitoneal liposarcoma, resembling a huge teratoma.

Case Report

A 34-year-old woman was referred to our hospital from a local clinic for a 2-week history of abdominal distention, pain, and palpable mass. She denied experiencing any vesical irritation. She had no history of surgery or medication and her family history was unremarkable. Her abdomen was markedly swollen and firm, but tender. Laboratory findings including tumor marker results within normal limits. Computed tomography (CT) showed a huge, inhomogeneous mass arising from the left ovary, consisting mainly of fat and calcified material. It was considered to be a mature teratoma (Figure 1). During laparotomy, the mass was seen as a large lipomatous tumor occupying almost the entire abdominal cavity. It was located in the retroperitoneal space, and it abutted on the left ovary. Both the ovaries, however, were grossly free. The mass was completely excised, but it was difficult to obtain clear margins sparing the major vessels and adjacent organs. The retroperitoneal tumor, including the left adnexa was removed, and microscopic analysis of frozen tumor sections showed liposarcoma. We decided to remove the uterus. The mass measured 22 x 15 x 11 cm, grossly appeared multinodular, and contained many whitish fatty areas (Figures 2 A, B, C). The histopathological diagnosis was of dedifferentiated liposarcoma (Figures 3). Positron emission tomography (PET) CT performed after surgery did not show metastasis or remnant tumor (Figure 4). The postoperative course was uneventful, and the patient was discharged nine days after the surgery. Presently she is undergoing radiation therapy.

Discussion

Liposarcomas originate from the mesoderm and are derived from adipose tissue. They account for 10~14% of all soft tissue sarcomas, and less than 1% of all malignancies. There are some types of sarcoma, liposarcoma (41%), leiomyosarcoma (28%), malignant fibrous histiocytoma (7%), fibrosarcoma (6%), etc. Liposarcoma is the most common tumor of the retroperitoneum accounting for 0.07-0.2% of all neoplasms [2]. The symptoms arise because of the compression of the organs, similar to that reported in our case. Patients mostly present with a mass only a few weeks or few months old, while metastasis at the first occurrence is not common. The histological type is the most important factor affecting survival rates for patients with liposarcoma [1].

Histologically the subtypes of liposarcomas are distinguished by the composition of lipoblasts. They are divided into four subtypes: well differentiated, dedifferentiated, myxoid/round cell, and pleomorphic. Dedifferentiated liposarcomas are characterized by the coexistence of well differentiated and poorly differentiated, non-lipogenic areas that are present either in a portion of the same tumor or in the primary and recurrent tumors. The dedifferentiated type is thought to be the transformation of well differentiated liposarcoma [3]. Conyers et al., reported that dedifferentiated liposarcoma is more aggres-

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Figure 1. — Computed tomography (CT) scan obtained before the surgery shows a mass containing a large amount of fat and calcified material. It appears as a mature teratoma.

Figures 2 A, B — Photographs of the mass obtained after surgery. C) In cross section, large masses of fat can be seen. Shows a section of a well differentiated area of the dedifferentiated liposarcoma in which lipoblasts are seen in fibrotic background (× 200).

Figure 3. — The pathological findings of the tumor. Shows a section of dedifferentiated liposarcoma composed of spindle shaped cells (× 200).

Figure 4. — Positron emission tomography (PET) CT scan obtained after surgery did not show distant metastasis or remnant tumor.
The present case was the dedifferentiated subtype of liposarcoma in a relatively young patient. To lower the possibilities of local recurrence and distant metastasis, we decided to give the patient radiation therapy, and she has been tolerant to the therapy.

Conclusion

Retroperitoneal liposarcomas are rare; however, they require an aggressive surgical approach, including multi-organ resection, if necessary, or multiple resections in the case of recurrence. In retroperitoneal liposarcoma, histological subtype, incomplete resection, contiguous organ resection, and older age are strongly associated with tumor-related mortality [5]. Especially in the dedifferentiated type local recurrence and distant metastasis rates are high. For liposarcoma, it is necessary to customize the treatment strategy on a case-by-case basis.

References


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Long-term disease-free survival in three ovarian cancer patients with a single relapse

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Summary

Recurrent ovarian cancer with long-term survival is uncommon and often associated with poor prognosis. We report three cases of patients with advanced ovarian cancer who have achieved long-term disease-free survival following a single prior relapse. Case 1 relapsed with a localized bulky tumor and received a complete surgical resection and chemotherapy. Case 2 had a persistent central pelvic tumor after debulking surgery and second-line chemotherapy, and yet achieved excellent control with concurrent chemoradiation to the true pelvis. Case 3 relapsed with paraaortic lymph node metastasis and probable lung metastasis (subsequently negated by positron emission tomography) and received chemotherapy alone. These three patients have since remained disease-free for 13, 12, and seven years, respectively, since their first relapse. We conclude that select patients can obtain long-term disease-free survival after the first relapse by accurate restaging and aggressive multimodality treatment.

Key words: Disease-free survival; Ovarian neoplasm; Recurrence; Treatment outcome.

Introduction

Ovarian cancer is the sixth most common cancer among women worldwide [1]. In Taiwan, ovarian cancer ranks third in incidence among all cancers and is the leading cause of death among gynecologic malignancies [2]. Debulking surgery followed by six courses of platinum-based chemotherapy is the standard treatment. Complete clinical remission can be achieved in > 50% of cases following first-line therapy. However, these responders will relapse within an average of 18 months, and the majority will experience a series of treatments, remissions and recurrences [3, 4]. Thus, management of recurrent ovarian cancer is a great challenge for oncologists.

