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Is there a role for neoadjuvant chemotherapy in early invasive cervical carcinoma?

G. Cormio, M.D., Ph.D.; V. Loizzi, M.D.; C. Carriero, M.D.; G. Putignano, M.D.; L. Selvaggi, M.D.
Department of Obstetrics and Gynecology, University of Bari, Bari (Italy)

Summary

Objective: The purpose of this study was to determine if a survival advantage may exist from neoadjuvant chemotherapy (NACT) followed by radical surgery in early invasive (Stage IB1 and IIA) cervical carcinoma. Methods: Using information from studies published on the topic of NACT in cervical carcinoma along with baseline control rates of standard treatment and patterns of failure, an estimate of how many patients with early invasive cervical cancer would benefit from this procedure was calculated. Results: NACT followed by tailored radical surgery could result in a significant decrease (about 40%) in recurrence rate (13 vs 22%) and ultimately in survival compared to conventional treatment in early invasive cervical cancer. Moreover the introduction of NACT in all patients should result in a 75% decrease of adjuvant radiotherapy (10 vs 40%), and probably in a decrease in surgical and radiation related complications. Conclusion: A fraction of patients with early invasive cervical cancer (high-risk Stage IB-IIA cervical cancer) could benefit from NACT followed by tailored radical surgery. A randomized controlled trial to test this research question is very difficult due to the large population required. A subset population is identified which may benefit from NACT.

Key words: Neoadjuvant chemotherapy; Cervical carcinoma.

Introduction

Invasive carcinoma of the uterine cervix is the fifth most common malignancy worldwide and is the second major cause of cancer-related death in women [1]. Although the introduction of the Pap smear has resulted in a 70% decrease in death rate in developed countries, the 5-year survival rate stage for stage has not essentially improved during the last decades [2].

Patients with early invasive cervical cancer (Stage IB-IIA) are appropriately treated by either radical surgery (i.e., radical hysterectomy plus systematic pelvic lymphadenectomy) or radiotherapy [3-5]; several studies have clearly shown similar cure rates (80-90% 5-year survival) with a different spectrum of complications [4, 5].

Since surgery yields a specimen for pathologic evaluation, several histopathologic factors associated with a poor prognosis, can be identified. Patients with unclear margins, parametrical involvement or lymph node metastasis are considered at significant risk of failure [6-8]. Moreover, lymphovascular space invasion, deep infiltration of the cervical stroma and an increasing lesion size adversely affect prognosis. It is common practice to administer postoperative radiotherapy to patients who have one or more of these poor prognostic factors [3]. No randomized trial has been conducted to evaluate the benefits of adjuvant radiotherapy, but a number of reports suggest that it does not significantly impact on survival, although it prolongs time to recurrence and improves pelvic control rates [7, 8].

Adjuvant pelvic radiotherapy following radical surgery is associated with a significant increase in short- and long-term complications [4], and for this reason it has been suggested that criteria for selecting early-stage cervical cancer patients to be treated with radical surgery is that postoperative adjuvant radiotherapy should be given to a maximum of 5% to 20% of these patients [9, 10].

Recently neoadjuvant chemotherapy has been increasingly used as a novel and promising therapeutic modality for the management of locally advanced cervical carcinoma [11]. Several phase II studies of chemotherapy before radical surgery have shown the feasibility and the efficacy of this approach [12, 13], and a randomized study has shown the superiority of neoadjuvant chemotherapy followed by radical surgery compared to radiotherapy alone in patients with FIGO Stage IB2-IIB [14].

The purpose of the present study was to answer the following questions:

- is there a role for neoadjuvant chemotherapy followed by tailored radical surgery in a subset of patients with early invasive (IB and IIA) cervical cancer?

- the application of this integrated treatment modality may result in a reduction of treatment-related complications compared to radical surgery followed by radiotherapy in high-risk patients?

- is it possible to select some patients for which this treatment modality may result in an increased cure rate?

- is it possible to design a prospective randomized study including an experiment arm with neoadjuvant chemotherapy followed by tailored radical surgery?
The evidence to support neoadjuvant chemotherapy in early invasive cervical cancer

Rationale and feasibility of neoadjuvant chemotherapy followed by radical surgery

After the discovery that cervical cancer is a chemosensitive tumor several factors have been advocated to support the rationale of the use of neoadjuvant chemotherapy in cervical cancer. Tumor size reduction is the most obvious. Tumor shrinkage, in fact, is associated with simplification of surgical procedures and transformation of inoperable tumors in radically resectable ones. In addition, chemotherapy given in the neoadjuvant setting might be more effective, partly because it is delivered to uncompromised tumor blood supply and to a population of chemosensitive tumor cells, in patients with intact bone marrow reserve. Theoretically chemotherapy may act on both local and distant disease thus eradicating possible subclinical metastasis. On the other hand potential drawbacks of neoadjuvant chemotherapy are toxicity, delay of primary treatment, potential selection of drug resistant clones and increased radioresistance [15].

A number of phase II studies have evaluated the efficacy of different drugs, different overall dose and different dose intensity regimens with satisfying results [11-13, 15, 16]. High clinical response rates to neoadjuvant chemotherapy (ranging between 66% and 100%) were seen in most reported series [15]. Pathological response to chemotherapy has also been evaluated on both primary tumor and lymph nodes, and complete response rates vary between 10% to 40% [15]. Another interesting observation is the reduced incidence of positive lymph nodes after neoadjuvant chemotherapy. The incidence of positive lymph nodes in Stage IB-IIA patients (all with tumor diameters larger than 4 cm) after NACT varied from 6% to 23% [12, 13]. These figures are much lower than 40% to 80% reported earlier in patients with bulky Stage IB-IIA who did not receive NACT. This suggests that chemotherapy also has an impact on tumor deposits in lymph nodes as it does on primary tumor.

In most of the reported series chemotherapy was usually well tolerated, toxicity was mild and manageable and very few toxic deaths have been reported [15].

Potential reduction of surgical radicality following neoadjuvant chemotherapy

The potential complications of radical hysterectomy are well recognized, and a number of strategies are currently being investigated to try to reduce some of them (nerve-sparing radical hysterectomy). Based on the hypothesis that neoadjuvant chemotherapy results in a remarkable reduction of tumor volume, the extent of resection of radical surgery could be modulated, in order to reduce surgery-related complications. There are a number of studies suggesting that in early invasive cervical cancer it is possible to tailor surgical radicality [17]. Moreover in the only prospective randomized study comparing class II and class III radical hysterectomy no significant difference in disease free survival, overall survival and recurrence rate could be detected in patients with Stage IB-IIA cervical cancer. In the same study it was demonstrated that the less extensive procedure was accomplished with a significantly lower blood loss, operative time and overall short- and long-term complications [18].

In most reported series surgery following neoadjuvant chemotherapy is usually accomplished easier due to tumor

RH = Radical hysterectomy; PLND = Pelvic lymph node dissection.

Figure 1. — Algorithm of cure and recurrence of Stage IB-IIA patients with cervical cancer after standard treatment.
Is there a role for neoadjuvant chemotherapy in early invasive cervical carcinoma?

Shrinkage and mean operative time, blood loss, hospital stay and complications appear to be reduced compared to patients with similar stage of disease who are submitted primarily to radical surgery [12, 13, 16]. However, in some situations the identification of an accurate dissection margin may be very difficult in previously treated patients and in presence of fibrotic tissue [15].

Reduction of adverse pathologic risk factors following neoadjuvant chemotherapy and radical surgery and thus the percent of patients requiring adjuvant radiotherapy

In a literature review of trials of neoadjuvant chemotherapy and surgery, around 30% of the cases also received adjuvant pelvic radiation [12], and this figure is even lower compared to adjuvant radiation rates following primary surgery reported for early invasive cervical cancer [5-8, 18].

It is obvious that if neoadjuvant chemotherapy results in a significant reduction of tumor volume, lymph node metastasis, vaginal and parametrial involvement the need for adjuvant radiotherapy could be remarkably decreased. In such instances we could also reduce the complication rate resulting from the association of the two treatments (surgery plus radiotherapy).

Moreover, sparing adjuvant radiotherapy might result in a possible salvage treatment if disease recurs.

Survival improvement

Data on survival are more limited. An Italian multicenter study comparing neoadjuvant chemotherapy followed by radical surgery with radical radiotherapy demonstrated that a statistically significant advantage in overall and disease-free survival could be found only for the Stage IB2-IIB group when treated with the combined modality [14]. All other retrospective studies have no statistical power to detect any difference in survival.

The results have also been subjected to a meta-analysis including 872 patients and 368 deaths. The overall results show a highly significant benefit of this modality compared to radiation alone, with a 36% reduction in the risk of death (HR = 0.64, 95% CI = 0.52 to 0.79, p = 0.00003). This is equivalent to an absolute improvement in survival of 15% (8-21%) at five years, increasing survival from 45% to 60%. Similar reductions are observed for progression-free survival, and local and systemic control [19]. This benefit is of the same magnitude as that achieved with the new standard of cisplatin-based chemoradiation [20]. Currently the EORTC is conducting a multicentric phase III study to compare cisplatin-based neoadjuvant chemotherapy followed by radical surgery, with or without adjuvant radiation, versus standard cisplatin chemoradiation in Stages IB2-IIB (EORTC # 55994).

Calculation of the possible magnitude of benefit from neoadjuvant chemotherapy in early invasive cervical cancer

With current standard therapies (radical surgery or radical radiotherapy or concurrent chemoradiotherapy), overall 5-year survival for Stage IB-IIA cervical carcinoma range approxi-
approximately between 75 and 95% based on clinicopathologic characteristics of the patient population. On the other hand the incidence of positive pelvic lymph nodes ranges between 7 to 30% and, despite adjuvant treatments, Stage IB patients with positive lymph nodes after radical hysterectomy have a 5-year survival ranging between 42 to 73% [2].

With this number we can hypothesize that of 100 patients submitted to radical hysterectomy and pelvic lymph node dissection for Stage IB-IIA cervical cancer about 40 will have one or more of the following adverse prognostic factors on pathologic examination: lymph node metastasis, parametrial or vaginal involvement, lymphovascular space involvement, unclear surgical margins or deep cervical stroma invasion. Despite all these, 40 patients will receive standard adjuvant pelvic radiation, and about 40% of them (16 patients) will experience recurrent disease and will probably die of their disease. Among the remaining 60 patients without poor prognostic factors after radical hysterectomy, about six (10%) will experience recurrent disease (Table 1).

On the other hand if we consider 100 Stage IB-IIA patients submitted to two to three cycles of neoadjuvant chemotherapy, with a response rate of approximately 80%, we can estimate that about 10% of the patients will be found to have adverse prognostic factors on pathologic examination following radical surgery. Again all these ten patients will receive adjuvant radiotherapy and only four (40%) would be expected to experience a recurrence. If we consider the same risk of recurrence (10%) for the remaining 90 low-risk patients not receiving adjuvant treatment we would expect about nine recurrences in this group (Table 2).

Thus on one side we have 100 patients operated on, 40 with adjuvant treatment and 22 recurrences, whereas on the other side we have 100 with neoadjuvant chemotherapy, 100 patients operated on, ten with adjuvant radiotherapy and approximately 13 recurrences. It means that with the experimental arm nine recurrences and 30 adjuvant radiotherapies are saved in face of 100 neoadjuvant chemotherapies. This could mean a 40% reduction of patients with recurrent disease and probably of death.

Feasibility of a randomized controlled trial assessing the value of neoadjuvant chemotherapy in early invasive cervical cancer

To develop a trial to address the question, a subset of patients with early invasive cervical cancer must be selected who most stand to benefit from neoadjuvant chemotherapy. In general these patients would have to have a high likelihood of adverse prognostic factors (nodal metastases or microscopic parametrial involvement), a high chance of successful chemotherapy response and a very low likelihood of complications related to the association of chemotherapy to surgery.

The proposed study would have to exclude those patients with a very low incidence of adverse prognostic factors (i.e. small tumor volume). Based on our calculation, with an α-error of 0.5% and a β-error of 95%, and if we expect a 10% difference in survival or recurrence rate, about 300 patients per arm are necessary to design a randomized study.

Discussion

Early invasive cervical cancer (Stage IB-IIA) shows a high 5-year survival rate of 75-95% when treated by either radiotherapy or radical surgery. Though there has been progress in the management of this disease with the addition of chemotherapy to radiation treatment [21], there is still a challenge to improve outcome in early invasive cervical cancer patients with histopathologic high-risk factors. In fact, while radiation and chemoradiation have a high chance of controlling disease in the pelvis, they are not successful in preventing distant spread. Moreover both procedures are associated with long-term radiation hazard complications to normal tissues.

Neoadjuvant chemotherapy has been applied in the last few years as a new therapeutic approach for locally advanced cervical carcinoma because of the disappoint-
ing results with conventional treatments, but there is no experience in the early invasive cervical cancer setting. A number of aspects concerning this novel approach, however, should be considered in detail: chemotherapy regimen, number of cycles to be delivered and patient selection.

A variety of regimens have been used, but there is still no evidence for a benefit advantage of platinum-based combinations to platinum alone. Currently, a new generation of active chemotherapeutic agents including taxanes [22] and vinorelbine [16] are being investigated, and the incorporation of these novel agents in future schedules with cisplatin might offer further opportunities for neoadjuvant strategies. A common finding is the fact that the number of chemotherapy cycles correlated with the response in the primary tumor [12, 13, 15], and this suggests that more cycles of chemotherapy are needed for an optimal result. Recently, Tierney et al. performed a systematic review and meta-analysis on neoadjuvant chemotherapy for locally advanced cervical cancer and found that chemotherapy cycle lengths of 14 days or shorter and cisplatin dose intensities greater than or equal to 25 mg/m^2 per week tended to show an advantage for neoadjuvant chemotherapy on survival [19]. With this in mind we should select an appropriate platin-based regimen with a proper dose intensity and correct number of cycles to achieve the best response.

In more than one study clinical stage and tumor size affected response to NACT and finally survival [12-15]. This could be satisfactorily explained by the direct correlations between disease volume, chemotherapy resistance, radical operability and outcome in many solid tumors including cervical cancer [15]. The greater the volume, the larger is the hypoxic and resting phase cell population with reduced or no chemosensitivity, and the probability of developing resistant clones. With this data one can assume that the smaller the tumor (early-stage cervical cancer) the higher the complete response rate to NACT, and that pathologic response rate reported for advanced cases (20-30%) might reach 40-60% in patients with early invasive cancers (Table 2).

The identification of patients who most likely can benefit from this combined approach (neoadjuvant chemotherapy followed by tailored radical surgery) can be based on three different aspects: common imaging techniques, serum tumor markers and chemosensitivity assay. In fact a number of reports have proven that magnetic resonance imaging (MRI) is able to identify with high accuracy those patients who will present at final pathologic examination extraterine spread, thus requiring adjuvant treatment [23]. Another possibility is preoperative use of one or more tumor markers. In a recent paper it was proven that determination of serum squamous cell carcinoma antigen allows more refined preoperative estimation of the likelihood for adjuvant radiotherapy than current clinical parameters, and simultaneously identifies patients at high risk for recurrence when treated with surgery only [24]. In the near future the development of chemosensitivity assay as for ovarian cancer might allow selection at the time of diagnosis of those patients who are most likely to achieve a complete response to neoadjuvant chemotherapy and can be safely managed with tailored radical surgery, sparing complications and morbidity resulting from the association of radical surgery with external radiotherapy.

There are however some potential pitfalls of neoadjuvant chemotherapy followed by tailored radical surgery in early invasive cervical cancer: toxicity associated with the addiction of neoadjuvant chemotherapy, delay in primary treatment and the lack of proof that chemotherapy will reduce distant disease.

Chemo- and radiotherapy-associated toxicity is usually mild to moderate, reversible and it is not cumulative with subsequent surgical complications. Three cycles of chemotherapy followed by radical surgery can be accomplished in about 60-70 days which is the usual length of external radiation. In some geographic areas, such as Southern Italy where radiotherapy facilities are not easily available this can be considered less than a waiting list for external radiation.

The demonstration of any activity of neoadjuvant chemotherapy on subclinical metastases is more difficult. The previously mentioned Italian multicenter study proved that about one-third of the failures showed a distant component, and that there was no statistically significant difference between the two arms with regard to the pattern of disease recurrence [14]. These data in accordance with those reported by the Argentine group suggest that the relatively short duration of NACT may be not enough to sterilize distant metastases [25].

Based on our calculation neoadjuvant chemotherapy followed by tailored radical surgery could result in a significant decrease (about 40%) in recurrence rate (13 vs 22%) and ultimately in survival compared to conventional treatment in early invasive cervical cancer. Moreover the introduction of neoadjuvant chemotherapy in all patients should result in a 75% decrease of adjuvant radiotherapy (10 vs 40%), and probably in a decrease in surgical and radiation-related complications. Of course these figures can be remarkably reduced if NACT should not give the expected results (80% response rate) and need to be confirmed in a prospective study.

In conclusion we believe that in the near future neoadjuvant chemotherapy followed by tailored radical surgery may become an alternative to conventional treatments in a selected population of patients with early invasive cervical cancer, especially in those regions where radiation facilities are not easily available. A prospective randomized study will demonstrate an eventual positive impact on survival of this combined treatment modality compared to the standard treatment.