We report three patients who achieved prolonged disease-free survival following a single relapse of ovarian cancer managed by accurate restaging and aggressive multimodality salvage treatment.

Case Reports

Case 1

A 63-year-old woman was diagnosed in 1993 with Stage IIc epithelial ovarian cancer (EOC) and underwent maximal debulking surgery at a medical center. The tumor was classified histologically as clear cell carcinoma. After six cycles of cisplatin/cyclophosphamide chemotherapy, the patient had no evidence of disease. In 1998, she developed a ventral hernia, and a large mass 17 cm in diameter mixed with solid and cystic components was discovered within the anterior abdominal wall. Another lobulated soft mass of 5 cm was detected in the right paracolic gutter of the ascending colon and hepatic flexure. No visible residual tumor was noted. After this surgery, the patient was treated with six courses of paclitaxel/carboplatin. The patient has been regularly followed at our clinic with no evidence of disease.

Case 2

A 68-year-old woman was diagnosed in 1996 with Stage IIc EOC and underwent maximal debulking surgery at another medical center. The tumor was classified pathologically as clear cell carcinoma. After four courses of carboplatin/cyclophosphamide, the patient had no evidence of disease.

The patient’s serum CA-125 level was found to be elevated to our clinic, the patient received maximal debulking surgery and shows no evidence of disease at the time of this report.

Case 3

A 63-year-old woman was diagnosed in 2002 with Stage IIIc EOC and underwent only hysterectomy and bilateral salpingo-oophorectomy at a local hospital. The tumor was classified pathologically as endometrioid adenocarcinoma. After referral to our clinic, the patient received maximal debulking surgery
and rectum. After six courses of paclitaxel/carboplatin the patient had complete remission.

In 2004, she presented with chronic cough and peri-umbilical tenderness and a serum CA-125 level elevated from 8.21 U/ml to 59.13 U/ml. Computed tomography (CT) showed multiple nodular lesions in the bilateral lung field (Figure 1a) and lymph node enlargement in the right paraaortic (PA) region (Figure 2a). F-18 fluorodeoxyglucose (18FDG) positron emission tomography-CT (PET-CT) showed metastasis of the right paraaortic lymph node at level L-4, of the paraaortic node at the bifurcation at level L-5, and of the right common iliac lymph node, but PET-CT did not show lung metastasis. The patient was treated with six courses of paclitaxel/carboplatin. After these treatments, she achieved complete remission (Figure 2b). She has been regularly followed without evidence of disease in the abdomen, and the lung lesions remain stationary (Figures 1b and c).

Discussion

Recurrent EOC is a chronic and lethal disease. The median survival ranges from 12 to 24 months after recurrence and the primary goal of management is palliation [4]. According to current guidelines, a carboplatin-based combination is strongly recommended for patients with platinum-sensitive disease rather than carboplatin monotherapy, although cumulative toxicity has been considered [5]. Some case reports suggest that aggressive treatment with cytoreductive surgery, chemotherapy, or
radiotherapy could improve survival even in patients with distant metastasis or with bulky tumors, if the therapy is tolerated by the patient [6, 7].

Secondary cytoreductive surgery for recurrent ovarian cancer remains controversial. Factors that affect survival after recurrence have been discussed. It is believed that secondary cytoreduction has a survival benefit in select platinum-sensitive patients. Chi *et al.* [8] suggested using the duration that the patient remained disease-free and the number of recurrence sites as selection criteria for offering secondary cytoreduction. They also suggested that the objective of secondary cytoreduction should be to achieve residual disease that measures less than 0.5 cm, which was associated with a significant survival benefit [3, 4, 8]. The disease-free interval from primary treatment to recurrence was over 50 months for Case 1 and 27 months for Case 2. The two cases had single-site recurrence and received optimal cytoreductive surgery.

The utility of PET-CT in detecting early recurrent ovarian cancer has been demonstrated. PET-CT had greater accuracy and less inter-observer variability than CT alone in detecting lesions in the abdomen (abdomen-pelvis) and in the body overall. PET-CT scanning may therefore have a significant impact on the clinical management of a patient [9, 10]. In Case 3, additional metastatic lymph nodes were detected by PET-CT, and over-diagnosis of lung metastasis was avoided.

Regional extra-peritoneal recurrence of ovarian cancer could be treated effectively by involving field radiation therapy (IFRT). IFRT in combination with optimal surgery can obtain 89% long-term control and 50% 5-year overall survival. IFRT induces less toxicity compared to traditional whole abdominopelvic radiation therapy [11]. Case 2 had excellent control of a persistent central pelvic tumor with concurrent chemoradiation to the true pelvis after the second debulking and chemotherapy.

In conclusion, long-term disease-free survival after the first relapse is achievable by tumor reductive surgery, platinum-based chemotherapy, and/or radiotherapy for selected patients with recurrent ovarian cancer.

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Endometrioid ovarian cancer arising from an endometriotic cyst in a young patient

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Summary

Objective: To present a case of a young woman with ovarian endometrioid adenocarcinoma arising from an endometriotic cyst and review of the literature. Case Report: A 33-year-old woman, gravid 2, para 2 was admitted to our department with a 5 cm adnexal mass. Diagnostic laparoscopy was performed and pathological examination demonstrated an endometriotic cyst with an area of ovarian endometrioid adenocarcinoma well differentiated, with no capsular invasion. One month after the operation the patient underwent MRI which revealed a 6 cm mass in the Douglas pouch. The multidisciplinary oncology council decided on exploratory laparotomy, which revealed no pathology. After that the multidisciplinary oncology council decided on adjuvant chemotherapy and the patient received four cycles of carboplatin/taxol. Conclusion: It should always be considered that even when there are no risk factors for malignancy occurrence, a high index of suspicion is necessary and will help to prevent delay in the diagnosis of this rare neoplasm.