Similarly to breast cancer, where multimodality treatment (QUART) has been demonstrated to reduce surgical radicality without compromising overall survival, also in cervix cancer this promising combined approach (neoadjuvant chemotherapy followed by radical surgery) might in the near future result in significant improvement in both survival and quality of life. In our opinion, the most
important point to be developed is the identification of those patients who are most likely to benefit from this combined modality.

References


Address reprint requests to:
V. LOIZZI, M.D.
Viale Kennedy, 80
70124 Bari (Italy)
e-mail: vloizzi@tiscali.it
Patterns of surgical care for uterine cancers in Ontario

L. Elit1, M.D. MSc. FRCS(C); S. Schultz2, MA MSc.; R. Prysbysz1, MSc.; J. Kwon1, M.D., MPh. FRCS(C); R. Saskin1, MSc.; N. Gunraj1, BSc MPH; A.S. Wilton2, MSc.; M. Simunovic1, M.D., MPh FRCS(C); D. Urbach1, M.D. MSc. FRCS(C) FACS

1Department of Obstetrics and Gynecology and Surgery, McMaster University, Hamilton
2Institute for Clinical Evaluative Sciences (ICES), Toronto
3Department of Gynecologic Oncology, MD Anderson, Houston
4Department of Surgery, University of Toronto, Toronto (Canada)

Summary

Objectives: To facilitate the planning of future resources for cancer services in Ontario, Cancer Care Ontario commissioned an evaluation of operative services delivered for uterine cancer. Methods: Women with an incident diagnosis of a uterine malignancy were identified from 1 April 2003 to 31 March 2004 using the Ontario Cancer Registry. Record linkages were created to other provincial health databases such as the Ontario Health Insurance Plan. Results: Uterine cancer affected 1,436 women. Disease specific rates of cancer were higher in rural areas and those from the highest income quintiles. Surgery occurred in 94.7% of women. Use of surgery did not appear to vary by SEC, urban/rural residence or LHIN. Gynecologists conducted 76.1% of the operations. Conclusions: There appear to be variations in incidence rates of uterine cancer with disease being more frequent in those of the highest SES. In two-thirds of the population, surgery is delivered in the region where the patient lives. Subspecialty care from gynecologic oncologists was provided to one-third of women. Rates of lymphadenectomy as part of a surgical attempt to assess disease spread appear low. These pilot data would be enhanced with further information such as comorbidity, treatment intent (palliative/curative), histology, grade and stage.

Key words: Uterine cancer; Health services.

Introduction

Canadians are concerned about timely access to cancer surgery [1]. To validate this concern, a formal provincial assessment of surgical waiting times has shown that there is geographic variation in access to care. Formulating health care policy around surgical waiting times and geographic variations in access to care begins with understanding what services are currently being accessed. The objective of this report is to provide information on surgical care for uterine cancer in Ontario. We will: 1. characterize the patients with uterine cancer; 2. inventory the components of the operative procedure; and 3. describe the health services women receive perioperatively.

Methods

Ethics approval for this study was obtained from the Research Ethics Board at Sunnybrook and Women’s College Hospital. This is a population-based study of all women with an incident gynecologic cancer from 1 April 2003 to 31 March 2004. The International Classification for Disease code (ICD-9) 182 was used for uterine cancer. The cohort was identified using the Ontario Cancer Registry (OCR). There was record linkage to other provincial health databases such as Ontario Health Insurance Plan (OHIP), Canadian Institute for Health Information (CIHI) discharge abstract database (DAD) and same day surgery (SDS), and National Ambulatory Care Reporting System (NACRS) to within one year of diagnosis. To be included the patient required a valid OHIP number and had to be 18 years or older at the time of diagnosis. Patient age and postal code at time of surgery were obtained from the Ministry of Health and Long-Term Care Registered Persons Database (RPDB).

Patients’ postal codes were used to obtain ecological income quintiles and conversion to Local Health Integration Networks (LHIN) using Statistics Canada conversion files. There are 14 LHIN in Ontario. These are non-for-profit corporations that work with local healthcare providers and community members to determine healthcare priorities for their region. Vital statistics information (socio-economic status, urban/rural residence) was available through RPDB. Spot checks on the procedure data by cancer site showed congruence between the CCI procedure codes and the OHIP billed procedure to within 5%. The top 20 CCI therapeutic and diagnostic procedure codes associated with the disease were identified to within 1% of the count for the period 2002-2005.

Statistical analysis was performed using SAS 9.1. Age was stratified into four cohorts (20-35, 36-49, 50-69, 70 years and over). All numbers reported here are age standardized per 100,000 women (ASR). Socioeconomic status was measured by the median household income in the neighborhood where the women lived. This was distributed into five quintiles. Rural vs urban residence was classified by one of three community population sizes (<100,000, 100,000-1,249,000 and over 1,249,000). Physician specialty was identified manually crosschecked using Scott’s Medical Database. Surgeon type was gynecologic oncologist, gynecologist, general surgeon and other.
Results

Demographics: incidence and treatment

From April 1, 2003 to March 31, 2004, 1,436 women in Ontario were diagnosed with an incident uterine cancer. Of these women, 94.7% underwent surgical procedures.

Age: This disease affects mostly postmenopausal women with the highest incidence in women 70 years and older (75.3 per 100,000 Ontario women). Rates of surgery did not vary by age.

SES: Those in the highest income quintile appear to have a higher likelihood of developing uterine cancer (33.2/100,000 highest highest income quintile vs 23.1/100,000 ASR in the lowest income quintile). Rates of surgery were slightly higher in the highest income quintile (95.4/100,000 vs 92.7/100,000 ASR).

Geography: The rates of disease and the access to surgery did not vary by geography.

LHIN: Seventy-nine percent of women received surgery in their LHIN of residence.

Definitive treatment

1. Hospitalization

Among the 1,360 women who had surgery for uterine cancer, there were a total of 1,938 surgical procedures (1.4 surgical procedures per woman) of which 29.1% were ambulatory (like D+C) and 70.9% involved overnight hospitalizations.

2. Operative therapy

There are four types of surgery for uterine cancer. Total abdominal hysterectomy with removal of the ovaries (TAH+BSO) is the standard of care. The addition of lymphadenectomy is especially useful in defining the need for adjuvant therapy in women with high-risk features such as grade 2 or 3, or deep myometrial involvement. Other procedures include biopsy of nodes only, biopsy of other gynecologic structures only, uterine biopsy only, and biopsy within the abdomen. The procedures were classified in terms of the most extensive procedure a woman received. Hysterectomy and bilateral salpingo-oophorectomy was the most common procedure and occurred in 71.9% of women. Pelvic and/or paraaortic lymphadenectomy were completed in an additional 18.7% of women.

Age: Younger women (26-39yr) were more likely to be treated by hysterectomy alone (25.0%). However, 50% of women in this age range did have TAH+BSO.

SES: The type of surgery did not vary across income quintiles.

Geography: Urban/rural location of residence did not influence type of surgery. Access to lymphadenectomy did vary by LHIN with the highest rate of 56.4% in the Champlain LHIN compared to the lowest rate of < 5% in two other jurisdictions.

Hospital type: 87.9% of uterine cancer surgeries were conducted in community hospitals compared to 12.1% of cases in academic centres.

3. Surgical discipline involved

Gynecologists provided primary surgery for 76.1% of Ontario women and in 80% of cases, the operation was a simple hysterectomy and bilateral salpingo-oophorectomy. In only 9.4% of cases, did the surgery by a gynecologist include a lymph node sampling procedure. Gynecologic oncologists operated on 21.3% of women. They conducted node sampling/dissection in approximately half of the patients on whom they operated. In total, 18.7% of women with uterine cancer had some form of operative nodal assessment. General surgeons conducted the primary surgery in 2.6% of cases. In the entire group, 4.6% of women received a simple hysterectomy without oophorectomy as their definitive surgery. The highest rate for simple hysterectomy without oophorectomy occurred when the general surgeons (16.7%) did the surgery compared to 3% if a gynecologists or gynecologic oncologists conducted the operation.

Perioperative workup

Preoperative assessments are most likely to include a pelvic ultrasound and some form of uterine biopsy. Pap smears were only done in one-third of the women. The radiologic assessments done either 12 months before or 12 months after surgery were CXRs (1.4/patient), and CT scan of the abdomen and pelvis (60%). MRI scans of the pelvis were infrequent (< 10%).

In the 12 months before or after the woman’s surgery, a gynecologist saw 92.4% of patients and gynecologic oncologists saw 38.1% of patients. A radiation oncology assessment occurred in 60.7% with radiation being delivered to 28.9%. A medical oncology assessment occurred in 6.3% of women with chemotherapy being provided to 7.6% of patients, usually after surgery.

The preoperative workup for women who did not receive surgery was very similar with the exception that a higher number of women (20%) also received an MRI of the pelvis. Only 5.3% of women with uterine cancer did not have surgery. Two-thirds of these women did not receive any cancer directed treatment (ie., radiation or chemotherapy).

(For a more detailed disclosure of tables that led to this report see reference [23]).

Discussion

In Ontario, this is the second review of the patterns of practice for uterine cancer [2-5]. It appears that the incidence of uterine cancer continues to increase with age and SES. This is consistent with the literature [6]. Being able to access an operation for the management of uterine cancer did not appear to vary by age, geography or SES. There is significant literature dealing with the impact of race and SES on incidence and mortality of uterine cancer [6-9]. However, little is known about the interplay between age, socio-economic and geographic factors on the incidence and delivery of uterine cancer care in the context of a socially funded medical system. Kwon et al.
[2] reported no difference in survival in 3,875 women receiving surgery in Ontario from 1996-2000. They showed that high-risk women in the lowest income quintile had the highest risk of death when compared to women from the highest income quintile. Our work did not address survival.

Three-quarters of the operations required a hospital stay. Three-quarters of patients had their surgery in their LHIN but one-quarter traveled outside of their region for surgical care. How the LHIN mechanism addresses planning for this resource and what fiscal responsibility the LHIN of residence has toward the LHIN of service is not clearly defined.

The type of surgery was influenced by age. In women with uterine cancer, it appears that younger women are receiving operations that are conservative with the aim of preserving ovarian function. The type of surgery did not appear to be influenced by SES. However the type of surgery was influenced by LHIN with lymphadenectomy rates being significantly higher in Champlain. The type of surgery was also influenced by type of surgeon. General surgeons were much more likely to do just a hysterectomy without removing the ovaries while gynecologic oncologists conducted a higher rate of lymphadenectomies than other specialists. This finding is consistent with work from Ontario [4] and the USA [10].

Lymphadenectomy is advocated in the surgical staging of endometrial cancer; however, there is much discussion concerning who should actually receive this procedure (i.e., all patients or just those at high risk for nodal involvement). It is not known whether completing the lymphadenectomy impacts survival. A preliminary result from MRC-UK ASTEC (A Study in the Treatment of Endometrial Cancer) which is the only randomized prospective study that has evaluated the role of lymphadenectomy in early-stage uterine cancer suggests that this procedure does not impact survival [11]. The reason for Ontario’s low lymphadenectomy rates (20.9%) cannot be discerned from the available data. Other information such as stage, histology, depth of invasion, and grade would be required to determine whether patients received an appropriate decision for surgery, and whether patients received the appropriate operative procedure. Kwon et al.’s [2] review of Stage 1 and 2 uterine cancer from 1996-2000 showed an 11% lymphadenectomy rate. The differences in our data may represent a change in practice or the fact that our rate is for all stages of disease while Kwon reports only for those with Stage 1 and 2 disease. That being said, both of these rates are much lower that the lymphadenectomy rates of 50-80% reported in the USA [12-16].

The current guidelines for working up a patient with abnormal vaginal bleeding focus on pelvic examination and uterine biopsy. There are no guidelines for the radiologic assessment of women diagnosed with uterine cancer. The role of routine CXR is to discern distant disease. The roles of CT scan and/or MRI are not clear. In our population 60% of women had a CT scan within 12 months of diagnosis.

Cancer Care Ontario and the Program in Evidence Based Medicine are moving toward better defining the medical care of women with uterine cancers through practice guidelines and practice standards. Currently the uterine cancer directed guideline involves the use of adjuvant radiation therapy in the intermediate risk Stage 1 patient [17], use of systemic therapy in advanced and recurrent disease [18] and follow-up care [19]. Currently there are no evidence-based guidelines or standards for uterine cancer surgery. As well, the quality indicators for uterine cancer care have not yet been identified in the literature.

There is a small body of literature on uterine cancer outcomes by region [20, 21]; much of this work is augmented by the massive undertaking of retrospective data collection [2-5]. Specific retrospective data on patterns of care for uterine [14, 15] cancer are available in Ontario. Addressing such questions on an ongoing basis for the purpose of assessing quality of care would be augmented by prospective collection of information on confounders (i.e., stage), cofactors (i.e., smoking) and pathologic details (grade, histology). The Canadian Partnership Against Cancer (CPAC) is looking at standardized operative reporting [22] as a means of prospectively collecting the details of the surgery and major confounders like body mass index and clinical stage.

Conclusion

As we move into the future, Ontario needs to define what constitutes evidence-based surgical care (in particular the role of lymphadenectomy) for women with uterine cancer and these standards need to be communicated throughout the province. The preoperative workup including indications for specific radiologic investigations needs to be better defined. Quality indicators need to be determined. Waiting times were not addressed in this review but would be part of any assessment of timeliness of care. As healthcare funding becomes more aligned to LHINs, we will need to better understand the LHINs expectation for the care of their constituents versus care of patients from other LHINs. Ultimately, quality of care assessments will require timely access to data, correction of the data for confounders and cofactors. Only then can we better discern any gap between the indicators and the care delivered.

Acknowledgement

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Address reprint requests to:
L. ELIT, M.D.
699 Concession Str.
Hamilton (Canada) L8V 5C2
e-mail: laurie.elit@jcc.hhsc.ca
Glucocorticoid receptor expression in cervical intraepithelial neoplasia and invasive squamous cell carcinoma of the cervix

F. Buxant¹, D. Bucella¹, V. Anafi¹, P. Simon¹, J.C. Noël²

Department of Gynaecology¹ and Pathology², Erasme Hospital, Free University of Brussels (Belgium)

Introduction

Glucocorticoids (GCs) are of great value in the treatment of inflammatory disorders, autoimmune diseases, and tissue oedema. GCs also possess antipyretic activity and are used after solid-organ transplantation [1] or in the therapeutic induction of apoptotic cell death in malignant lymphoid cells [2]. Before, during and after chemotherapy of solid tumours, GCs are given at various doses to reduce toxicity, particularly hyperemesis, and to protect normal tissue of cancer patients against the long-term effects of genotoxic drugs [3].

GCs act also as cofactor with human papillomaviruses in the etiology of cervical cancer. Moreover, recently GCs were described as inhibitors of some chemotherapy or radiation-induced apoptosis. The presence or not of a glucocorticoid receptor (GR) in normal and abnormal exocervices is thus interesting. Methods: To clarify the issue, we tested by immunohistochemistry the expression status of GR in normal cervix epithelium (n = 30), in low-grade cervical intraepithelial neoplasia (LSIL) (n = 30), in high-grade cervical intraepithelial neoplasia (HSIL) (n = 30) and in invasive squamous cell carcinoma (ISCC) (n = 30). All the patients with these lesions have a corresponding liquid-based cytology and were proved to be HPV-positive by using hybrid capture 2 methodology with probes against high-risk oncogenic HPVs. The evaluation of GR expression was performed by using the H-score system and an H-score > 50 was considered positive. Result: GR expression was observed in normal epithelium, LSIL, HSIL and ISCC. No statistically significant difference concerning this expression was observed. Conclusion: Because GCs could play a positive role in the progression of cancer, our demonstration of GR persistence in cervix cancer cells raises concern about the widespread combined use of GCs with antineoplastic drugs or agents in the clinical management of cervix cancer in women.

Summary

Objectives: Glucocorticoids (GCs) are used in cancer treatment to cause programmed cell death in transformed cells of the hematopoietic system and to lessen side-effects as nausea, vomiting, edema formation and allergies to specific chemotherapeutic agents. GCs act also as cofactor with human papillomaviruses in the etiology of cervical cancer. Moreover, recently GCs were described as inhibitors of some chemotherapy or radiation-induced apoptosis. The presence or not of a glucocorticoid receptor (GR) in normal and abnormal exocervices is thus interesting.

Methods: To clarify the issue, we tested by immunohistochemistry the expression status of GR in normal cervix epithelium (n = 30), in low-grade cervical intraepithelial neoplasia (LSIL) (n = 30), in high-grade cervical intraepithelial neoplasia (HSIL) (n = 30) and in invasive squamous cell carcinoma (ISCC) (n = 30). All the patients with these lesions have a corresponding liquid-based cytology and were proved to be HPV-positive by using hybrid capture 2 methodology with probes against high-risk oncogenic HPVs. The evaluation of GR expression was performed by using the H-score system and an H-score > 50 was considered positive. Result: GR expression was observed in normal epithelium, LSIL, HSIL and ISCC. No statistically significant difference concerning this expression was observed. Conclusion: Because GCs could play a positive role in the progression of cancer, our demonstration of GR persistence in cervix cancer cells raises concern about the widespread combined use of GCs with antineoplastic drugs or agents in the clinical management of cervix cancer in women.