Key words: Endometriosis; Endometriosis-associated cancer; Ovarian endometrioid adenocarcinoma.

Introduction

Endometriosis is the presence of endometrial glands and stroma outside the uterus. It is a common problem among women in reproductive age having an incidence up to 10% in the general female population [1, 2]. Endometriosis is an estrogen-dependent disease and is a usual cause of pelvic pain and infertility.

Endometriosis is very common in the ovaries, fallopian tubes, uterosacral ligaments and lateral pelvic peritoneum. Less commonly it can be found in the vagina, rectovaginal septum and the colon and rectum.

Many studies correlate endometriosis with clear cell and endometrioid cancer in premenopausal women with large endometriomas. We present a case of a 33-year-old woman with a 5 cm endometrioma who had ovarian endometrioid adenocarcinoma together with a review of the literature.

Case Report

A 33-year-old woman, gravid 2, para 2 was admitted to our department with a 5 cm adnexal mass. The patient had had chronic pelvic pain for the previous two years, but she did not complain about dysmenorrhea or dyspareunia. Her physical examination revealed a soft mass in the area of the left adnexa and no pain during palpation of the uterosacral ligaments. An abdominal ultrasonography showed a 5 x 3 cm endometrioma of the left ovary. The serum concentration of CA 125 was within normal limits (19.8 IU/ml, normal range < 35 IU/ml).

After written and informed consent of the patient was obtained, a diagnostic laparoscopy was performed. Intraoperative observation demonstrated an endometrioma of the left ovary with a maximum diameter of about 5 cm; the rest of the abdominal organs were normal. Peritoneal washing was obtained and the mass was removed intact and sent for frozen section examination which suggested no existence of malignancy. Pathological examination demonstrated an endometriotic cyst with an area of well differentiated ovarian endometrioid adenocarcinoma and no capsular invasion. It consisted of confluent glands and cribriform areas. The neoplastic cells included rounded nuclei, clumped chromatin and small nucleoli. Mitotic figures were scanty. One month after the operation the patient underwent magnetic resonance imaging (MRI), which revealed a 6 cm mass in the Douglas pouch. The multidisciplinary oncology council decided on exploratory laparotomy which revealed no pathology. Nevertheless, total hysterectomy with bilateral oophorectomy, omentectomy, appendectomy and pelvic lymphadenectomy were performed. Recovery was uneventful and pathology examination revealed no cancer cells. After that the multidisciplinary oncology council decided on adjuvant chemotherapy and the patient received four cycles of carboplatin/taxol.

Discussion

Criteria to identify malignant tumors arising from endometriosis were first proposed by Sampson in 1927 [3] and in 1953 Scott [4] added stricter criteria. The frequency of malignant transformation of endometriosis is not exactly evaluated, but it is estimated that 1% or less of women will develop neoplasms associated with endometriosis [5].

The ovary is by far the most common site where malignancy arises in association with endometriosis, accounting for about 75% of such cases [6]. Endometrioid adenocarcinoma and clear cell adenocarcinoma are the most common malignancies found to arise in ovarian endometriosis, accounting for roughly two-thirds of all such reported cases [7]. While endometrioid adenocarcinoma is the more common of the two entities by a ratio between 1.3:1 and about 4:1, women who have clear cell...
Endometrioid ovarian cancer arising from an endometriotic cyst in a young patient

Progress in imaging techniques such as magnetic resonance imaging (MRI) have changed the approach to diagnosis of cystic endometriosis. Our case report shows that even when there are no risk factors for malignancy occurrence, a high index of suspicion is necessary and will help to prevent delay in diagnosis of this rare neoplasm. Furthermore, the surgeon must be very careful to avoid intraoperative rupture of the ovarian cyst.

Recent papers show a role of K-ras and PTEN in the development of endometriosis and ovarian cancer in a mouse model [11]. It seems though that there is a long way to go, in order to completely understand the pathophysiology and the biologic mechanisms involved in the malignant transformation process.

References

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Figure 1. — Well differentiated endometrioid adenocarcinoma arising in an ovarian endometriotic cyst (H-E x 200).
Removal of a vaginal leiomyoma presenting as tumor previa allowing vaginal birth

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Summary

Leiomyomas of the vagina are very rare tumors of the female genital tract with only 300 cases reported so far. A case of removal of the vaginal leiomyoma presenting as tumor previa in advanced pregnancy is described. Removal of the tumor allowed vaginal birth three weeks after surgery.

Key words: Leiomyoma; Vagina; Birth.

Introduction

Benign or malignant neoplasms of the vagina are rare. Most vaginal tumors are asymptomatic until a significant size is reached. Symptoms and signs may include a sensation of pressure, dyspareunia, obstruction of the vagina or urethra, or vaginal bleeding. Most of these lesions can be detected during routine examination of an asymptomatic patient. Vaginal leiomyomas or fibromyomas are rare lesions usually located in the anterior vaginal wall. Only between 250 and 300 cases have been reported in the world literature [1, 2]. These lesions are benign smooth muscle neoplasms, usually solitary and in many cases asymptomatic. Histologically, they resemble leiomyoma of other origins. Sites of origin include vaginal smooth muscle, local arterial musculature, or smooth muscle of the bladder or urethra. As uterine leiomyomas, vaginal lesions are also estrogen dependent. Malignant conversion is extremely rare. When large, symptoms can include vaginal discharge or bleeding, dyspareunia, or urinary retention. The differential diagnosis of a midline anterior vaginal mass includes urethral diverticulum, fibroepithelial polyp, cystocele, Skene duct abscess, or vaginal malignancy. Therapy involves excision in symptomatic patients. Recurrence is uncommon but reported [3, 4]. Vaginal neoplasms are divided into cystic or solid lesions and biopsy provides a definitive diagnosis.

Case Report

Our patient, aged 45 years, tertiparous, presented in the 32nd week of gestation with a tumor on the right vaginal wall which was first noticed three months before. During that time the patient was asymptomatic. On examination a tender, solid, elastic tumor 60 x 70 mm in diameter was found protruding through the vaginal vestibule. The pedunculated tumor originated from the right vaginal wall, emerging through the defect in the vaginal wall mucosa. The tumor obstructed the vaginal outlet as tumor previa. The distal part of the tumor was necrotic. Obstetric findings were normal, consistent with eight months of pregnancy. Tumors of this localization can cause obstruction of the birth canal preventing normal vaginal birth. After preparation of the operative field for vaginal surgery, enucleation of the tumor was performed, and hemostasis was achieved with sutures placed at the pedicle of the tumor. The defect on the vaginal wall was drained and sutured. The postoperative course was normal and the patient was treated with antibiotics, tocolytic therapy and dexamethasone to achieve fetal lung maturity in case of premature labor. She was discharged from the hospital the sixth postoperative day. She gave birth three weeks later (breech presentation) to a healthy infant weighing 2,650 g. Histopathological findings were: an oval node weighing 130 g, size 75 x 75 x 70 mm, yellow color, tenacious to soft consistency, spindle fiber structure, with marked swelling. Histopathological diagnosis was: leiomyoma with edema.

Discussion

Leiomyomas are benign, mesenchymal, monoclonal tumors that typically originate from the myometrium smooth muscle cells, although atypical sites such as the vagina, lungs, vascular structures, and retroperitoneal area have been reported [5]. Smooth muscle tumors are the most common tumors of the adult vagina, but also bizarre (atypical, sympathetic, or pleomorphic) leiomyomas can be found in this localization [6]. Vaginal fibromyomas (leiomyomas and rhabdomyomas) are rare; approximately 300 cases have been reported in the literature. Surgical excision through the vaginal route has been the traditional approach, but the abdominoperineal route may be necessary for huge tumors [2]. Recurrences occur infrequently. Leiomyomas of the vagina may have variable clinical presentation. They are asymptomatic or present with pain, dyspareunia or urinary tract pressure symptoms. Usually they are slow-growing, but rapidly growing vaginal leiomyomas may mimick a prolapsed uterus [7]. If presenting as a mass they are most often diagnosed clinically and treated surgically by excision. These hypervascular tumors may sometimes cause life-threatening hemorrhage. Preoperative embolization may be helpful in devascularization of the tumors before surgical excision, minimizing perioperative blood loss [8]. Sørensen and Chauhan [9] presented a case of vaginal wall leiomyoma of a posterior vaginal wall which was mistaken for a cer-
Removal of a vaginal leiomyoma presenting as tumor previa allowing vaginal birth

It is suggested that whenever there is a clinical suspicion of a vaginal leiomyoma, magnetic resonance imaging (MRI) or translabial ultrasound (US) are the recommended imaging modalities to achieve a proper diagnosis. Preoperative imaging and biopsy are helpful to rule out possible malignancy. Uncommon presentation may necessitate imaging studies. The lesion usually has MRI and US features similar to its uterine counterpart [10]. Leiomyoma may also originate from the vaginal cuff after total abdominal hysterectomy and bilateral salpingo-oophorectomy [9]. Sometimes the size of the tumor necessitates an abdominoperineal approach and hysterectomy for better surgical access. Govri et al. [11] reported a case of a vaginal leiomyoma arising from the right lateral wall that presented as a gluteal swelling with pus discharging per rectum, creating a clinical dilemma in diagnosis.

Our patient presented with a large vaginal leiomyoma in advanced pregnancy. Such tumor may cause obstruction of the birth canal as tumor previa. We performed excision of the tumor in order to liberate the birth canal which allowed normal vaginal birth three weeks after surgery.

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Figure 1. — Tumor of the right vaginal wall obstructing the vaginal outlet.

Figure 2. — Relation of the tumor to the vaginal outlet.

Figure 3. — Cross section of the tumor after removal.
Endometrial stromal sarcoma in a 29-year-old patient. Case report and review of the literature

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Summary

Objective: Endometrial stromal sarcomas are rare tumors accounting for about 0.2% of all genital tract malignancies. They are considered to occur more often in premenopausal women. Endometrial stromal sarcomas are hormone sensitive tumors. A state of hyper-oestrogenemia could act as a growth stimulus. Given the rarity of these tumors there are limited reports in the literature referring to the clinical management and final outcome of these cases. Case: The patient, a 29-year-old woman, had a surgical history of myomectomy in another hospital three months before her referral to our department. The histological examination of the removed myoma showed an endometrial stromal sarcoma. Total abdominal hysterectomy, with bilateral salpingo-oophorectomy, omentectomy and elective pelvic lymphadenectomy were then performed as a second radical surgical approach. Conclusion: Endometrial stromal sarcomas are uncommon and their differential diagnosis from typical submucosal uterine myomas or benign endometrial polyps could be difficult. The hysteroscopic features of uterine sarcomas are often similar to those of endometrial polyps or submucosal myomas. The histological examination of the specimen is necessary to exclude malignancy and establish the final diagnosis. Total abdominal hysterectomy, bilateral salpingo-oophorectomy with pelvic lymphadenectomy is the optimal treatment in cases of endometrial stromal sarcomas.