Key words: GR expression in cervical neoplasia.
Materials and Methods

Cervical tissue samples from patients undergoing biopsy, cervical conisation or radical hysterectomy were selected sequentially from the Department of Pathology, Erasme University Hospital, Brussels, Belgium, and consisted of 30 cases of normal exocervix, 30 cases of low-grade squamous intraepithelial lesions (LSIL corresponding to CIN1), 30 cases of high-grade squamous intraepithelial (HSIL corresponding to CIN2-3) and 17 cases of invasive squamous cervix carcinoma (ISCC). All the patients with LSIL, HSIL and ISCC obtained a corresponding liquid-based cytology (Cytic Corp, Marlborough, MA), and they were proved to be HPV positive by using hybrid capture 2 DNA HPV methodology (Digene Corp, Gaithersburg, MD) with probes against HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59 and 68 as previously described [16-18].

Immunohistochemistry

Four-micrometer sections were cut sequentially and mounted onto superfrost-treated slides (Menzel-Glasser, Braunschweig, Germany). The slides were dried overnight at 37°C before deparaffinization in xylene and rehydration through graded ethanol. For epitope retrieval, the slides were immersed in a waterbath at 95°C to 99°C for 90 min with an ethylenediaminetetraacetic acid buffer, pH 9.0 (S236, Dako Corp, Glostrup, Denmark). Then the slides were cooled in their buffer for 20 min at room temperature. H2O (0.3%) was added to the slides and incubated at room temperature for 30 min. The tissues were then incubated one hour at room temperature with a monoclonal antibody against the N-terminus of the glucocorticoid receptor (clone NCL-L-GCR, dilution 1/25, Novocastra Laboratories Ltd, Newcastle, UK).

The evaluation of GR was performed independently by two pathologists by using the H-score system as previously described for ER receptors [19]. The H score is based on a summation of the proportion of tumour cells showing different degrees of reactivity: 0 x % tumour cells negative + 1 x % tumour cells weakly positive + 2 x % tumour cells moderately positive + 3 x % tumour cells strongly positive. This gives a maximum total score of 300 if 100% of the tumour cells show a strong reactivity. In all cases, 500 cells were randomly counted. Positive control consisted of tonsil tissue. To control the non specific binding of the primary antibody, non immune mouse serum was substituted as the first layer of the serial sections.

Statistical analysis

The data were compared with Student’s t-test (2-tailed) to test for equality of means.

Results

The staining pattern of GR was only nuclear and GR was demonstrated in normal exocervices, LSIL, HSIL, and ISCC (Figure 1). No difference was found to be significant (t-test for equality of means, p > 0.02) between normal exocervices, LSIL, HSIL, and ISCC (Table 1).

Discussion

Squamous cell carcinoma accounts for 75 to 85% of cervical cancers and develops in the background of increasing grades of dysplasia. HR-HPV is detected in more than 90% of malignant cervical tumours and it is widely accepted that HPV plays an essential role in the pathogenesis of cervical cancer. The association of hormones and HPVs with cervical cancer was clearly established [20]. Hormones are intricately associated with the growth and differentiation of cervical epithelium and induce a higher level of the HPV E6-E7 oncoproteins in human ectocervical cells [14, 17]. HPV E6-E7 degrades the p53 gene. Loss of function of the normal p53 gene and thus its tumour suppressor action is a possible biological mechanism for the role of steroids in cervical carcinogenesis [21]. In our study, we demonstrate the persistence of expression of GR during the progression of CIN to ISCC. GC could thus act during the first transformation of normal cells but also could continue to play a role after HPV infection. For this reason, before and during GC administration to women with chronic inflammatory disease or organ transplant, cervical screening is needed and recommended. Screening for premalignant conditions should be included in pretreatment evaluation and HPV testing should be performed. For HPV-negative women, HPV vaccine should be discussed with the patient.

For nearly 50 years, physicians have relied on GCs to treat several types of cancer (lymphoid cancer). There are hormones are widely used in combination with chemotherapy and radiation of patients with solid tumours due to several other beneficial effects. Concomitantly, concerns about the widespread use of GCs during therapy of solid tumours have been expressed repeatedly [22-24]. Zhang and colleagues [25] found that dexamethasone inhibits cisplatin and 5-fluorouracil-induced apoptosis and promotes growth in several established and primary carcinoma cancer cells (bone, brain, breast, cervix, melanoma and neuroblastoma) while the opposite effect occurs in lymphoid cells. In vitro, GCs upregulate c-fms in breast cancer cells, via a GR-dependent pathway, associated with an increase in cancer invasiveness [26, 27]. Rutz and Herr [28] address several additional mechanisms through which glucocorticoids may influence the result of cytotoxic therapy of cancer, such as inducible interference of apoptosis signalling, interference with immune response against malignant cells, and issues relating to their impact on glucose metabolism in cancer patients. Herr et al. [29] describe molecular evidence of a general induction of survival signalling in epithelial cells and carcinoma cells by GC.

Moreover, the synthetic steroid dexamethasone inhibits radiation-induced apoptosis in cervix carcinoma by E6 modulation of p53 expression [30, 31] and may adversely affect treatment negatively.

Chemotherapy and radiation are the cornerstone of advanced cervix cancer treatment. We demonstrated the persistence of GR in cervix cancer cells.
Figure 1. — Immunohistochemical expression of GR. A: Normal epithelium, B: LSIL (CIN1), C: HSIL (CIN2-3), D: ISCC.
These data therefore raise concern about the widespread combined use of GCs with antineoplastic drugs or agents in the clinical management of women with cervix cancer.

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Address reprint requests to: F. BUXTANT, M.D.
Department of Gynaecology
CUB Hospital Erasme
1070 Brussels (Belgium)
e-mail: fbuxant@ulb.ac.be
Histone deacetylase inhibitor trichostatin A modulates cell cycles in A2780 human ovarian cancer cell lines

X.X. Ma, Y.N. Jin, Q. Miao, S. Li, Y.Q. He, H.W. Lv
Department of Obstetrics and Gynecology, Shengjing Hospital, China Medical University, Shenyang (China)

Summary

Purpose of investigation: To analyze the effect and mechanism of TSA on cell cycles in human ovarian cancer cells. Methods: cells were cultured in RPMI 1640 supplemented. Flow cytometry analysis and RT-PCR were used to examine the distribution of cell cycles and the level of P21\textsuperscript{W AF/CIPI} mRNA. Results: TSA induced increase of the G\textsubscript{2}/M phase accompanied by decrease of the S phase and enhanced level of P21\textsuperscript{W AF/CIPI} mRNA in a concentration and time-dependent manner in A2780 cells. Conclusion: Trichostatin A affects the activity of cyclin-dependent kinase through increased expression of P21\textsuperscript{W AF/CIPI} mRNA. TSA causes A2780 cell blockage in the G\textsubscript{2}/M phase and inhibits cell proliferation of A2780 cells. The minimum level of active TSA is 100nM and the minimum time is 12 hours. The effect relies on time and concentration.

Key words: TSA; A2780 cells; Flow cytometry; cell cycle; P21\textsuperscript{W AF/CIPI}.

Introduction

Ovarian cancer is a common gynecologic cancer with high malignancy. Some cancers are curable, but many become refractory to therapies. So far, the 5-year survival rate is still less than 30%-40%. In ovarian cancer and other malignancies, a common character is cell cycle disorder and excessive proliferation. Epigenetics is the study of chromatin modifications that affect gene expression without altering DNA nucleotide sequences. Epigenetic abnormalities, including DNA methylation and histone hypoacetylation around the promoter region of genes, can be changed by some reagents. Thus, expression of tumor suppressor genes silenced by epigenetic mechanisms can be restored, leading to cell cycle arrest and/or apoptosis of the cancer cells.

Histone is the basic structure of nucleosome in chromatin. One of the most important mechanisms in chromatin remodeling is the post-transcriptional acetylation of N-terminal tails of histones. Acetylation is a capital covalent modification of histone, which is regulated by the opposing activities of histone acetyltransferases (HATs) and histone deacetyltransferases (HDACs). HATs catalyze histone acetylation and activate transcription while HDACs catalyze removal of acetyl groups on the amino-terminal lysine residues of core nucleosomal histones, associated with transcriptional repression [1]. Aberrant recruitment of HDAC activity has been associated with development of certain human cancers.

Many HDAC inhibitors (HDACIs) have been identified inhibiting cell proliferation through restoring expression of some genes silenced by epigenetic mechanisms [2]. HDAC inhibitors inducing tumor cell growth arrest, differentiation and/or apoptosis are currently the focus of intensive research. Several HDAC inhibitors have shown impressive antitumor activity in vivo with remarkably little toxicity in preclinical studies and are currently in a phase I clinical trial. Trichostatin A, a specific inhibitor of HDAC, induced histone hyperacetylation followed by blocking cell cycle progression at G1, resulting in apoptosis of the cancer cells.

P21\textsuperscript{W AF/CIPI} is best known as a broad-specificity inhibitor of cyclin/cyclin-dependent kinase complexes, and it is essential for the onset of cell cycle arrest in damage response and cell senescence. Numerous laboratory and epidemiological studies in vitro and in vivo show that the reduction or disappearance of P21\textsuperscript{W AF/CIPI} mRNA was related to the oncogenesis and development of ovarian cancer [3].

The effect of HDACIs on ovarian cancer has not been fully examined as of yet. We focused particularly on TSA, and examined whether it was able to accommodate the expression of P21\textsuperscript{W AF/CIPI} mRNA, inhibit cell growth, cell cycle arrest, and apoptosis in ovarian cancer cell line.

Materials and Methods

Cell lines and reagents

The human ovarian cancer cell line A2780 was provided by China Medical University. It was maintained in RPMI1640 supplemented with 10% fetal calf serum, 100 U/ml penicillin-G, and 100 U/ml streptomycin. TSA was purchased from Sigma. The cell line was incubated at 37°C in 5% CO\textsubscript{2}. Cells were seeded at a density of 1x10\textsuperscript{5} cells/ml of medium in tissue culture dishes and allowed 24 hours before drug treatment to attach. After 24 hours, the medium was replenished with fresh medium containing TSA. Cells were harvested after exposure to various concentrations of TSA for various times, and saved for the following experiments.
Flow cytometry analysis

To determine cell apoptosis and cell cycle distribution after treatment with TSA, ovarian cancer cells were cultured in 10-cm in diameter tissue culture dishes and treated with various concentrations (0, 50, 100, 200 and 400 nmol/l) for 24 hrs. Then a group of cells treated with TSA 100 nmol/l were measured at 6, 12, 24, 36 and 48 hours. Floating and adhered cells were harvested, pooled, and washed twice with cold PBS. After centrifugation (1000 rpm, 5 min), the cells were fixed in cold 75% ethanol at 4°C for a night; they were centrifuged (1000 rpm, 5 min) again and washed twice with cold PBS. Then they were resuspended in PBS containing 10 μg/ml RNase A at 37°C for 30 min, and stained with 100 μg/ml of PI for 30 min in the dark. Cell populations in the G1/G0, S and G2/M phases were analyzed using a FACScan flow cytometer. In each sample 10,000 fluorescent cells were counted.

RNA preparation and reverse transcriptase polymerase chain reaction (RT-PCR)

Cells were treated as described. Total cellular RNA was extracted using TRizol reagent according to the manufacturer’s instructions. Then, PCR analyses were performed on the aliquots of the cDNA preparations to detect P21W AF/CIPI and β-actin (as an internal standard) gene expression using a thermal cycler. The reactions were carried out in a volume of 20 μl containing Taq DNA polymerase 0.2 μl, dNTP (2.5 mM) 2 μl, 10 × buffer 2.5 μl, and 0.1 μl of 5’ and 3’ primers. After initial denaturation for 3 min at 94°C, 35 amplification cycles were performed (40 sec of 94°C denaturation, 1 min of 51.5°C annealing, and 1 min of 72°C extension), then another 7 min of 72°C extension. The PCR primers used in this study are as follows: outer forward 5'-GAG CCC AGA CGT TAT TCT-3'; outer reverse 5'-AGT CCC ACA ACT GCC TAT-3'; inter forward 5'-GGC ATC GTG ATG GAC CCC AGA CGT TAT TCT-3'; outer reverse 5'-GCT GGA AGG TGG ACA GCG-3'. After amplification, portions of the PCR reactions were electrophoresed on 2% agarose gel. The expression amount was determined using an auto-analysis system.

Results

Effects of TSA on cell cycle phase distribution

Using a conventional flow cytometer, the effects of various concentrations and various times of TSA on the cell cycle phase distribution were determined. As shown in Table 1 the A2780 cell line accumulated at the G2/M phase 11.04% after exposure to TSA (100 nmol/l, 24 hrs) vs 4.57% in the control cells (0 nmol/l, 24 hrs). It accumulated at the S phase 19.76% after exposure to TSA (100 nmol/l, 24 hrs) vs 4.57% in the control cells (0 nmol/l, 24 hrs). TSA induced an increased G2/M phase accompanied by decrease of the S phase in a concentration-dependent manner in A2780 cells. As shown in Table 2 the A2780 cell line accumulated at the G2/M phase 20.04% after exposure to TSA (100 nmol/l, 24 hrs) vs 10.25% in the control cells (0 nmol/l, 24 hrs). It accumulated at the S phase 10.17% after exposure to TSA (100 nmol/l, 24 hrs) vs 10.25% in the control cells (0 nmol/l, 6 hrs). These variances of cell cycle distribution were in a time-dependent manner. However the cell line in the G2/M phase 28.38% (100 nmol/l, 48 hrs) vs 27.91% (100 nmol/l, 36 hrs), and in the S phase 5.66% (100 nmol/l, 48 hrs) vs 5.36% (100 nmol/l, 36 hrs) showed no significant differences.

Table 1. — Cells were treated with various concentrations (0, 50, 100, 200 and 400 nmol/l) for 24 hrs.

<table>
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<th>Cell cycle phase</th>
<th>0 nmol/l</th>
<th>50 nmol/l</th>
<th>100 nmol/l</th>
<th>200 nmol/l</th>
<th>400 nmol/l</th>
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<tbody>
<tr>
<td>1 G1/G0</td>
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<td>63.28</td>
<td>70.41</td>
<td>65.37</td>
<td>67.13</td>
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<tr>
<td>2 G2/M</td>
<td>4.41</td>
<td>6.26</td>
<td>10.23</td>
<td>23.84</td>
<td>29.33</td>
</tr>
<tr>
<td>S</td>
<td>36.29</td>
<td>30.46</td>
<td>19.36</td>
<td>10.79</td>
<td>3.54</td>
</tr>
<tr>
<td>1 G1/G0</td>
<td>55.21</td>
<td>62.34</td>
<td>64.03</td>
<td>70.48</td>
<td>62.66</td>
</tr>
<tr>
<td>2 G2/M</td>
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<td>34.36</td>
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<td>8.31</td>
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<td>69.08</td>
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<tr>
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</tr>
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<td>16.84</td>
<td>10.14</td>
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Table 3. — Expression of P21WAF/CIPImRNA with various concentrations (0, 50, 100, 200 and 400 nmol/l) for 24 hrs.

<table>
<thead>
<tr>
<th>n</th>
<th>0 mM</th>
<th>50 mM</th>
<th>100 mM</th>
<th>200 mM</th>
<th>400 mM</th>
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<td>3.491</td>
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Table 4. — Expression of P21WAF/CIPImRNA with 100 nmol/l TSA at various times (6, 12, 24, 36, 48 hrs).

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</table>

Effects of TSA on expression of P21WAF/CIPImRNA

RT-PCR analysis was performed to determine whether the level of P21WAF/CIPImRNA was related to TSA treatment. As shown in Table 3 the expressions of P21WAF/CIPImRNA were 3.451 (100 nmol/l, 24 hrs) vs 1.523 in the control cells (0 nmol/l, 24 hrs). They were in a concentration-dependent manner. TSA treatment obviously enhanced the level of P21WAF/CIPImRNA. As shown in Table 4 the A2780 cell line was stimulated for 12, 24, 36 and 48 hours, respectively. The expressions of P21WAF/CIPImRNA were 3.322 (100 nmol/l, 12 hrs) vs 2.556 (100 nmol/l, 24 hrs). TSA treatment enhanced the level of P21WAF/CIPImRNA in a time-dependent manner.

Discussion

Epigenetics including DNA methylation and histone hypoacetylation around the promoter region of genes can silence the expression of tumor suppressor genes without
altering DNA nucleotide sequences and becomes a capital way of regulating gene expression. Epigenetic abnormalities are now considered as a reason for oncogenesis. Acetylation is a capital covalent modification of histone, which is regulated by the opposing activities of HATs and HDACs. HATs catalyze histone acetylation and activate transcription while HDACs repress transcription. Regulating the balance between HATs and HDACs can modulate gene expression in cells [4].

Many inhibitors (HDACIs) have been identified, including TSA, sodium butyrate, depsipeptide (FR901228, FK228), trapoxin, valproic acid (VPA), and suberoylanilide hydroxamic acid (SAHA) [5]. Some of these reagents on cancer cells have been examined and have revealed that expression of selected genes were upregulated significantly by HDACIs. These genes encode transcriptional factors, which can upregulate their downstream target genes, leading to cell cycle arrest and/or apoptosis of the cancer cells [6, 7]. Recent reports showed that some HDACIs mediated a prominent cellular growth arrest and/or apoptosis of the cancer cells [8]. Recent studies showed that it could inhibit the activity of HDACs and cause acetylated nuclear histones to accumulate selectively and reversely in mammals by Zn⁺ chelation. As an exciting new anticancer agent, TSA could inhibit proliferation and induce differentiation and/or apoptosis of tumor cells with high potency.