Key words: Endometrial sarcoma; Leiomyoma; Myomectomy.

Introduction

Endometrial stromal sarcomas (ESS) are extremely rare malignant tumors that represent approximately 10% of all uterine sarcomas but only around 0.2% of all uterine malignancies [1-3]. The annual incidence of ESS is 1-2 per million women accounting for 400 to 700 new cases each year in Europe [4].

The differential diagnosis of ESS from other lesions such as leiomyomas is often preoperatively difficult and the final diagnosis is mainly postoperatively given after histological examination [5, 6]. The typical gross appearance of ESS includes a single nodule, multiple solid and cystic masses, and a poorly demarcated lesion with occasional cystic degeneration, or rarely a cystic multinodular lesion [7].

The staging of uterine sarcomas is based on the International Federation of Gynecology and Obstetrics (FIGO) Staging System for uterine corpus cancer [8, 9]. According to the World Health Organization’s (WHO) classification, uterine sarcomas are classified into four main histological subtypes in an order of decreasing incidence: carcinosarcomas, leiomyosarcomas, endometrial stromal sarcomas and other sarcomas [10, 11]. Unfortunately, clinical-trial reports and literature reviews often include a broad range of histological subtypes of sarcoma which restrict interpretation and application of results. Response rates from protocols with multiple subtypes should consequently be interpreted with caution; therefore the effort to tailor the approach to patients seems mandatory.

We report a case of a 29-year-old patient diagnosed with ESS together with a literature review based on pubmed databases.

Case Report

The patient, a 29-year-old nulliparous woman with a history of abnormal uterine bleeding and surgical removal of a uterine leiomyoma three months before, was referred to our department. After receiving three cycles of gonadotrophin releasing hormone (GnRH) analogues the patient underwent (in another hospital) surgical excision of a uterine tumor with a maximum diameter of 6 cm which was located in the frontal region of the uterus and had ultrasound characteristics of a typical leiomyoma. Histological examination of the lesion revealed an endometrial stromal sarcoma with 10 mitotic figures (MF)/10 high power fields (HPF). Immunohistochemistry showed: CD 10 (+), SMA (-), caldesmon (-), desmin (-), PanCK (+), vimentin (+) and ER (+ ≥ 80%), PGR (+ ≥ 90%).

A more radical surgical treatment of the disease was considered necessary and the patient was re-operated in our department. The serous neoplastic markers were within normal ranges.

Total abdominal hysterectomy with bilateral salpingo-oophorectomy, omentectomy and elective pelvic lymphadenectomy were performed. The final histological examination revealed an endometrial stromal sarcoma. Within the uterine cavity, a yellowish polypoid lesion measuring 1 x 0.4 x 0.3 cm was detected. At microscopic examination the tumor was characterized by the presence of multi nodule lesions throughout the myometrium trying to penetrate to the myometrium vessels. The cervix was found to have no malignancy but histology revealed lesions of chronic cervicitis and tubal metaplasia.

The adnexae, omentum and all removed pelvic lymph nodes were negative for malignancy. Immunohistochemical markers were as follows: CD10 (+), vimentin (+), SMA locally (+), caldesmon (-) and ER (+) > 90%, PGR (+) > 90%.

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Endometrial stromal sarcoma in a 29-year-old patient. Case report and review of the literature

Due to the high recurrence risk even with localized tumors, many clinicians advocate adjuvant chemotherapy, radiation therapy and/or hormones to suppress tumor growth [23]. There is no firm evidence coming from a prospective study that adjuvant chemotherapy or radiation therapy is of substantial benefit for patients with uterine sarcoma [24]. Postoperative pelvic radiotherapy reduces local recurrence but has not been consistently shown to prolong survival [25].

Low-grade endometrial stromal sarcomas are estrogen and progesterone receptor-positive tumors [26, 27]. In the past, hormonal therapy consisting of progestins was given for advanced or recurrent metastatic ESS. Hydroxyprogesterone acetate (MPA) and megestrol acetate are synthetic derivates of progesterone receptor [28]. Aromatase inhibitors and GnRH analogues have become new effective alternatives for first- and second-line treatment [29-31]. Recently, hormonal therapy has been introduced for the prevention of recurrences.

Uterine sarcomas have a poor prognosis and survival is much worse than that reported for endometrial adenocarcinoma, with an overall 2-year survival less than 50% even when presenting at an early stage [32]. A higher survival probability for patients with ESS is often reported [33]. Prognostic factors in patients with ESS are still controversial [34]. The negative prognostic influence of a high mitotic index was revealed in previous studies [35]. In the present study survival probabilities have been calculated by the product limit method of Kaplan and Meier which showed patients with no myometrial invasion and low mitotic count ≤ 5 MF/HFP to have longer disease-free survival but the p value was not statistically significant.

Conclusions

ESS is a malignant tumor that shows endometrial stromal differentiation and is histologically characterized by uniform small to medium sized cells and a distinctive

The patient had an uneventful recovery. Follow-up 24 months after initial diagnosis and surgical treatment with clinical examination and magnetic resonance imaging (MRI) showed no signs of recurrence.

Discussion

Uterine sarcomas are relatively rare tumors of mesodermal origin representing 2-6% of all uterine malignancies [12]. Depending on mitotic activity, vascular invasion or prognosis there are three categories of endometrial stromal tumors: endometrial stromal nodule, high-grade endometrial stromal sarcoma and undifferentiated endometrial sarcoma. ESS represents a very rare class of malignant tumors that comprises approximately 10% of all uterine sarcomas but only around 0.2% of all uterine cancers [13]. The typical gross appearance of ESS includes a single nodule, multiple solid-cystic masses and a poorly demarcated lesion with occasional cystic degeneration, or rarely cystic multilocular lesion [14].

Endometrial stromal sarcoma is characterized by proliferative lesions composed of cells with endometrial stromal cell differentiation and typically extensive worm-like vessel invasion [15]. Treatment of ESS is surgical [16, 17]. It includes total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and cytological examinations of washings from the abdominal cavity [18, 19]. If the tumor is palpable in the parametrium, a more extensive procedure such as a radical hysterectomy should be performed [20].

Lymphatic invasion is well established and pathognomic for ESS formally designated as endolymphatic stromal myosis [21]. Despite this, lymph node involvement is not considered as a clinical problem and pelvic lymphadenectomy is not generally added to hysterectomy as the cornerstone of the treatment [22]. However recent data reported a higher incidence of lymph node metastases, showing the need for an extensive lymph node sampling.

Due to the high recurrence risk even with localized tumors, many clinicians advocate adjuvant chemotherapy, radiation therapy and/or hormones to suppress tumor growth [23]. There is no firm evidence coming from a prospective study that adjuvant chemotherapy or radiation therapy is of substantial benefit for patients with uterine sarcoma [24]. Postoperative pelvic radiotherapy reduces local recurrence but has not been consistently shown to prolong survival [25].

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Uterine sarcomas have a poor prognosis and survival is much worse than that reported for endometrial adenocarcinoma, with an overall 2-year survival less than 50% even when presenting at an early stage [32]. A higher survival probability for patients with ESS is often reported [33]. Prognostic factors in patients with ESS are still controversial [34]. The negative prognostic influence of a high mitotic index was revealed in previous studies [35]. In the present study survival probabilities have been calculated by the product limit method of Kaplan and Meier which showed patients with no myometrial invasion and low mitotic count ≤ 5 MF/HFP to have longer disease-free survival but the p value was not statistically significant.

Conclusions

ESS is a malignant tumor that shows endometrial stromal differentiation and is histologically characterized by uniform small to medium sized cells and a distinctive
arterial vasculature that resembles spiral arteries of the normal endometrium. The clinical presentation of ESS is usually abnormal uterine bleeding in premenopausal women and shows an indolent clinical behavior.

Optimal therapy of ESS is not well established. The standard surgical procedure includes total abdominal hysterectomy, bilateral salpingo-oopherectomy and radical cytoreductive surgery of extra uterine disease. Moreover, despite traditional recommendations to include bilateral salpingo-oopherectomy in the primary surgical management of ESS, same investigators have advocated preserving ovarian function, particularly in younger women. The role of pelvic, paraaortic lymphadenectomy and adjuvant treatment with radiation therapy, chemotherapy or hormonal treatment remains controversial.

References


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Primary ovarian leiomyosarcoma

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Summary

Background: Primary ovarian leiomyosarcoma is an extremely rare subtype of ovarian sarcomas. It most commonly occurs in postmenopausal women and has unfavorable prognosis. Case: The patient, a 58-year-old postmenopausal woman, presented with a complaint of abdominal pain. Preoperative examination revealed an intraabdominal mass 25 x 17 x 14 cm in the right adnexa. She underwent bilateral salpingo-oophorectomy, total omentectomy, appendectomy and bilateral pelvic lymphadenectomy. The histopathology revealed leiomyosarcoma of the right ovary Stage Ia. She did not receive any postoperative adjuvant therapy. Follow-up 21 months after initial surgery, showed no evidence of recurrence. Conclusion: Additional studies are needed to understand more about the nature, clinical behavior and treatment of this very rare tumor.

Key words: Primary ovarian leiomyosarcoma; Treatment; Surgery; Radiotherapy; Chemotherapy; Prognosis.

Introduction

Primary ovarian sarcoma is a very rare tumor accounting for less than 3% of all ovarian malignancies [1]. The most common histologic subtypes are carcinosarcoma, endometrial stromal sarcoma, fibrosarcoma and rhabdomyosarcoma [2-4]. Leiomyosarcoma is an extremely rare subtype of ovarian sarcomas [5].

Until now, about 64 cases of primary ovarian leiomyosarcoma (POLMS) have been reported in the English literature [6]. POLMS most commonly occurs in postmenopausal women and has unfavorable prognosis [2, 5-7]. We present a case of POLMS and review the literature.

Case Report

The patient, a 58-year-old, gravida 1, para 1 postmenopausal woman presented with a complaint of abdominal pain. She had a history of hysterectomy for uterine leiomyomas without salpingo-oophorectomy 15 years before. Her family history revealed no evidence of cancer among the first-degree relatives.

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

Preoperative computed tomography (CT) of the abdomen and pelvis, and abdominal ultrasound (US) revealed an intraabdominal mass 25 x 17 x 14 cm in the right adnexa. Preoperative CT of the chest, chest X-ray, colonoscopy and urethrocystoscopy were normal. Preoperative intravenous pyelography (IVP) revealed bilateral distention of the ureter and hydronephrosis. Preoperative CA-125 was elevated to 63.4 U/ml.