In mammals, P21wAF/CIPI is one of the earliest cyclin-dependent kinase inhibitors, CDK1, after which, P16, P27KIP, P18, P19 and so on were recognized. Under surrounding stimulation, CDKI implies negative regulation by combining with a cyclin-CDK compound or CDK directly to inhibit the combination of cyclin and CDK. In a number of cell systems, histone deacetylase inhibitors, such as TSA have previously been shown to modulate P21wAF/CIPI promoter activity through these proximal Sp1/Sp3 sites [9, 10]. Recruitment HDACs could inhibit P21wAF/CIPI transcription while HDACIs can effectively disrupt the interactions between HDACs, Sp1/Sp3, resulting in releasing HDACs from the Sp1/Sp3 complexes, concomitant with increased histone acetylation and P21wAF/CIPI gene transcription. Then P21wAF/CIPI mediates arrest of the G1 and G2/M cell cycle transition by P21wAF/CIPI-CDK2 and P21wAF/CIPI-PCNA protein interaction.

Recent studies have shown that the expression of P21wAF/CIPI decreased in ovarian cancer, which indicated P21wAF/CIPI decreased work as a factor of oncogenesis and development in ovarian cancer.

HDAC inhibitors are emerging as an exciting new class of potential anticancer agents for the treatment of solid and hematological malignancies. Recently, an increasing number of structurally diverse HDAC inhibitors have been identified which inhibit proliferation and induce differentiation and/or apoptosis of tumor cells in culture and in animal models. HDAC inhibition causes acetylated nuclear histones to accumulate in both tumor and normal tissues, providing a surrogate marker for the biological activity of HDAC inhibitors in vivo. The effects of HDAC inhibitors on gene expression are highly selective, leading to transcriptional activation of certain genes such as the cyclin-dependent kinase inhibitor P21wAF/CIPI but repression of others. HDAC inhibition not only results in acetylation of histones but also transcription factors such as p53, GATA-1 and estrogen receptor-alpha. The functional significance of acetylation of non-histone proteins and the precise mechanisms whereby HDAC inhibitors induce tumor cell growth arrest, differentiation and/or apoptosis are currently the focus of intensive research. In this study, we treated A2780 cells with different concentrations of TSA in 24 hours. We observed that, with the concentration of 100 nM, TSA induced cell cycle arrest and increased the expression of P21wAF/CIPI mRNA. With the increase of concentration, the G2/M phase cells increased, while the S phase cells decreased, presenting a dose-dependent arrest at the G2/M phase. We then treated TSA with 100 nM TSA at different times. The result showed a time-dependent form with a peak level of P21wAF/CIPI mRNA at 24 hours, and after 48 hours its expression descended with an increase of the G2/M phase, and decrease of the S phase.

TSA affects the activity of cyclin-dependent kinase through increasing the expression of P21wAF/CIPI mRNA. TSA causes A2780 cells to block the G2/M phase and inhibit proliferation of A2780 cells. The minimum level of active TSA is 100 nM and the minimum time is 12 hours. The effect relies on time and concentration.

In summary, our data showed that TSA could induce P21wAF/CIPI acetylation, increase its transcriptional activation and P21wAF/CIPI mRNA in A2780 cells with increasing of the G2/M phase, and decreasing of the S phase. The finding in this report can serve as a good approach to elucidate further functions of P21wAF/CIPI, and discover a novel pathway involved in apoptosis and gene products. These data could aid our understanding of the cellular activity of P21wAF/CIPI and augment the role of P21wAF/CIPI in cell cycle regulation.

Conclusion

TSA affects the activity of cyclin-dependent kinase through increasing the expression of P21wAF/CIPI mRNA. TSA causes A2780 cells to block the G2/M phase and to inhibit cell proliferation of A2780 cells. The minimum level of active TSA is 100 nM and the minimum time is 12 hours. The effect relies on time and concentration.

References


Address reprint requests to:
X.X. MA, M.D.
Department of Obstetrics and Gynecology
Shengjing Hospital of China Medical University
Shenyang 110004 (China)
e-mail: maxiaoxin666@yahoo.com.cn
Aberrant DNA hypermethylation of hMLH-1 and CDKN2A/p16 genes in benign, premalignant and malignant endometrial lesions

M. Guida¹, F. Sanguedolce², P. Bufo², A. Di Spiezio Sardo¹, G. Bifulco¹, C. Nappi¹, G. Pannone²

¹Department of Gynaecology and Obstetrics and of Pathophysiology of Human Reproduction, University of Naples “Federico II”
²Department of Surgical Sciences, Institute of Pathology and Cytopathology, University of Foggia (Italy)

Introduction

Endometrial carcinogenesis is a complex process requiring progressive acquisition of genetic mutations as well as epigenetic alterations of cancer-related genes, thus making cells amenable to malignant transformation [1]. Since promoter gene hypermethylation may affect several small regions of DNA, scattered throughout the whole human genome, so far we still do not know all the genes where methylation may have consequences regarding potential malignancy. Several studies have been carried out to elucidate the role of epigenetic inactivation in endometrial cancer initiation and progression to assess methylated loci as targets for selective pharmacologic agents [2].

Most recent works have focused on DNA mismatch repair and Wnt signal-related genes, whose epigenetic inactivation has been detected in many human cancers [3, 4].

The DNA mismatch repair gene human MutL homolog-1 (hMLH1) plays a significant role in repairing base-pair mismatches, which can occur in gene amplification during cell division, and its dysfunction leads to microsatellite instability (MSI) (i.e., increase of DNA replication errors at microsatellite sites) [5]. The CDKN2A/p16 protein inhibits cyclin-dependent kinases 4 and 6, key regulators of progression through the G1 phase of the cell cycle. Both genes have been investigated with regard to their epigenetic alterations in endometrial cancer [3, 6], showing conflicting results.

Endometrial polyps (EP) represent benign lesions characterised by an abnormal proliferation of both epithelial and mesenchymal components of the endometrium; a few cases of malignant EP have been reported, mostly associated with tamoxifen use [7-10]. Although the putative association of EP with premalignant and malignant endometrial lesions has been hypothesised, there are no consistent data supporting this hypothesis [11]. On the other hand, AH is a well established premalignant condition usually sharing genetic alterations (i.e., MSI and loss of PTEN function) with subsequent Type I EC [12].

Therefore, in order to provide a precise “methylation profile” of endometrial lesions by assessing multiple genes, we sought to comprehensively study epigenetic alterations of hMLH1 and CDKN2A/p16 genes in benign, premalignant and malignant endometrial lesions, and their relationship with clinicopathological features.

Materials and Methods

Clinical samples

Formalin-fixed, paraffin-embedded tissue sections of endometrial adenocarcinomas (EC, n = 5), atypical hyperplasias (AH, n = 8) and endometrial polyps (EP, n = 13), were obtained from patients aged 34 to 77 years old (median age 53), who were scheduled for outpatient hysterectomy due to abnormal uterine bleeding. No patient had any personal history of breast carcinoma or tamoxifen treatment. All patients gave oral informed consent to the study. Pathological analysis of tumour specimens revealed endometrioid adenocarcinoma in all cases. All tumours were graded according to the TNM/FIGO grading...
system [13]. At least two H&E-stained sections were examined by a single pathologist skilled in gynaecological oncology. Clinical and pathological data of all patients are summarised in Table 1.

DNA isolation and sodium bisulfite conversion

After careful examination of H&E-stained slides, we selected tumour sections with the greatest proportion of malignant tissue. DNA was isolated from five consecutive 10-μM sections of each formalin-fixed, paraffin-embedded tissue sample. Genomic DNA was extracted using a proteinase K (Qiagen, Valencia, CA) digestion followed by DNA isolation using the Wizard DNA clean-up kit (Promega, Madison, WI) according to the manufacturer’s protocols. Sodium bisulfite modification of the DNA was performed using the EZ DNA Methylation Kit (Zymo Research, Orange, CA) following the manufacturer’s protocol, initially incubating for 5 min at 95°C before denaturation. The decrosslinking steps in the extraction as well as 95°C incubation ensured a complete melting of the DNA and thus a complete sodium bisulfite conversion for these highly cross-linked formalin-fixed specimens.

Methylation specific PCR (MSP) assay

Methylation-specific polymerase chain reaction (PCR) for the analysis of promoter hypermethylation was carried out, following a matched analysis between fresh-frozen and formalin-fixed, paraffin-embedded tissue samples. Genomic DNA was extracted using a proteinase K (Qiagen, Valencia, CA) digestion followed by DNA isolation using the Wizard DNA clean-up kit (Promega, Madison, WI) according to the manufacturer’s protocols. Sodium bisulfite modification of the DNA was performed using the EZ DNA Methylation Kit (Zymo Research, Orange, CA) following the manufacturer’s protocol, initially incubating for 5 min at 95°C before denaturation. The decrosslinking steps in the extraction as well as 95°C incubation ensured a complete melting of the DNA and thus a complete sodium bisulfite conversion for these highly cross-linked formalin-fixed specimens.

Methylation-specific PCR (MSP) assay

Methylation-specific polymerase chain reaction (PCR) for the analysis of promoter hypermethylation was carried out, following a matched analysis between fresh-frozen and formalin-fixed, paraffin-embedded tissue samples. Genomic DNA was extracted using a proteinase K (Qiagen, Valencia, CA) digestion followed by DNA isolation using the Wizard DNA clean-up kit (Promega, Madison, WI) according to the manufacturer’s protocols. Sodium bisulfite modification of the DNA was performed using the EZ DNA Methylation Kit (Zymo Research, Orange, CA) following the manufacturer’s protocol, initially incubating for 5 min at 95°C before denaturation. The decrosslinking steps in the extraction as well as 95°C incubation ensured a complete melting of the DNA and thus a complete sodium bisulfite conversion for these highly cross-linked formalin-fixed specimens.

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Statistics

The methylated/unmethylated status of the hMLH1, CDKN2A/p16, and simultaneous hMLH1-CDKN2A/p16 genes was analysed as a dichotomous variable. Fisher’s exact tests and one-way analysis of variance (ANOVA) were used to evaluate pairwise differences in the proportions of women with EP versus AH and EC. Statistically significance was set at p < .05. All data were analysed by Stanton Glantz statistical software 3 (MS-DOS) and GraphPad Prism4.

Results

Good quality amplifiable DNA was retrieved from 22 out of 26 cases. Twelve cases were EP, six were AH and four cases were EC. In those 22 cases, the methylation status of the gene promoters of CDKN2A/p16 and hMLH1 was analysed (Figures 1 and 2). At least one of the two promoters was methylated in 13 of 22 cases (59%) (cumulative methylation index, CMI).

Aberrant methylation of hMLH1 was observed in two out of four EC (50%), one out of six AH (16%), and five out of 12 EP (42%), respectively. Conversely, epigenetic alteration of CDKN2A/p16 was seen in three out of four EC (75%), three out of six AH (50%) and two out of 12 EP (16%), (Table 1). Thus, there was a clear trend
towards increased hypermethylation of CDKN2A/p16 from benign up to malignant lesions. The simultaneous methylation of either gene was not detected in any cases of EP, but in 16% of AH and in 50% of EC, thus showing a trend for a higher frequency of epigenetic alterations in pre- and malignant lesions.

Differences in methylation frequencies between benign (EP) and premalignant/malignant lesions (AH+EC) were not statistically significant in any case (p > .05).

Discussion

A recent comprehensive review has pointed out the key role of epigenetic analysis in managing EC owing to its potential advantages over cancer prevention, diagnosis and treatment [2].

Data available among the international literature showed MSI presence in 20 to 40% of patients with EC, mainly those of endometrioid type which show a better biological behaviour [18, 19]; this suggests that mutations of the DNA mismatch repair genes might be associated with endometrial carcinogenesis, especially when occurring in the familiar setting (hereditary nonpolyposis colon cancer (HNPCC) syndrome). On the other hand, MSI in sporadic EC is mostly due to epigenetic silencing of the hMLH1 gene by hypermethylation [20].

A large series of gynaecological cancers analysed for gene methylation patterns [21], using a panel of 34 different genes, showed that aberrant methylation of hMLH1 was present only in the group of EC, as compared with ovarian and cervical tumours.

Additionally, a higher prevalence of aberrant methylation of hMLH1, in comparison with APC, E-cadherin, RAR-β and CDKN2A/p16 was found, and epigenetic analysis of normal endometrium disclosed no epigenetic alterations of any of the five genes [22].

Our present study is the first to analyse DNA methylation of selected genes both in benign and premalignant/malignant endometrial lesions. According to the available literature, our preliminary results show a higher frequency of hMLH1 hypermethylation in both pre-malignant and malignant lesions with an increased frequency in those lesions with a worse biological behaviour.

This suggests that, in a well known molecular setting (i.e., KRAS mutations, loss of PTEN function) [10], such epigenetic events may contribute to genomic instability in type I EC progression.

Frequencies of p16 mutation and deletion in endometrial cancer are only 5-6% and 3%, respectively [23, 24]; however reduced levels of the encoded protein have been found in 19% of cases [24], thus suggesting an involvement of aberrant DNA methylation.

While p16 is usually ubiquitous in endocervical adeno-carcinomas in that it represents a surrogate marker for the presence of HPV [25], the expression of the encoded protein has been shown to be patchy or negative in EC [22, 26]. However population biases (i.e., different races), simultaneous analyses of tumour tissues from different sites, as well as technical reasons may represent potential limiting factors, thus impairing the results of these studies [22, 25, 26].

Expression of tumour suppressor genes is quite difficult to investigate, since it requires the application of different methods. Indeed inactivation of CDKN2a/p16 may occur as a result of several molecular events (i.e., deletion, mutation, or methylation); therefore, multiple comparative analyses are needed in order to precisely quantify the inactivation degree of such tumour suppressor gene.

Analysis of proteic expression of CDKN2a/p16 may disclose an increased expression due to either transcription of the corresponding locus on the inactivated allele or lack of ubiquitylation in case of point mutations, which is commonly seen for p53, another cell-cycle related gene. In order to avoid such technical biases, we selected nested MSP-PCR as the method of choice in p16 expression assessment; our preliminary results seem to suggest that the lack of protein expression in EC is possibly due to inactivation of one allele at the CDKN2a/p16 locus by aberrant promoter methylation.

Interestingly, pooled analysis of the two genes showed a significant increase of the discrimination capability between benign and pre-malignant/malignant endometrial lesions, though not statistically significant.

The study findings seem to support the concept that simultaneous hMLH1-CDKN2a/p16 methylation status in endometrial lesions is associated with the development of carcinogenesis, suggesting that an orderly progression of epigenetic silencing by hypermethylation of selected genes may finally lead to EC through AH.

Such data not only provide a further insight into tumour biology, but also point out the potential role of these frequently methylated genes as early diagnostic markers to be assessed on screening assays.

Moreover, cancer-specific epigenetic changes are currently being evaluated as new clinically relevant therapeutic targets, in order to avoid conventional cancer treatments and their associated side-effects.

Conclusion

The methylation status of hMLH1 and CDKN2A/p16 promoters has been investigated in benign, premalignant and malignant endometrial lesions by means of methylation-specific PCR. Our preliminary results seem to indicate that simultaneous hypermethylation of both genes may represent an early event in endometrial carcinogenesis. However further studies are needed to confirm such preliminary findings.

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References


Address reprint requests to:
A. DI SPIEZIO SARDO, M.D.
Department of Gynaecology and Obstetrics
and of Pathophysiology
of Human Reproduction
University of Naples “Federico II”
Naples (Italy)
e-mail: cdispie@tin.it
Role of HPV DNA testing for detection of high-grade cervical lesions in women with atypical squamous cells of undetermined significance: A prospective study in a Korean population

Department of Obstetrics and Gynecology, Ajou University School of Medicine, Suwon (Korea)

Summary
Objective: The purpose of this study was to determine if HPV DNA testing improves the accuracy of detecting possible high-grade cervical lesions in women with atypical squamous cells of undetermined significance (ASC-US). Methods: Cervical cytology and Hybrid Capture II (HCII) assay for HPV DNA detection was performed in 10,526 women from July 2005 to July 2007. Two hundred and sixty women with ASC-US underwent colposcopy-directed biopsy to determine the final histologic diagnosis. They were divided into two groups according to the positivity of the HPV DNA test, and the respective biopsy results were compared. Results: Positive HCII was significantly more associated with CIN 2, CIN 3, and invasive cancer than negative HCII (p < 0.001). The odds ratio of positive HPV DNA testing in detecting high-grade lesions was 7.0 (95% CI; 2.8-17.7). Conclusion: The HPV DNA test is useful for predicting the severity of lesions of the uterine cervix and formulating decisions with regard to treatment plans.

Key words: ASC-US; HPV DNA test; Cervical intraepithelial neoplasia (CIN).