On exploratory laparotomy, the right ovary was markedly distended, measuring 25 x 17 cm. Frozen section showed malignancy and the patient underwent bilateral salpingo-oophorectomy, total omentectomy, appendectomy and bilateral pelvic lymphadenectomy.

Histopathology revealed leiomyosarcoma of the right ovary (Figures 1, 2). Tumor cells had high mitotic activity (11 mitotic figures per 10 high-power fields). Tumor was limited to the right ovary without penetrating the serosal surface. The peritoneal washing smear was negative for malignant cells. Histologic diagnosis was confirmed by positive immunostaining. Tumor cells were positive for smooth muscle actin (SMA), vimentin, desmin and ki-67, weakly positive for S-100 protein and negative for epithelial membrane antigen (EMA), cytokeratin, inhibin, CD10 and CD99. The final diagnosis was Stage Ia leiomyosarcoma of the right ovary.

The patient did not receive any postoperative adjuvant therapy. Follow-up 21 months after initial surgery with CT of the chest, abdomen and pelvis, abdominal US, chest X-ray, IVP, colonoscopy and urethrocystoscopy showed no evidence of recurrence.

Discussion

POLMS is a very rare tumor [5]. Until now, about 64 cases of POLMS have been reported in the English literature [6]. Among them, 41 were POLMS without heterologous elements and 23 were POLMS with heterologous elements [5-8].

The precise histogenesis of POLMS is still uncertain [5]. It probably originates from smooth muscle present in the walls of the blood vessels in the cortical stroma, in the corpus luteum, in the ovarian ligaments at their point of attachment to the ovary, in remnants of Wolffian ducts and in totipotential cells of ovarian mesenchyme [5, 9-11]. Malignant transformation of an ovarian leiomyoma and migration of a uterine leiomyoma are very rare mechanisms of histogenesis [12, 13].

POLMS usually occurs in postmenopausal women, although sometimes younger women may be affected [2, 7, 14]. It has been reported in patients between 12 and 84 years of age (mean age 52.6 years) [7, 8]. It is usually unilateral and may reach more than 10 cm in diameter [6, 7, 11, 12]. In our case, the patient was 58 years old and POLMS was unilateral.

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However, the role of postoperative radiotherapy or chemotherapy remains controversial, especially in patients with disease confined to the ovary [2, 5, 7, 13, 16, 18]. Postoperative adjuvant therapies should be attempted for unresectable residual disease because those patients have a dismal prognosis [7]. Our patient was Stage Ia, so she did not receive any postoperative adjuvant therapy.

Most patients with POLMS usually have nonspecific symptoms and signs such as pelvic pain and abdominal bloating [6, 13]. Also they have symptoms of pressure on the bladder and bowel [13]. Our patient had abdominal pain with no other symptoms and signs.

POLMS is typically present as a solitary, large, lobular, soft, fleshy, solid mass with hemorrhage and cystic degeneration [6, 12]. The diagnostic criteria for POLMS are hypercellularity, nuclear atypia, pleomorphism, coagulative necrosis and high mitotic activity (> 5 mitotic figures per 10 high-power fields) [6, 9, 12]. According to these criteria, our case is POLMS, as it fulfills all of them.

Immunohistochemical staining for POLMS is generally positive for muscle specific actin, SMA, desmin, vimentin, p53 and proliferation markers and negative for cytokeratins and S-100 [2, 8, 11, 12, 15]. Mitotic activity and stage have a direct correlation with the malignant potential and aggressiveness of POLMS [5]. In our patient, tumor cells were positive for SMA, desmin, vimentin and ki-67, weakly positive for S-100 protein and negative for EMA, cytokeratin, inhibin, CD10 and CD99.

Due to the rarity of POLMS it is very difficult to determine the optimal therapy [5]. Surgery remains the mainstay of treatment for POLMS and complete resection should be attempted whenever possible [7, 13]. Initial debulking surgery usually consists of total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and extirpation of all resectable tumor masses within the pelvis and abdomen [5, 7, 16, 17]. In our case, the patient underwent bilateral salpingo-oophorectomy, total omentectomy, appendectomy and bilateral pelvic lymphadenectomy.

Postoperative radiotherapy is used for local disease control [13]. Postoperative chemotherapy is used for prevention of distant metastases [7, 13]. Various chemotherapeutic regimens including cisplatin have been applied [5, 7]. However, the role of postoperative radiotherapy or chemotherapy remains controversial, especially in patients with disease confined to the ovary [2, 5, 7, 13, 16, 18]. Postoperative adjuvant therapies should be attempted for unresectable residual disease because those patients have a dismal prognosis [7]. Our patient was Stage Ia, so she did not receive any postoperative adjuvant therapy.

POLMS most commonly recurs in the abdomen and pelvis [8]. Less frequently recurrence is in the lung, bone, liver, mediastinum and brain [8]. The prognosis of POLMS is generally unfavorable, depending on mitotic activity and stage at diagnosis [2, 5]. Most reported cases recurred within one year and patients died within two years after initial diagnosis [7]. Patients die with extended local disease and multiple distant metastases [13]. Our patient was Stage Ia and, 21 months after initial surgery, she is well with no evidence of recurrence.

Conclusion

POLMS is a very rare tumor and has unfavorable prognosis. Additional studies are needed to understand more about the nature, clinical behavior and treatment of this tumor.