Introduction
Approximately 50 million women undergo Pap tests, and more than 2 million women receive a cervical cytologic diagnosis of atypical squamous cells of undetermined significance (ASC-US) each year in the United States. Among them, 10-20% of women are found to have underlying high-grade cervical lesions [1]. The management of ASC-US is still a controversial issue in the gynecologic field [2]. Some gynecologists prefer routine colposcopic evaluation as an initial management modality to identify underlying high-grade cervical lesions, but others recommend repeat cervical cytology, and perform colposcopy for women with persistent cytologic abnormalities. In the early 1990s, some investigators reported that human papillomavirus (HPV) infection caused most cervical intraepithelial neoplasia (CIN) and the presence of an oncogenic HPV type strongly correlated with a high-grade lesion and invasive cancer [3, 4]. The Hybrid Capture II (HC II) assay detects DNA of 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). Previous studies have shown that HPV DNA analysis using semi-quantitative polymerase chain reaction (PCR) could be useful in determining HPV load [5-7].

The Food and Drug Administration (FDA) has approved the HC II using a liquid-based cervical smear preparation. In 2007, the American Society for Colposcopy and Cervical Pathology (ASCCP) proposed consensus guidelines for the management of women with abnormal cervical cytology and recommended the HPV DNA test as the preferred method for women with ASC-US [8]. In Korea, the use of the HPV DNA test has recently increased and several studies have been done on the efficacy of HPV DNA testing in the management of women with ASC-US [9-11]. They showed that the HPV DNA test was a reliable triage method for the diagnosis of high-grade CIN, however, did not have sufficient power to prove the efficacy of the HPV test because of the small numbers of enrolled cases and retrospective nature. The purpose of this study was to determine if HPV DNA testing by HC II assay of liquid-based cervical cytology improves the accuracy of detecting possible high-grade cervical lesions in smears reported as an ASC-US diagnosis.

Materials and Methods
Our region (Suwon), in the middle of Korea, is an area of 120,000 km² with about 1,060,000 inhabitants - 503,000 men and women, respectively. From June 2005 to May 2007, a total of 10,526 women visited the Health Promotion Center at Ajou University Hospital for health screening. General gynecologists performed the initial gynecologic examination and all women were screened by ThinPrep® (Cytyc, Boxborough, MA, USA) and tested for HPV DNA determination by HCII (Digene, Gaithersburg, MD, USA) for gynecologic screening. HCII testing was performed according to the manufacturer’s recommendations, including adequate controls. Viral load was expressed as the relative light unit (RLU) ratio of specimens. Solutions of high-risk HPV at 10 pg/ml served as positive controls. All RLU measurements of specimens were divided by RLU of appropriate positive controls to provide a ratio. Specimen ratio of ≥ 1.0 was regarded as positive for HPV DNA, while a ratio < 1.0 was regarded as negative. Cervical samples were taken from the exo- and endocervix using the ThinPrep...
cytology collection system according to the manufacturer’s protocol. All slides were reviewed by six pathologists and final cytological diagnoses were made according to the 2001 Bethesda System [12], which were classified as no intraepithelial lesion or malignancy (NILM), atypical squamous cells (ASC), low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial lesion (HSIL). The cytologists involved in the study were unaware of the results of the HPV DNA tests. From a cohort of 10,526 women tested for ThinPrep® and HCII, 260 women were found to have ASC-US and were referred for colposcopy and biopsy. Experienced colposcopists performed the procedures. Colposcopic patterns were classified according to the International Federation of Cervical Pathology and Colposcopy (IFCPC) [13].

Pathologic diagnoses based on the presence or absence of CIN were made according to the World Health Organization criteria [14] and classified as negative, CIN 1, CIN 2 or CIN 3. HPV DNA results were evaluated and compared to the pathologic diagnoses of the cervical biopsy for their ability to accurately predict the presence or absence of CIN. Colposcopy was considered as the gold standard. Women with negative colposcopy results or biopsy results were considered disease negative. Women with a pathologic diagnosis of CIN 1 or higher were considered as disease-positive and women with CIN 2 or higher were considered as having high-grade lesions. To evaluate the screening performances of the HPV DNA test, we calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). We also calculated the odds ratio (OR) for HPV DNA-positive women to show the presence of CIN. The prevalence of ASC-US was 2.5%. In the ASC-US cohort, the median age was 46 years with a range of 26 to 67 years. The overall rate of positive HPV DNA prevalence among women with ASC-US on liquid-based cytology was 43.5%. An approximately 68.1% of positive HPV DNA prevalence was prominent for the age groups 35-55 years old (Table 1). The relationship between histologic diagnoses and HPV DNA status are indicated in Table 2. The overall rate of CIN and invasive carcinoma was 76.5% (199/260). The prevalence of high-grade lesions was significantly higher in the HPV DNA-positive group (23%) than the HPV DNA-negative group (4.1%). Two women (1.8%) were found to have invasive carcinoma. In the ASC-US population, the sensitivity, specificity, PPV, and NPV of HCII for detecting high-grade lesions were 81.3%, 61.8%, 23.0%, and 95.9%, respectively (Table 3). Receiver operating characteristic (ROC) curve analysis showed the area under the curve for HCII was 0.715 (95% CI, 0.627-0.804) (Figure 1). Multivariate analysis using a logistic regression model indicated that positive HPV DNA on HCII was the only significant factor for detecting high-grade cervical lesions in women with ASC-US on cytology (OR, 7.02 [95% CI, 2.78-17.75]; p < 0.001).

Results

The prevalence of ASC-US was 2.5%. In the ASC-US cohort, the median age was 46 years with a range of 26 to 67 years. The overall rate of positive HPV DNA prevalence among women with ASC-US on liquid-based cytology was 43.5%. An approximately 68.1% of positive HPV DNA prevalence was prominent for the age groups 35-55 years old (Table 1). The relationship between histologic diagnoses and HPV DNA status are indicated in Table 2. The overall rate of CIN and invasive carcinoma was 76.5% (199/260). The prevalence of high-grade lesions was significantly higher in the HPV DNA-positive group (23%) than the HPV DNA-negative group (4.1%). Two women (1.8%) were found to have invasive carcinoma. In the ASC-US population, the sensitivity, specificity, PPV, and NPV of HCII for detecting high-grade lesions were 81.3%, 61.8%, 23.0%, and 95.9%, respectively (Table 3). Receiver operating characteristic (ROC) curve analysis showed the area under the curve for HCII was 0.715 (95% CI, 0.627-0.804) (Figure 1). Multivariate analysis using a logistic regression model indicated that positive HPV DNA on HCII was the only significant factor for detecting high-grade cervical lesions in women with ASC-US on cytology (OR, 7.02 [95% CI, 2.78-17.75]; p < 0.001).

Discussion

The present study demonstrates that the HPV DNA triage is an efficient tool for detecting high-grade cervical lesions in women with ASC-US on cervical cytology, and the HPV DNA status is the only independent factor after adjusting the effects of various confounding factors. The ASC-US/LSIL Triage Study (ALTS) group in the United States has evaluated three alternative methods of management including immediate colposcopy, repeat cytology, and triage by HPV DNA testing in patients with ASC-US or LSIL to detect CIN 3 or higher grade lesions [1]. They demonstrated HPV testing in women with ASC-US had greater sensitivity to detect CIN 3 or higher grade lesions and similar specificity to a single follow-up Pap smear indicating ASC-US or higher grade lesions. Wright et al. proposed “interim guidance” for the use of HPV DNA testing in the screening setting [15]. Recent cost-effective simulation studies indicate that HPV DNA

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Table 1. — Prevalence of HPV (+) among 260 women with ASC-US on liquid-based pap smears, stratified by age.

<table>
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</tr>
<tr>
<td>Total</td>
<td>147 (56.5)</td>
<td>113 (43.5)</td>
<td>260</td>
</tr>
</tbody>
</table>

Table 2. — Biopsy results following HPV DNA tests.

<table>
<thead>
<tr>
<th>Biopsy diagnosis</th>
<th>HPV DNA test, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Benign conditions</td>
<td></td>
</tr>
<tr>
<td>(CC with SM, atrophy)</td>
<td>45 (30.6)</td>
</tr>
<tr>
<td>CIN 1 (including flat condyloma)</td>
<td>96 (65.3)</td>
</tr>
<tr>
<td>CIN 2</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>CIN 3</td>
<td>4 (2.7)</td>
</tr>
<tr>
<td>Invasive squamous cell carcinoma</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table 3. — Diagnostic performance of the HPV DNA test (HCII) for ASC-US women detecting high-grade cervical lesions.

<table>
<thead>
<tr>
<th>HPV DNA test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCII (95% CI)</td>
<td>81.3 (76.6-86.0)</td>
<td>61.8 (55.9-67.7)</td>
<td>23.0 (17.9-28.1)</td>
<td>95.9 (93.5-98.3)</td>
</tr>
</tbody>
</table>

Table 4. — Logistic regression analysis of factors associated with high-grade cervical lesions.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (continuous)</td>
<td>–</td>
<td>NS</td>
</tr>
<tr>
<td>Marital status</td>
<td>–</td>
<td>NS</td>
</tr>
<tr>
<td>Parity</td>
<td>–</td>
<td>NS</td>
</tr>
<tr>
<td>Positive HPV DNA</td>
<td>7.02 (2.78-17.75)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

CI: confidence interval; NS: not significant.
Role of HPV DNA testing for detection of high-grade cervical lesions in women with atypical squamous cells of undetermined etc. 273

Figure 1. — Receiver operating characteristic (ROC) curve analysis of the sensitivity and specificity of HCII in detecting high-grade cervical lesions. Area under the curve for HCII = 0.715 (95% CI, 0.627-0.804; p < 0.001).

The NPV was found to be extremely high (95.9% [95% CI, 93.5-98.3]) and, on ROC analysis, the area under the curve for HCII was 0.715 (95% CI, 0.627-0.804; p < 0.001).

Several investigators reported that HCII showed a high sensitivity and specificity for detecting CIN 2 or higher lesions [23, 24]. Our study showed similar results. The sensitivity and specificity of HCII were 81.3% (95% CI, 76.6-86.0) and 61.8% (95% CI, 55.9-67.7), respectively. The NPV was found to be extremely high (95.9% [95% CI, 93.5-98.3]) and, on ROC analysis, the area under the curve for HCII was 0.715 (95% CI, 0.627-0.804; p < 0.001). In this study, CIN 2 or higher lesions were more frequently found in HPV DNA-positive women compared to HPV DNA-negative women. Invasive carcinoma was found only in the HPV-positive group (1.8%). A positive HPV DNA test was an independent predictor of high-grade cervical lesions.

Our study is a hospital-based study and not a population-based randomized study. However, women enrolled in this study visited our institution for health screening without any gynecologic symptoms or diseases and took the HPV DNA test on their own initiative without any concern of HPV infection. Thus, it seems that selection bias by subjects and investigators could be minimal.

In conclusion, it seems that HPV DNA-positive women diagnosed as having ASC-US are associated with high-grade cervical lesions and the HPV DNA test is useful for detecting CIN 2 or higher lesions of the uterine cervix. In a practical strategy for the triage of women with ASC-US, it is important to reduce unnecessary colposcopies and subsequent biopsies. Our data suggest that HCII could play a pivotal role in formulating decisions with regard to treatment plans.

References


Address reprint requests to:
S.J. CHANG, M.D.
Department of Obstetrics and Gynecology
Ajou University School of Medicine
San 5, Wonchon-dong, Youngtong-gu
Suwon, Gyunggi-do 442-721 (Korea)
e-mail: drchang@ajou.ac.kr
Antiproliferative effects of 2-methoxyestradiol alone and in combination with chemotherapeutic agents on human endometrial cancer cells

C.H. Chen¹, M.D.; W.J. Lee¹, M.D.; T.C. Chang¹, M.D.; R.J. Chen¹, M.D., Ph.D.; C.H. Chien², Ph.D.; S.N. Chow¹,³, M.D., Ph.D.

¹Department of Obstetrics and Gynecology, College of Medicine and National Taiwan University Hospital, National Taiwan University, Taipei
²Institute of Biochemistry and Molecular Biology, School of Life Sciences, National Yang-Ming University, Taipei
³Department of Obstetrics and Gynecology, Far Eastern Memorial Hospital, Taipei (Taiwan)

Summary

Objective: 2-methoxyestradiol (2-ME), an endogenous estradiol metabolite, has potent antiproliferative effects on cancer cells. However, its usefulness for treating endometrial cancer has not yet been fully explored. We investigated for the first time whether in vitro combinations of 2-ME with various chemotherapeutic agents might result in a synergistic inhibitory effect on the proliferation of human endometrial cancer cells. Methods: As a model, two different human endometrial cancer cell lines, HEC-1-A and RL95-2, were used. These cells were treated with 2-ME alone or in combination with paclitaxel, cisplatin, or doxorubicin. Measurements to detect an antiproliferative effect were performed after 24, 48, and 72 hours using the MTT assays. Results: In both endometrial cancer cell lines a significant synergistic effect of 2-ME with paclitaxel was observed. The combination of 2-ME and cisplatin was not synergistic and provided only additive effects. The antiproliferative effect of 2-ME was somewhat antagonized by doxorubicin. Conclusions: Our study shows that 2-ME has a direct antiproliferative effect on endometrial cancer cells. Our results also show a potential anticancer synergy between 2-ME and paclitaxel in vitro. On the other hand, no remarkable synergistic actions were observed between 2-ME and doxorubicin, suggesting that 2-ME may selectively enhance the anticancer actions of certain chemotherapeutic agents in human endometrial cancer. Therefore, combination therapy should be investigated further as an additional therapeutic option for advanced or recurrent endometrial cancer.

Key words: 2-methoxyestradiol; Paclitaxel; Endometrial cancer; Proliferation.

Introduction

Endometrial cancer is the most common gynecologic malignancy and the fourth most common cancer in American women [1]. It is a common disease of peri- and postmenopausal women. The median age at diagnosis is about 65 years [2]. Although the incidence of endometrial cancer in Taiwan is lower than in the United States and Europe, it is still the second most common gynecologic cancer and has been increasing in recent years because of changing dietary habits. If discovered early, surgery, often combined with radiation therapy, usually eliminates all of the cancer. However, not all cases of endometrial cancer can be successfully treated with surgery. Systemic chemotherapy or hormone therapy is used for patients with more advanced or recurrent endometrial disease. Unfortunately, the prognosis for these patients is poor, with a median survival of less than one year [3]. Therefore, there is a need to develop better therapeutic modalities for patients with more advanced or recurrent endometrial cancer.

Recent research has provided evidence that 2-methoxyestradiol (2-ME), an endogenous metabolite of 17b-estradiol, may be a candidate as a therapeutic agent for the treatment of several cancers because of its antiproliferative properties and low systematic toxicity. Moreover, 2-ME has been reported to have high antiangiogenic activity [4] and it suppresses the growth of various cancer cells in vitro [5-7] as well as solid tumors, such as breast cancer, melanoma, and prostate cancer, in vivo [8-10]. 2-ME inhibits tumor proliferation by induction of apoptosis and cell cycle arrest at the G2/M phase in a wide range of cancer cells [6, 11-13]. Besides the antiproliferative actions of 2-ME, another potential application of 2-ME is its use as an adjunct to enhance the anticancer effect of other commonly used chemotherapeutic agents or hormone deprivation when they are given in certain combinations [9, 14, 15].

Although the molecular basis of the antitumorigenic effect of 2-ME has been widely studied, as yet little data are available from investigating the effects of 2-ME when used for endometrial cancer treatment [7, 16]. Therefore, the effects of 2-ME alone or in combination with other anticancer drugs with activity on endometrial cancer cells are worth studying. In the present study, we evaluated the antiproliferative effects of 2-ME alone and in combination with paclitaxel, cisplatin, or doxorubicin (three clinically relevant agents used to treat advanced or recurrent endometrial cancer) on human endometrial cancer cells to investigate its potential use in the treatment of endometrial cancer.
Materials and Methods

Drugs and reagents. For in vitro studies, 2-methoxyestradiol (2-ME), paclitaxel, cisplatin and doxorubicin were all purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Due to their high lipophilicity, the stock solutions of 2-ME (10 mmol/l) and paclitaxel (0.2 mmol/l) were prepared in pure ethanol (200 proof). The stock solutions of doxorubicin (5 mmol/l) and cisplatin (5 mmol/l) were prepared in phosphate buffer (pH 7.4). All these stock solutions were stored at -20°C in tightly sealed sterile tubes. Shortly before introducing the anticancer agents to the cultured cancer cells, each chemical was freshly diluted with a buffer to the desired concentrations and an aliquot of the drug-containing solution was added to each well.

Culture of human endometrial cancer cell lines. A previously published study has demonstrated that 2-ME inhibits the growth of human endometrial cancer HEC-1-A and RL-95-2 cells in vitro [7]. As a model, we also used these two human endometrial cancer cell lines in our study. The endometrial cancer cell line HEC-1-A was maintained in medium consisting of McCoy’s 5A medium with 10% FBS; RL95-2, another endometrial carcinoma cell line, was grown in a 1:1 mixture of Dulbecco’s modified Eagle’s medium (DMEM) and Ham’s F12 medium with 10 mM HEPES, 2 mM L-glutamine, 2.0 g/l sodium bicarbonate, and 5 μg/ml insulin, 10% FBS. All cell lines were grown in 10 cm² culture dishes in 5% CO₂ in humid air at 37°C.

MTT assays. Cells were seeded in 96-well culture plates at a density of 5000 viable cells for HEC-1-A and 7000 cells for RL95-2 per well and incubated for 24 hours. Every eight duplicate wells were exposed to various concentrations of 2-ME, paclitaxel, cisplatin, and doxorubicin, respectively. Each concentration of drug alone and drug combination was completed in triplicate. Cell proliferation and viability were measured after drug treatment for 24, 48, and 72 hours using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. MTT was added to each well of the plates, and then the plates were incubated for four hours at 37°C. To stop the reaction, MTT was removed and 200 μl DMSO was added to the plates, which were then incubated at 37°C for 10 min. MTT absorbance at 570 and 630 nm were measured using a microtiter enzyme-linked immunosorbent assay (ELISA) plate reader. The cytotoxicity was evaluated with reference to the inhibitory concentration 50 (IC₅₀).

Statistical analysis. The inhibition rate of drug-treated cells was expressed as mean ± standard deviation (SD) of the values obtained from three replicate experiments. The Kruskal-Wallis test was applied for comparison of continuous variables, which was nonparametric in this study. A p value less than 0.05 was considered statistically significant. All the statistical analyses were performed by using Stata/SE 8.0 for Windows, 2003 (Stata Corporation, College Station, TX, USA).

Results

Effects of 2-methoxyestradiol on human endometrial cancer cell lines

The growth inhibitory effects of 2-ME were determined in HEC-1-A and RL-95-2 endometrial cancer cells. Following in vitro treatment of the cells with increasing concentrations of 2-ME for three days, cell proliferation was evaluated using MTT assays and dose-response curves were plotted. The results showed that the cell survival rate was reduced by 2-ME treatment in a dose- and time-dependent manner (data not shown). Our results are consistent with those reported in another previously published study [7]. The effects of each compound singly and in combination using the concentration indicated for each time period are shown in Figure 1.

Effects of 2-methoxyestradiol in combination with paclitaxel.

To determine the nature of the responses of cells to 2-ME and paclitaxel in combination, cells were treated with concentrations of 2-ME or paclitaxel that caused moderate antiproliferative effects ranging from of 5%-20% inhibition. Because inhibition greater than 100% cannot be measured, synergistic interactions are best identified by using low antiproliferative concentrations. Thus, although the greatest antiproliferative effect was reached at a concentration of 5×10⁻⁰ mol/l 2-ME for HEC-1-A cells and 1×10⁻⁰ mol/l 2-ME for RL95-2 cells, we used a concentration of 1×10⁻⁰ mol/l 2-ME in this combination study.

When the human endometrial cancer cell line HEC-1-A was treated with 10⁻⁰ mol/l paclitaxel, this drug alone only produced rather weak growth inhibition (about 20%); however, the combination of 10⁻⁰ mol/l 2-ME with paclitaxel resulted in synergistic antiproliferative effects (about 50%) compared with each drug used alone. The inhibitory effects of each drug alone, the mathematical sum of the two drugs in combination, and the actual experimentally observed combination effect were plotted as shown in Figure 2A. Similarly, treatment with 10⁻⁰ mol/l paclitaxel alone on the RL95-2 cell line produced an inhibition of about 7%, but the additional presence of 10⁻⁰ mol/l 2-ME resulted in about 22% growth inhibition (Figure 2A). In both the HEC-1-A and RL95-2 cell lines, 2-ME and paclitaxel were found to act synergistically, i.e., the resultant inhibition of proliferation was significantly greater than the sum of their individual effects. The results suggest that 2-ME and paclitaxel have synergistic antiproliferative actions in these two cell lines.

Effects of 2-methoxyestradiol in combination with cisplatin.

To compare the inhibition abilities of 2-ME in combination with other chemotherapeutic agents, we investigated the antiproliferative effects of 2-ME in combination with cisplatin. The inhibitive effects of 2-ME and cisplatin on both of the two cell lines, HEC-1-A and RL95-2, were determined as shown in the bar graphs in Figure 2B, respectively. After concomitant exposure to low concentrations of each drug (10⁻⁶ mol/l 2-ME and 10⁻⁷ mol/l cisplatin), there was only one additive effect observed on both cell lines, when compared with the inhibitory effects of the two drugs alone. On the HEC-1-A cell line, there was 34% inhibition with the two-drug combination, 24% inhibition with 2-ME alone, and 9% inhibition with cisplatin alone. On the RL95-2 cell line, there was 22% inhibition with the two-drug combination, 16% inhibition with 2-ME alone, and 11% inhibition with cisplatin alone.
Antiproliferative effects of 2-methoxyestradiol alone and in combination with chemotherapeutic agents on human endometrial cancer cells.

Figure 1. — Cell growth curves of 2-ME, paclitaxel, cisplatin, and doxorubicin alone or in combination using the concentration indicated on human endometrial cancer cells from 24 to 72 hours. Data are shown as mean ± SD (n = 3).

(A) 2-ME (10⁻⁶ mol/l) and paclitaxel (10⁻⁹ mol/l).
(B) 2-ME (10⁻⁶ mol/l) and cisplatin (10⁻⁷ mol/l).
(C) 2-ME (10⁻⁶ mol/l) and doxorubicin (10⁻⁸ mol/l).
Figure 2. — (A) Antiproliferative effects of 2-ME in combination with paclitaxel. (B) Antiproliferative effects of 2-ME in combination with cisplatin. (C) Antiproliferative effects of 2-ME in combination with doxorubicin. HEC-1-A and RL95-2 cells were treated with low inhibitory concentrations of the two compounds either singly or in combination for 72 hours. The predicted value is the sum of the effects of each agent used alone. The actual value is the experimentally measured value obtained when the agents were used in combination. Data are shown as mean ± SD (n = 3). *: p < 0.05.
Effects of 2-methoxyestradiol in combination with doxorubicin.

The other chemotherapeutic agent, doxorubicin, was also used to evaluate the anticancer effects of the drug alone or in combination with 2-ME. With treatment using the two drugs in combination (10^6 mol/l doxorubicin and 10^6 mol/l 2-ME) on both the HEC-1-A and RL95-2 cell lines, antagonistic actions were observed between 2-ME and doxorubicin compared with the drugs used alone. The representative data are shown in Figure 2C. The same experiments were repeated multiple times, and highly consistent patterns were observed.

Discussion

In this study, we investigated for the first time whether in vitro combinations of 2-ME with various chemotherapeutic agents might result in a synergistic inhibitory effect on the proliferation of human endometrial cancer cells (HEC-1-A and RL95-2). Our data revealed that 2-ME, an endogenous metabolite of 17β-estradiol with strong anticancer and apoptotic actions in cancer cells, in combination with paclitaxel in vitro produced a synergistic anticancer effect compared with the anticancer activity of each drug alone. Previously published studies with breast cancer cells and ovarian cancer cells in vitro have also reported a certain degree of synergistic effects between the anticancer actions of 2-ME and paclitaxel [14, 15]. Our observations were similar to the findings of those reports.

It has been found that 2-ME inhibits cancer cell growth by the mechanism of promoting G2/M cell cycle block and its effects on microtubules, apoptosis, and angiogenesis [4, 17]. 2-ME is also well known for its unique characteristics such as oral activity [4], nontoxicity [8], selective inhibition of proliferating cells [18], and the ability to overcome resistance to other conventional drugs in tumor cells [19]. Paclitaxel is a well-studied chemotherapeutic agent that disrupts microtubules and has clinical efficacy in a variety of cancers including endometrial cancer [20]. Other studies have also shown that combination therapies including paclitaxel with other chemotherapeutic drugs have higher efficacy or reduced adverse effects in patients with endometrial cancer [21]. Thus, in our study, the synergy between the anticancer effects of 2-ME and paclitaxel in vitro suggests the possibility that 2-ME in combination with paclitaxel may efficiently increase the anticancer activity or maintain the effective anticancer activity of paclitaxel without increasing the drug dose in human endometrial cancer treatment.

Although the mechanism of the anticancer actions of 2-ME at pharmacologic concentrations may result from the suppression of microtubule dynamics [22], the exact mechanism underlying the synergistic effects between 2-ME and paclitaxel is not yet really understood. Han et al. assumed that the observed synergism may be due to the similar mechanisms of action shared by these anticancer agents through the disruption of the normal microtubule functions during mitosis, however, 2-ME may have a different target site on the microtubule to exert its anticancer effect than paclitaxel [14].

Considering other anticancer agents that have different mechanisms of anticancer actions and through the combination testing of these drugs, it may be possible to provide useful information for determining whether 2-ME can broadly or selectively enhance the anticancer actions of chemotherapeutic agents in human endometrial cancer. According to our study, when cisplatin was used in combination with 2-ME, an additive effect was observed. However, when doxorubicin was used in combination with 2-ME, it was found to antagonize the growth-inhibiting effect of 2-ME. The same experiments were repeated multiple times in our study and highly consistent patterns were observed. A previous study also reported a similar antagonistic effect in human breast cancer cells [14]. More studies are needed to investigate the mechanism of such an antagonistic action.

2-ME is currently being used in a phase II trial for patients with hormone-refractory prostate cancer and the tolerability has been shown to be very good [23]. Another phase I study of 2-ME in combination with docetaxel in patients with locally recurrent or metastatic breast cancer also has shown no serious drug-related adverse effects [24]. In our study, the drug combination of 2-ME and paclitaxel produced a synergistic anticancer effect in human endometrial cancer cell lines in vitro, suggesting that 2-ME may selectively enhance the anticancer actions of certain chemotherapeutic agents in human endometrial cancer. Because of the nontoxic characteristic of 2-ME as reported in previous studies, 2-ME may be used as an effective adjunct to enhance the chemotherapeutic effects of certain anticancer agents such as paclitaxel in the treatment of endometrial cancer. However, further phase I and II studies are needed to confirm the present results as others have already done in breast and prostate cancer. We believe that the activity exhibited by 2-ME merits future clinical investigation of this drug as a new agent for the treatment of advanced or recurrent endometrial cancer.

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References


Address reprint requests to equal corresponding authors:

S.N. CHOW, M.D., Ph.D.  
Department of Obstetrics and Gynecology  
National Taiwan University Hospital  
7, Chung-Shan South Road, Taipei (Taiwan)  
e-mail: snchow@ntu.edu.tw  
or C.H. CHIEN, Ph.D.  
Institute of Biochemistry and Molecular Biology  
School of Life Sciences  
National Yang-Ming University  
155, Section 2, Linon Street, Taipei (Taiwan)  
e-mail: chiench@ym.edu.tw
Elevated blood active ghrelin and normal total ghrelin and obestatin concentrations in uterine leiomyoma

A. Markowska¹, A. Ziolkowska², K. Nowinka³, L.K. Malendowicz²

¹Department of Perinatolog and Gynaecology, ²Department of Histology and Embryology, ³Department of Oncology
Poznan University of Medical Sciences, Poznan (Poland)

Summary

Ghrelin and obestatin originate from the same peptide precursor, preproghrelin. Both peptides are secreted in the blood. We investigated serum active and total ghrelin and obestatin concentrations in women with uterine myomatosis. Serum concentrations of active ghrelin in uterine leiomyoma were significantly higher compared to women in the control group (86 ± 3 vs 56 ± 9 pg/ml, respectively; p < 0.02). On the other hand, serum concentrations of total ghrelin and obestatin in uterine leiomyoma did not differ from those in the control group. In the control group the ratio of active to total ghrelin concentrations amounted to 0.62, while in women with uterine myoma it was 0.95, pointing to a prevalence of the active form of ghrelin in women with uterine myoma. Also the ratio of active ghrelin concentration to obestatin concentration was higher in the latter group while the ratio of total circulating ghrelin to obestatin concentrations was similar in the two groups. The data may suggest a role of active ghrelin in the development of a myoma. Moreover, the results indicate that increased blood ratios of active to total ghrelin and to obestatin concentrations are not specific for cachexia.

Key words: Uterine leiomyoma; Ghrelin; Obestatin.

Introduction

Ghrelin and obestatin originate from the same peptide precursor, preproghrelin. Both peptides are secreted in the blood. We investigated serum active and total ghrelin and obestatin concentrations in women with uterine myomatosis. Serum concentrations of active ghrelin in uterine leiomyoma were significantly higher compared to women in the control group (86 ± 3 vs 56 ± 9 pg/ml, respectively; p < 0.02). On the other hand, serum concentrations of total ghrelin and obestatin in uterine leiomyoma did not differ from those in the control group. In the control group the ratio of active to total ghrelin concentrations amounted to 0.62, while in women with uterine myoma it was 0.95, pointing to a prevalence of the active form of ghrelin in women with uterine myoma. Also the ratio of active ghrelin concentration to obestatin concentration was higher in the latter group while the ratio of total circulating ghrelin to obestatin concentrations was similar in the two groups. The data may suggest a role of active ghrelin in the development of a myoma. Moreover, the results indicate that increased blood ratios of active to total ghrelin and to obestatin concentrations are not specific for cachexia.

Numerous evidence indicates that it participates mainly in the short-term control of energy homeostasis, demonstrating orexigenic activity [8, 9].

Ghrelin and its GHS-R1a receptor have been noted to be expressed in several hormone-dependent tumours, including cancers of the ovary, testes, breast and prostate [7, 10-12]. Moreover, studies on the potential relationship between ghrelin concentration and uterine myomas have been justified by demonstration that its receptors are present in the uterus [4] and that:

- ghrelin controls cell proliferation and growth of tissues [6];
- it affects steroidogenesis [13];
- similarly to leptin it is linked to control of energy homeostasis and the relationship between myoma development and leptin has been proven [14].

Obestatin is a recently discovered peptide composed of 23 amino acids, originating from proteolytic cleavage of the ghrelin preprohormone. Obestatin has been thought to represent a ligand of GPR39, an orphan receptor belonging to the family of ghrelin receptors [2], but recent studies have failed to confirm it [15]. Also, the results of studies are divergent in the search of obestatin effects that would be opposite to those of ghrelin, related to food intake, growth control, and energy expenditure [2, 16]. Studies on an animal model demonstrated that obestatin may modulate activity of endogenous ghrelin [17]. Studies by Vicennati et al. [18] demonstrated a lowered ratio of blood ghrelin to obestatin in obese females. Studies on obestatin in uterine myomas have been motivated by its relationship to ghrelin and obesity, which is more frequent in women developing uterine myomas [19, 20]. In the available literature no reports have been identified on blood ghrelin and obestatin concentrations in cases of uterine myoma.
Material and Methods

The Ethics Committee of the Poznan University of Medical Sciences approved the study protocol and before the study, each participant signed an informed consent form. Concentrations of total ghrelin, active ghrelin and obestatin were measured in the blood serum of 39 women aged 24-59 years (mean: 45.3 years) who underwent surgery at the Department of Oncology, Poznan University of Medical Sciences, due to uterine myoma. The procedure, depending on size of the tumours, their location and on age of the patient included extirpation of the myoma or hysterectomy. In all the patients histopathological examination permitted a diagnosis of uterine leiomyoma. The control group consisted of 32 healthy women aged 24-65 years, with a mean of 42.2 years. In all cases body weight, height and body mass index (BMI) were established routinely. Content of adipose tissue in the body (BF%) was calculated according to the formula of Deurenberg [21]. Characteristics of the patients are given in Table 1.

Table 1. — Basic clinical data of studied patients with uterine myomatosis and of controls: BMI - body mass index [$kg/m^2$], BF% - body fat percentage. Results are means ± SE. Number of studied cases shown in brackets. Statistical evaluation of differences, in relation to the control group – unpaired Student’s t-test: * p < 0.05.

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>Height (cm)</th>
<th>BMI</th>
<th>BF%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>42.2 ± 1.7</td>
<td>62 ± 2</td>
<td>163 ± 1</td>
<td>23.4 ± 0.7</td>
<td>32.2 ± 1.2</td>
</tr>
<tr>
<td>Uterine myomomas</td>
<td>45.3 ± 1.2</td>
<td>67 ± 2</td>
<td>163 ± 1</td>
<td>25.2 ± 0.6</td>
<td>35.3 ± 0.9*</td>
</tr>
</tbody>
</table>

In all patients blood was sampled from the cubital vein, centrifuged and the serum was frozen at -80°C for later estimation of ghrelin and obestatin concentrations. Serum total and active ghrelin concentrations were measured using immunoenzymatic tests, produced by Linco (Linco Research, Inc., St. Charles, MO, USA). Test parameters for total ghrelin: specificity for active ghrelin: 80%, for des-octanoyl human ghrelin: 100%, sensitivity: 5 pg/ml, test reproducibility: intra-assay CV - 0.9-1.9% and inter-assay CV - 5.2-7.8%. Parameters of the test for active ghrelin: specificity for active ghrelin 100%, for des-octanoyl human ghrelin 0%, sensitivity: 8 pg/ml, reproducibility: intra-assay CV - 0.9-3.6%, inter-assay CV - 3.6-13.0%. Obestatin concentrations were determined using LLC tests of Peninsula Laboratories (San Carlos, CA), with a sensitivity of 100 pg/ml, and specificity for human obestatin: 100%. Individual stages of the tests were performed as recommended by the manufacturers. Absorbance was measured using a photometer with microplate reading attachment (Multiscan, Labsystem) at the wavelengths of 450 nm and 620 nm.

Data are expressed as the mean ± SEM and their statistical comparison was done by the unpaired Student’s t-test.