References

Primary ovarian leiomyosarcoma


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Primary retroperitoneal mucinous cystadenoma adjacent to the kidney: report of two cases and review

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Summary
We report two cases of primary retroperitoneal mucinous cystadenomas mistaken as renal cysts and discuss the differential diagnosis and management of this tumor.

Key words: Retroperitoneal tumors; Mucinous cystadenoma; Renal cyst.

Introduction
Primary retroperitoneal mucinous cystadenomas (RMCs) are extremely rare tumors and the exact etiology is unclear. The diagnosis is never made preoperatively and preoperative abdominal imaging studies can only detect a cystic mass in the retroperitoneal space. A large retroperitoneal mucinous cystadenoma adjacent to the kidney region always presents as a renal cyst.

Case Reports
Case 1: A 52-year-old woman was admitted to the Department of Urology for right lumbago of one month's duration. No quadrant mass was palpable at the abdominal examination. The percussion tenderness of the right kidney region was positive. Ultrasound (US) examination of the abdomen revealed a cystic mass measuring 14 cm in diameter associated with the right kidney. Intravenous urography (IVU) showed a huge soft tissue mass located below the right kidney and the right kidney was pushed up (Figure 1). Abdominal computed tomography (CT) revealed a 10 × 9.0 × 10.5 cm homogeneous cystic mass located in the inferior pole of the right kidney (Figure 2). The cystic mass was thought to be a right renal cyst. Therefore in March 12, 2009 laparoscopic marsupialization of the right renal cyst was performed. However the operation revealed that the retroperitoneal mass was separated from the right kidney. In May 2009 abdominal CT revealed that the retroperitoneal mass was separated from the right kidney region and extended into the right iliac fossa. The cystic retroperitoneal mass was completely removed and the cystic cavity was filled with a pallide-flavens fluid. Pathologic examination showed a cyst measuring 5.0 × 4.5 cm. Its wall measured 0.2 cm in thickness and the luminal surface was smooth. Microscopic exam showed that the cyst consisted of fibrous connective tissue lined by a single layer of benign mucinous columnar epithelium (Figure 5). The postoperative course was uneventful and the patient has remained asymptomatic after five years follow-up.

Discussion
The most common retroperitoneal mucinous cystadenomas (RMCs) are frequently ovarian tumors which share a histological similarity to ovarian mucinous cystadenomas but can arise in any location in the retroperitoneum without attachment to the ovary [1]. Primary RMCs are rare and occur only in female patients [2]. The histogenesis of primary mucinous cystadenomas of the retroperitoneum is not very clear, however some hypotheses have been proposed to explain the origin. The most plausible theory is that these tumors arise from inclusions of mesothelial cells with mucinous metaplasia [1, 3-5]. Pennel and coworkers that mucinous cystadenomas can arise from ectopic supernumerary ovaries [3, 6] or from teratomas [3].

To our knowledge, no more than 30 cases have been reported as primary RMCs in the literature. Since primary RMCs have the potential toward a phase of progression to malignancy, early diagnosis is very important [7]. With the use of US, CT and MRI the detection of retroperitoneal cysts is possible. However the diagnosis is difficult preoperatively since RMCs are often mistaken for ovarian cysts, cystic lymphangiomas, mesenteric cysts, hydatid cysts and renal cysts [3, 8].
Figure 1. — IVU showed a huge soft tissue mass located below the right kidney and the right kidney was pushed up.

Figure 2. — Abdominal CT revealed a 10 × 9.0 × 10.5 cm homogeneous cystic mass located in the inferior pole of the right kidney.

Figure 3. — Wall of the cyst consisting of fibrous connective tissue lined by a single layer of benign mucinous columnar epithelium.

Figure 4. — Abdominal CT revealed a 6 × 5.0 × 5.5 cm homogeneous hypodense mass located in the middle pole of the right kidney.

Figure 5. — Wall of the cyst consisting of fibrous connective tissue lined by single layer of benign mucinous columnar epithelium.
A retroperitoneal mucinous cystadenoma associated with the kidney can easily be mistaken for a huge renal cyst. Interestingly, among these cases, two cases were considered as renal cysts preoperatively [3, 9-11]. The two cases we reported here both presented as renal cysts preoperatively.

Preoperative diagnosis of these tumors is very difficult. There is no relationship between the age of patients and the size of tumors. The symptoms are nonspecific and most of the patients complained of an asymptomatic mass or abdominal discomfort [12]. Aspiration is a good method to delineate the nature of the cyst, but cytology of the aspirated fluid frequently fails to reveal the cell type of the epithelial cells of the cyst lining. Progression to malignancy cannot be prevented, these it is not a very suitable method for diagnosis and treatment. As for the management of primary RMCs, complete surgical excision is recommended to eliminate the risk of infection, recurrence, and malignant degeneration [2, 13]. Today with the development of the laparoscopic technique, exploratory laparotomy with complete enucleation of the cyst is usually used for both diagnosis and treatment. Laparoscopic resection of primary RMCs was accomplished in our cases. The advancement of laparoscopic surgery offers the surgeon a useful option to remove a retroperitoneal cystic mass with further advantages including less postoperative pain, lower morbidity, shorter hospitalization, and an earlier recovery [5]. However when malignancy is suspected, laparoscopic excision may not be appropriate as decompression of the cystic mass is inevitable when it is removed through a trocar.

In conclusion, retroperitoneal mucinous cystadenoma adjacent to the kidney region always presents as a renal cyst. When confronted with a cystic mass in the retroperitoneum, a primary RMC should be included in the list of differential diagnoses.

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