Results

Plasma concentrations of active ghrelin, total ghrelin and obestatin in patients with leiomyoma and in women in the control group are presented in Figure 1. Serum level of active ghrelin in uterine leiomyoma was significantly higher as compared to the control group (86 ± 3 vs 56 ± 9 pg/ml, respectively, p < 0.02). On the other hand, concentrations of total ghrelin and obestatin did not differ between either group (control vs leiomyoma: total ghrelin: 94 ± 19 vs 88 ± 19 pg/ml; obestatin: 538 ± 31 vs 575 ± 26 μg/ml, respectively).

In the control group the ratio of active to total ghrelin concentrations amounted to 0.62, while in women with uterine myoma it was 0.95, pointing to a prevalence of active ghrelin in women with leiomyoma (Figure 2). Also the ratio of active ghrelin to obestatin concentrations was higher in the group with uterine leiomyoma while the ratio of total circulating ghrelin to obestatin was similar in both groups of women.

Discussion

Levels of ghrelin in the blood demonstrate significant alterations linked to nutrition and hormonal changes. In obesity levels of circulating ghrelin are known to be depressed while they are elevated in anorexia nervosa [22-27]. Elevated blood ghrelin levels are detected in polycystic ovary syndrome while they are depressed in hyperthyroidism [28-32].

Literature on the subject draws attention to elevated ghrelin levels in the serum of patients with cachexia of various origins, i.e., in cachexia associated with chronic heart failure [33], and in cancer cachexia [34-37]. Furthermore, elevated ghrelin levels are noted in chronic liver disease [38].

In this study we have shown that the plasma concentration of total ghrelin in women with uterine leiomyomas resembled that of the control group while the concentration of active ghrelin was significantly higher in the group with uterine leiomyomas than in the control group. The changes result in a markedly higher ratio of active to total ghrelin in the sera of women with leiomyoma than in women of the control group. In the absence of alterations in total ghrelin levels, changes in the ratio indicate that a mechanism other than increased secretion must be responsible for the increase in active ghrelin levels in uterine myomatosis [35]. As proven by the values of BMI, the observed changes in the ratio of active to total ghrelin have not been linked to cachexia, which means that the increased ratio of active to total ghrelin in the blood is not specific for cachexia.

Few reports only have dealt with obestatin levels in human blood [39-42]. Obestatin levels are significantly lower in obese than in normal weight and anorectic women, and in obese and anorectic patients an increased total ghrelin to obestatin ratio was observed [27, 39]. In our study, no differences were observed in obestatin levels between women in the control group and women with uterine leiomyoma, which suggests simultaneous secretion of both hormones from the common precursor. In parallel, in females with myoma the ratio of active ghrelin to obestatin was higher than in the control group. The result suggests again that a mechanism other than increased secretion must be responsible for the increase in circulating active ghrelin levels in uterine myomatosis [35].
Elevated blood active ghrelin and normal total ghrelin and obestatin concentrations in uterine leiomyoma

Hormones and peptides of the growth hormone axis are frequently involved in neoplastic growth of tissues [12]. Ghrelin and its receptor have been shown to control cell proliferation, growth of tissues and to inhibit apoptosis [4, 12, 43]. A number of studies indicate that ghrelin also affects proliferation of neoplastic cells and that it may also play an important role in the growth of some tumours, acting, i.e., in an auto/paracrine manner [12, 44]. Expression of ghrelin and its active receptor, GHS-R1a, has been documented in certain hormone-dependent cancers, such as cancers of the ovary, testes, breast and gastrointestinal stromal tumours [7, 45, 46]. Ghrelin receptors are also present in the uterus which justifies the hypothesis that circulating active ghrelin may possibly participate in the development of uterine leiomyoma. Nevertheless, this hypothesis requires further investigations. Moreover, lack of changes in circulating total ghrelin and obestatin in myomatous females as compared to the control group suggests that those peptides are not linked to growth of uterine leiomyoma.

The present report seems to be the first attempt to evaluate the potential role of ghrelin and obestatin in the development of uterine myoma.

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Address reprint requests to:
A. MARKÓWSKA, M.D.
Department of Perinatology and Gynecology
Poznan University of Medical Sciences
33 Polna St. 60-535 Poznan (Poland)
e-mail: annamarkowska@vp.pl
Comparison of three vascular endothelial markers in the evaluation of microvessel density in breast cancer

B.B. da Silva¹, M.D., Ph.D.; P.V. Lopes-Costa¹, M.D.; A.R. dos Santos¹, M.D.; E.C. de Sousa-Júnior¹, M.D.; A.P. Alencar², M.D.; C.G. Pires³, M.D.; M.A. Rosal¹, M.D.

¹Department of Gynecology, Mastology Division, Federal University of Piauí, Teresina, Piauí
²Department of Statistics, University of São Paulo, São Paulo (Brazil)

Summary

Purpose: To evaluate the microvessel density by comparing the performance of anti-factor VIII-related antigen, anti-CD31 and anti-CD34 monoclonal antibodies in breast cancer. Methods: Twenty-three postmenopausal women diagnosed with Stage II breast cancer submitted to definitive surgical treatment were evaluated. The monoclonal antibodies used were anti-factor VIII, anti-CD31 and anti-CD34. Microvessels were counted in the areas of highest microvessel density in ten random fields (200 x). The data were analyzed using the Kruskal-Wallis nonparametric test (p < 0.05). Results: Mean microvessel densities with anti-factor VIII, anti-CD31 and anti-CD34 were 4.16 ± 0.38, 4.09 ± 0.23 and 6.59 ± 0.42, respectively. Microvessel density as assessed by anti-CD34 was significantly greater than that detected by anti-CD31 or anti-factor VIII (p < 0.0001). There was no statistically significant difference between anti-CD31 and anti-factor VIII (p = 0.4889). Conclusion: The density of stained microvessels was greater and staining was more intense with anti-CD34 compared to anti-CD31 and anti-factor VIII-related antigen.

Key words: Microvessel density; Anti-CD34, Anti-CD31; Anti-factor VIII; Neovascularization; Mammary neoplasia.

Introduction

Angiogenesis is an essential factor for the growth and dissemination of the metastases of malignant tumors [1-4]. Tumors are able to absorb sufficient nutrients and oxygen by simple diffusion up to a size of 1-2 mm. Upon reaching this size, additional growth depends on supplementary vascularization supplied by the formation of new capillaries from mature vessels in the host, which subsequently grow and invade the tumor mass [5, 6].

Angiogenesis has been associated with the prognosis of many neoplasias [7], among them breast cancer [8-10]. It has been suggested that the intensity of angiogenesis may be inversely correlated with the time of overall survival and disease-free time in breast cancer patients [2, 8, 9, 11]; however, not all studies have confirmed this association [12-14]. Such discrepancies may be the result of various methodological issues such as patient selection, sample size and the methods used to quantify microvessels and identify vascular endothelial cells [15, 16].

Using immunohistochemical markers of endothelial cells to calculate microvessel density is the most widely applied technique for the quantification of angiogenesis [15]; however, no consensus as yet exists with respect to which endothelial cell marker provides optimal sensitivity and specificity [2,17]. The first study in which immunohistochemistry was used to examine microvessel density in breast cancer was performed by Weidner et al. [8] in 1991. These investigators used an antibody against the factor VIII-related antigen as a vascular endothelial marker, and reported an almost linear relationship between the increase in microvessel count and breast cancer metastases [8]. The following year, Horak et al. [18] tested an anti-CD31 monoclonal antibody and showed a strong association between microvessel density and the overall survival of patients with breast cancer. Later, anti-CD34 became widely used in various studies for the immunohistochemical quantification of microvessels in breast cancer samples, the results having been found to correlate with the prognosis of these patients [19-21]. The importance of this subject has motivated attempts to identify an endothelial marker that would provide greater sensitivity and specificity.

Studies comparing the three vascular endothelial markers most commonly used in breast cancer, anti-factor VIII-related antigen, anti-CD31 and anti-CD34, are few and those published in the literature are controversial, which led us to design the present study.

Materials and Methods

Twenty-three women receiving care at the Mastology Clinic of the Department of Gynecology, Getúlio Vargas Hospital, Federal University of Piauí, diagnosed with Stage II breast cancer and submitted to segmentectomy or mastectomy between January 2005 and December 2007, were included in this study. All patients had an infiltrating ductal carcinoma and had been menopausal for at least one year.

Patients who had previously been submitted to any type of treatment for neoplasia were excluded from the study. The protocol was approved by the Internal Review Board of the Federal University of Piauí and all patients gave their written consent prior to initiation of the study. The size of the tumor varied from 3 to 5 cm (mean 3.7 cm) and the mean age of patients was 61.2 years (range 46-88 years). Tumor specimens were obtained by incisional biopsy at the time of definitive surgery.

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Tumor samples were fixed in buffered formalin for a period of 12-24 hours and cut into 3-μm-thick sections. Next, the samples were processed and stained with hematoxylin-eosin for confirmation of the diagnosis of infiltrating ductal carcinoma, after which they were deparaffinized in xylol for five minutes, dehydrated in absolute ethanol and washed in buffered saline solution at pH 7.4 for five minutes.

Immunohistochemical evaluation of factor VIII, CD31 and CD34 markers was performed using the Envision™ (Dako, USA) detection system with antigen recovery. For this, the sections were treated with 3% hydrogen peroxide (H2O2) diluted in buffered solution for 5 min to block the endogenous peroxide. After recovery of the epitopes, the tissue samples were incubated with the following primary monoclonal antibodies: anti-CD34 (anti-human hematopoietic progenitor cell, CD34 class II, Clone QBEnd-10, DAKO Corporation, Dakopatts, Carpinteria, CA) at a dilution of 1:25 with bovine serum albumin (BSA); anti-factor VIII (anti-human factor VIII-related antigen, DAKO Corporation) at a dilution of 1:200 with bovine serum albumin (BSA), anti-CD31 (anti-human endothelial cell, Clone JC70, DAKO Corporation) at a dilution of 1:30 with bovine serum albumin (BSA) for a period of 16 hrs that included an overnight period, in a refrigerator at a temperature of approximately 4°C. Next, following washing with buffered saline solution, the sections were incubated for 45 min with the Envision™ detection system. To read the reaction, all the sections were treated with a solution of DAB [(3-3)- diaminobenzidine tetrahydrochloride] at a concentration of 1 mg/ml of TRIS buffered solution and hydrogen peroxide solution for 5 min. Next, the sections were counterstained with Harris hematoxylin or methyl green for 5 min followed by dehydration in ethanol and xylol baths. Endothelial cells were considered positive when stained a brownish color.

Quantification was carried out by two observers blinded with respect to the identity of the patients and with no previous knowledge of any of the cases. It was performed using a light microscope (Nikon Eclipse E-400, optical microscope, Tokyo, Japan) connected to a color videocamera (Samsung digital camera CHC-370N, Seoul, Korea), which captured the image and transmitted it to a computer equipped with the Imagelab® software program, version 2.3, developed by Softium Informática Ltda. (São Paulo, Brazil) for image analysis. To count the microvessels, the areas of highest microvessel density (hotspots) were selected at 40 x magnification, following which the image was captured and the microvessels counted in ten randomly selected fields per slide (200 x magnification). Countable microvessels were defined according to the criteria established by Weidner et al. [8] as any brown-stained endothelial cell or endothelial cell cluster, clearly separated from each other and from adjacent microvessels, with or without a vessel lumen. In each case, microvessel density was the mean number of vessels counted in ten fields.

Microvessel densities obtained with the three endothelial cell antibodies were compared using the Kruskal-Wallis nonparametric test [22]. The 2 x 2 multiple group comparisons were carried out using the Mann-Whitney test [22], taking the Bonferroni correction into consideration. Statistical significance was established at p < 0.05.

**Results**

At light microscopy, the density of stained microvessels was greater and staining was more intense with anti-CD34 compared to anti-CD31 and anti-factor VIII (Figure 1). The mean density (with standard error) of microvessels in breast cancer specimens of these postmenopausal patients was 4.16 ± 0.38, 4.09 ± 0.23 and 6.59* ± 0.42.
6.59 ± 0.42 for anti-factor VIII, anti-CD31 and anti-CD34, respectively (Table 1). According to the Kruskal-Wallis nonparametric test, the mean density of microvessels as assessed by anti-CD34 was significantly greater than the mean microvessel density found with anti-CD31 or anti-factor VIII (p < 0.0001). There was no statistically significant difference between the microvessel density found with anti-CD31 and anti-factor VIII (p = 0.4889) (Table 1 and Figure 2).

Discussion

In this study carried out on postmenopausal women, the antibody against the endothelial antigen CD34 was found to be the most sensitive vascular endothelial marker compared to the antibodies against factor VIII-related antigen and CD31 in samples of infiltrating ductal breast carcinoma routinely fixed in paraffin, since the density of stained microvessels was greater and staining was more intense with this antibody.

In addition to the proliferation of blood vessels, tumors also induce alterations in the expression of endothelial cell molecules [23]. Moreover, many antigens lose their antigenicity when stored in paraffin [24]. Therefore, the ideal marker of blood vessels should take into account not only quantitative changes but also these qualitative alterations, and, preferentially, it should also bind to antigens that maintain their antigenicity when stored in paraffin blocks. Up to the present time, no ideal endothelial marker has been found, and many antibodies are being studied and used for this purpose [25, 26]. Among the most commonly used are the antibodies to factor VIII-related antigen, anti-CD31 and anti-CD34, however reports on studies comparing these three markers in breast cancer are sparse and controversial in the literature [17, 23, 27].

Factor VIII was one of the first endothelial markers to be used [24]. Factor VIII-related antigen, or the Von Willebrand factor, plays an important role in platelet adhesion and aggregation and is located in the Weibel-Palade bodies in the cytoplasm of vascular endothelial cells [26, 28]. An antibody against factor VIII generally results in adequate staining of large blood vessels; however, in many tissues this antibody frequently fails to stain capillaries and sinus endothelial cells [23, 28], and a reaction may occur with the endothelium of lymphatic vessels [2, 18, 24]. This has resulted in numerous studies in which staining for factor VIII-related antigen has been found to be inconsistent both in quantitative and qualitative terms, perhaps affected by the degree of maturation of endothelial cells, mostly tumoral, that could be at a maturation level unidentified by its antibody [23, 28]. Consequently, the reliability of the antibody to factor VIII-related antigen for pathological research has been questioned, stimulating a search for more sensitive markers.

CD31 is a glycoprotein and a member of a family of adhesion molecules, being expressed during the differentiation of myelomonocytic cells [26]. It appears to be involved in platelet adhesion and also in the initiation of inflammation and wound healing [18, 9]. Of the antibodies tested against CD31, only JC70 recognizes an epitope that is resistant to formalin [2, 24, 28]. This antibody has a potential value in practical diagnosis since the spectrum of its reactivity is broader than that of anti-factor VIII, also staining immature blood vessels. In addition, it does not react with lymphatic vessels [2, 24]. Consequently, the count achieved with the use of this marker tends to be around 30% greater than that found with anti-factor VIII [17, 25]. The disadvantages of anti-CD31 are that it cross-reacts weakly with fibroblasts and some plasma cells, and is rarely expressed to any great extent in neoplastic cells [17, 26, 28]. Another drawback of anti-CD31 that may impede its use as a routine marker is that its failure to stain may be as high as 20% in routinely fixed breast samples [17, 25]. Using the clone JC70, Horak et al. [18] compared anti-CD31 with anti-factor VIII in 12 cases of breast cancer, reporting that the staining found with the JC70 antibody to CD31 consistently revealed a greater number of microvessels and proved to be a more sensitive marker of endothelial cells than anti-factor VIII-related antigen.

In turn, CD34 is a transmembrane glycoprotein present in immature hematopoietic precursor cells and endothelial cells and is believed to be involved in leukocyte adhesion and in the migration of endothelial cells during angiogenesis [26]. Of the monoclonal antibodies against CD34, QBEnd-10 results in clear, strong staining in cryostat sections, and has the additional advantage of recognizing its antigen in paraaffin-fixed sections [27]. Marking of CD34 results in counts similar to those found with CD31 without the high staining failure rate, and for this reason it is currently the most widely used endothelial marker [17].

Siitonen et al. [26], in only 19 cases of breast cancer, reported a greater intensity of staining with anti-CD34 and a greater microvessel count with anti-factor VIII. For this reason, these investigators preferred these two endothelial cell antibodies to anti-CD31. Martin et al. [19] reported similar results to those found by Siitonen et al. [26]. Nevertheless, in the present study, despite the current controversies, a higher density of microvessels and a greater intensity of staining were found when anti-CD34 was used in comparison with anti-CD31 and anti-factor VIII-related antigen in paraffin-fixed samples of infiltrating ductal breast carcinoma from postmenopausal women. These findings may justify an increasing use of this monoclonal antibody in the study of angiogenesis, not only in breast cancer but also in other neoplasias.

References

Ovarian cancer: lymph node metastases

G. Balbi, M.A. Manganaro, A. Monteverde, I. Landino, C. Franzese, F. Gioia

Department Obstetrics and Gynecology, Second University of Study of Naples, Naples (Italy)

Summary

Purpose of investigation: To analyze pelvic and paraaortic lymph node involvement in epithelial ovarian cancer. Methods: Between 1995 and 2006, 60 patients with FIGO Stages II, III, IV epithelial ovarian cancer underwent surgical treatment, including systematic pelvic and paraaortic lymphadenectomy. Results: Aortic lymph node metastases were documented in 45 (75%) patients and pelvic nodal metastases in 42 (70%). The incidence of paraaortic nodal involvement was 20% (12/60) in the absence of positive pelvic nodes while the incidence of pelvic nodal involvement was 15% (9/60) in the absence of paraaortic disease; both pelvic and paraaortic lymph node involvement occurred in 55% of patients. The most frequent groups for nodal metastases are paracaval (56%), external iliac (60%), and obturator (55%). Conclusion: The rate of nodal involvement is important in ovarian cancer and there is a high prevalence of both pelvic and paraaortic lymph node metastases. For this reason bilateral pelvic and paraaortic lymphadenectomy is necessary for staging and as treatment for micrometastases, also in patients with unilateral tumors.

Key words: Ovarian cancer; Lymph node metastases; Aortic lymphadenectomy; Pelvic lymphadenectomy.

Introduction

Epithelial ovarian cancers are gynecologic tumors that spread frequently through the lymphatic system [1] and therefore, in recognition of the prognostic importance of retroperitoneal interest, the International Federation of Gynecology and Obstetrics (FIGO) staging classification includes a stage for node involvement [2].

Systematic pelvic and paraaortic bilateral lymphadenectomy is an important component of surgical staging for epithelial ovarian cancer (EOC) to determine the nodal extension of disease [3] and to decide which patients with retroperitoneal involvement should undergo adjuvant chemotherapy.

The purpose of our study was to prove that systematic lymphadenectomy for EOC should be both pelvic and paraaortic, because lymph node involvement is present in both stations lymph nodes in over 50% of patients N+. Also lymphadenectomy should be bilateral independently from the unilaterality of the tumor.

Materials and Methods

Between 1995 and 2006 at our Institute 60 patients with FIGO Stages II, III and IV primary epithelial ovarian carcinoma underwent surgical treatment, including systematic pelvic and paraaortic lymphadenectomy.

For these patients, lymphadenectomy was associated with total hysterectomy, bilateral salpingo-oophorectomy, omentectomy and appendicectomy. Pelvic lymphadenectomy was carried out along the external iliac, internal iliac, common iliac and obturator vessels on both sides and paraaortic node dissection was performed from the bifurcation of the aorta to the origin of the renal vessels. No patient received neo-adjuvant chemotherapy.

Patients’ age, clinical disease stage, tumor grade, number of resected lymph nodes, number of metastatic lymph nodes, laterality, and distribution and sites of metastatic nodes were the variables analyzed in this study.

Results

Clinical characteristics of the study patients are shown in Table 1.

The mean age was 50 years (range 30-74). Patients with clinical Stage III disease accounted for 55% (33/60) of patients in this study. Histologic grade of differentiation was described as a universal grading system for grade 1, 2 and 3. All patients underwent a complete lymphadenectomy. The frequency of lymph node metastases according to the stage of disease (II, III, IV) were: 42.8% (9 of 21), 63.7% (21 of 33) and 83.4% (5 of 6), respectively.

Aortic lymph node metastases were documented in 45 (75%) patients. The total number of paraaortic lymph nodes removed was 1,060; of these 424 (40%) contained metastatic disease. The minimal number of paraaortic nodes removed to consider lymphadenectomy appropriate for patients was 15 [4]. The incidence of paraaortic nodal involvement was 20% (12/60) in the absence of positive pelvic nodes and 55% (33/60) in the presence of pelvic disease.

Pelvic nodal metastases were documented in 42 (70%) patients. The total number of pelvic lymph nodes removed was 1,680, of these 420 (25%) were positive. The minimal number of pelvic nodes removed for patients was 25 to consider lymphadenectomy appropriate [4]. The incidence of pelvic nodal involvement was 15% (9/60) in the absence of paraaortic disease (Table 2).

In the pelvic area the common iliac (45%), external iliac (60%) and obturator nodes (55%) were the most frequently involved node groups. The distribution of paraaortic positive nodes was: paracaval (56%), intercavoaortic (46%) and supramesenteric (36%).

The frequency of nodal involvement according to tumor grade was: 23% (2/9) for grade 1, 55.6% (10/18) for grade 2 and 60.6% (20/33) for grade 3.

As for laterality of primary tumors, 40% (24/60) were bilateral, 26.7% (16/60) were right-sided and 33.3% (20/60) left-sided. In 16 patients with unilateral right...
In 1992, Panici nodes, most of which are sited in the abdomen and pelvis ovarian cancer target lymphadenectomy. lymph nodes, is fundamental to understand the disseminating the routes of lymphatic channels and location of genital cancers. Therefore the lymphatic anatomy, including the routes of lymphatic channels and location of ovarian tumors, eight (50%) had nodal involvement; of these four (50%) patients had ipsilateral nodal involvement, three (37.5%) had bilateral and one (12.5%) had contralateral positive nodes. In 20 patients with unilateral left ovarian tumors, 11 (55%) had nodal involvement: five (45.5%) patients had ipsilateral positive nodes, four (36.4%) bilateral and two (18%) patients had contralateral lymph node involvement (Table 3).

Table 1. — Clinical characteristics of the study patients.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Median age (range)</th>
<th>Stage</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>50 (30-74)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>II</td>
<td>21 (35)</td>
<td>1</td>
<td>9 (15)</td>
</tr>
<tr>
<td>III</td>
<td>33 (65)</td>
<td>2</td>
<td>18 (30)</td>
</tr>
<tr>
<td>IV</td>
<td>6 (10)</td>
<td>3</td>
<td>33 (55)</td>
</tr>
</tbody>
</table>

Table 2. — Lymph node involvement. No. of patients: 60.

<table>
<thead>
<tr>
<th>Pelvic Aortic</th>
<th>Nodal involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic</td>
<td>Only N + right</td>
</tr>
<tr>
<td>Positive</td>
<td>9 (15)</td>
</tr>
<tr>
<td>Negative</td>
<td>6 (10)</td>
</tr>
</tbody>
</table>

Table 3. — Lymph node involvement in relation to the laterality of tumor.

<table>
<thead>
<tr>
<th>Laterality of tumor</th>
<th>Only N + right</th>
<th>Only N + left</th>
<th>N + bilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right (N+ = 8)</td>
<td>4 (50%)</td>
<td>1 (12.5%)</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td>Left (N+ = 11)</td>
<td>2 (18%)</td>
<td>5 (45.6%)</td>
<td>4 (36.4%)</td>
</tr>
</tbody>
</table>

Discussion

Even if in the last decades much progress has been made in gynecology-oncology, EOC still represents the most aggressive neoplasia among gynecologic tumors, with the poorest prognosis. In 70% of the cases advanced stage (FIGO Stage IIB-IV) is diagnosed because it is asymptomatic for a long time [5].

EOC is the most lymphoproliferative tumor among genital cancers. Therefore the lymphatic anatomy, including the routes of lymphatic channels and location of lymph nodes, is fundamental to understand the dissemination of retroperitoneal lymph nodes and, consequently, of ovarian cancer target lymphadenectomy.

In the normal adult body there are about 450 lymph nodes, most of which are sited in the abdomen and pelvis [6]. In 1992, Panici et al. [7] verified a new detailed nomenclature for lymph node groups: paraaortic nodes were distinguished as paracaval, preaortic and retrocaval, depending on their relationship with the vena cava, and pelvic lymph nodes were divided into six groups as common, internal and external iliac, presacral, obturator and paraaortic depending on the relationship with the pelvic blood vessels. Ferraris et al. [8] considered systematic lymphadenectomy in cervical cancer when at least 20 positive pelvic lymph nodes were collected. Our findings indicate that the minimal number of pelvic lymph nodes removed should be 25, and for paraaortic nodes 15 should be removed to consider lymphadenectomy appropriate.

Additionally, in agreement with previous reports [9, 10] indicating that the intraoperative assessment of retroperitoneal lymph nodes by palpation is erroneous in 33% to 60% of patients, in our study, to avoid inaccurate staging and improper management, complete lymph node dissection was executed in all patients with EOC.

The dissemination of malignant cells in EOC happens in two ways: exfoliation and peritoneal implantation or retroperitoneal lymphatic drainage of the ovary, which increase as the disease spreads intraperitoneally [11].

The lymphatic pathway of spread has been extensively described by Plentl and Friedman [12]. They described three major diffusion lymphatic ways for EOC: 1) bilaterally along the ovarian blood vessels to the aortic area, terminating at the vena cava on the right side and between the aortic and renal vessels on the left side (the main route). 2) Through the broad ligament towards the lateral pelvic wall and terminating at the external iliac and interiliac nodes, then reaching the common iliac nodes and the aortic region. 3) Less frequently involved, along the round ligament to the external iliac and inguinal lymph nodes.

We, like other authors [13, 14], observed a higher rate of positive paraaortic nodes (75%) than pelvic node metastases (70%) in EOC.

However aortic node metastases are commonly the initial route for the spread of EOC, followed by pelvic and inguinal node metastases [15]. In our study 15% of patients had negative aortic nodes but positive pelvic nodes, and 20% of patients had negative pelvic nodes but positive aortic nodes. Moreover, no patient in this series with positive pelvic and aortic nodes had inguinal lymph node involvement because it appears to be a late manifestation in EOC.

Onda et al. [16] described the distribution of pelvic and aortic node metastases in EOC and concluded that the high aortic nodes (above the inferior mesenteric artery), the internal-external iliac nodes and obturator would yield the best sensitivity and negative predictive value. Also our data confirm that the paraaortic nodes constitute the crucial site of nodal involvement in EOC, even in patients with unilateral tumors. Our findings indicate that the most frequent groups for nodal metastases are paraaortic (56%), external iliac (60%) and obturator (55%).

The findings of this study confirm that there is a high prevalence (55%) of both pelvic and paraaortic lymph node metastases in patients with advanced ovarian cancer, as in the study of Aletti et al. [17].

A growing rate of nodal involvement with increasing stage of tumor is well established in EOC. Burghardt et al. [18] were the first authors who analyzed this subject and they found that metastatic lymph node number was greater in advanced stages. Indeed, the incidence of positive lymph nodes was 24% in Stage I, 50% in Stage II, 74% in Stage III and 73% in Stage IV. Suzuki et al. [19] also found metastatic lymph node numbers to correlate with clinical stage. In our study a significant correlation between clinical stage and lymph node involvement was also found: 42.8% of lymph node metastases in Stage II, 63.7% in Stage III and 83.4% in Stage IV.

Another important factor that influences the rate of nodal involvement is the tumor grade. Nodal involvement in our
date were: 23% for grade 1, 55.6% for grade 2 and 60.6% for grade 3. This rate of nodal involvement is in accordance with the literature [20, 21]. Only Morice et al. observed that the incidence of nodal metastases was higher in grade 2 disease (51%) than in grade 3 (47%) [22].

In regard to contralateral metastatic disease in patients with unilateral EOC, Benedetti-Panici et al. [23] initially indicated that lymphatic spread was ipsilateral and that ipsilateral lymphadenectomy was appropriate for staging. However, later studies [24, 25] have suggested that bilateral pelvic and paraaortic lymphadenectomy should be performed for adequate staging also in the presence of unilateral EOC because contralateral nodal involvement has been reported in 30% of patients with unilateral clinical Stage I disease. Ipsilateral lymphadenectomy is also inadequate in patients with unilateral EOC and clinical Stage II to IV [26]. Indeed, in the study of Pereira et al. [27], only 50% of patients with unilateral disease had ipsilateral pelvic nodal involvement and 53% ipsilateral aortic nodal involvement while the rest had contralateral or bilateral disease. Our results confirm similar findings: 50% of patients with unilateral right ovarian cancer had ipsilateral nodal involvement, 37.5% bilateral and 12.5% had contralateral positive nodes; 45.6% of patients with unilateral left ovarian cancer had ipsilateral positive nodes, 36.4% bilateral and 18% contralateral lymph nodes involvement.

Conclusion

The results of this study confirm that the rate of nodal involvement is important in EOC and that there is a high prevalence of both pelvic and paraaortic lymph node metastases. Consequently bilateral pelvic and paraaortic lymphadenectomy are necessary for staging and as treatment for micrometastases.

For a definitive conclusion, larger studies may be needed to evaluate prognostic significance in terms of progression-free and overall survival.

References


Address reprint requests to:
G. BALBI, M.D.
Department of Obstetrics and Gynecology
Second University of Naples
Via Cimbrones, 84 - 80127 Naples (Italy)
email: giancarlo.balbi@unina2.it
ERBB2 (HER2) protein expression in uterine sarcomas

M. Zafrakas¹, L. Zepiridis¹, T.D. Theodoridis¹, I.D. Venizelos²,
A. Papanicolaou¹, T. Agorastos¹, J.N. Bontis¹

¹1st Department of Obstetrics & Gynecology, Aristotle University of Thessaloniki, Papageorgiou General Hospital
²Department of Pathology, Hippokrateio General Hospital, Thessaloniki (Greece)

Summary

Purpose: Multiple clinical trials in recent years have shown that breast cancer patients with primary tumors overexpressing ERBB2 can be effectively treated with specific forms of modern anti-ERBB2-targeted therapy. The aim of the present study was to analyze the expression of the ERBB2 (HER2) protein in uterine sarcomas, in order to investigate the possibility of applying this treatment modality in uterine sarcomas. Methods: The expression of ERBB2 has been analyzed immunohistochemically in formalin-fixed paraffin-embedded primary uterine sarcomas (n = 11). Results: Using a semi-quantitative immunohistochemical score, we found that ERBB2 expression was very weak in the majority of tumors, with only three sarcomas showing moderate ERBB2 expression. Published studies evaluating the same issue in small numbers of uterine sarcomas reached similar findings. Conclusion: Overall, ERBB2 expression appears to be weak in uterine sarcomas. However, targeted treatment might still be feasible in a subgroup of patients with uterine sarcomas overexpressing ERBB2.

Key words: Uterine sarcoma; ERBB2; HER2; Targeted therapy; Trastuzumab; Herceptin®; Lapatinib; Tykerb®.

Introduction

Uterine sarcomas are rare malignant tumors of mesenchymal origin with a poor prognosis [1, 2]. Though they represent 2-5% of the total, they account for more than 25% of mortality from malignancies of the uterine corpus [1-3]. Moreover, the majority of uterine sarcomas are diagnosed in advanced inoperable stages, and most cases respond poorly to chemotherapy and radiation [4-6]. Thus, development of new therapeutic strategies for the treatment of these tumors is needed.

In recent years, a major advance in the treatment of malignant disease has been the administration of novel therapeutic modalities targeting specific molecular characteristics of tumors [1]. ERBB2, also widely known as HER-2 or HER-2/neu, belongs to a gene family of four genes encoding transmembrane receptor tyrosine kinases, mediating cell growth, differentiation, and survival [7, 8]. Overexpression of ERBB2, occurring in 20-25% of breast cancers, was found to be associated with reduced disease-free and overall survival, and this has led to the development of therapeutic strategies targeting the HER-2 protein [9-11]. To evaluate the possibility of applying ERBB2-targeted treatment in uterine sarcomas, we have analyzed immunohistochemically its expression in a series of these tumors.

Materials and Methods

 Archived tumor specimens from 11 patients with uterine sarcomas were obtained from the Pathology Department of the Hippokration General Hospital in Thessaloniki Greece. All tumor specimens were fixed in formalin and embedded in paraffin. All patients were surgically treated at the 1st Department of Obstetrics and Gynecology. Patient age ranged between 40 and 69 years. Hematoxylin-eosin stained slides were reviewed to confirm histological diagnosis. Tumors were classified according to the World Health Organization (WHO) classification (2003) for uterine sarcomas. The tumors included four leiomyosarcomas, two low-grade endometrial stromal sarcomas (LGiESS), two high-grade endometrial stromal sarcomas (HiGESS), and three mixed Mullerian mesenchymal tumors (MMMT).

Representative tissue blocks were selected for immunohistochemistry. Immunoperoxidase staining for ERBB2 (HER2) was performed in 4.0 μm thick tissue sections from all tumors. The BioGenex Automatic Staining System (BioGenex, San Ramon, CA) was used, as previously described [1]. 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 (BioGenex, San Ramon, CA) for 25 min followed. Tissue sections were incubated with the polyclonal rabbit antibody anti-ERBB2 antibody A0485 (Dako, Glostrup, Denmark) at a dilution of 1:250 for 30 min. Incubation with a peroxidase-streptavidin conjugate (BioGenex, San Ramon, CA) for 20 min followed. Diaminobenzidine tetrahydrochloride was then used as a chromogen and sections were counterstained with hematoxylin, dehydrated and mounted. Tissue sections from breast tumors with strong staining for ERBB2 were used as positive controls.

For evaluation of immunohistochemical data, a semi-quantitative scoring system was used, as described previously [12]. In brief, staining intensity was characterized using the following scale: 0 = negative, 1+ = low, 2+ = middle and 3+ = strong. The percentage of stained cells varied between: 0 = negative, 1 = < 10%, 2 = 10-50%, 3 = 51-80% and 4 = > 80% positive cells. According to the scores, tissues were classified as having low (0 to 2 points), middle (3 to 6 points) or strong (8 to 12 points) ERBB2 expression.

Results

Immunostaining results for ERBB2 expression are summarized in Table 1, according to sarcoma histological type. ERBB2 expression was detected in all four leiomyosarcomas (two
1+/:5%, one 2+/:10%, and one 3+/:30%), and both LGESS (one 1+/:5%, and one 2+/:80%). One of the two HGEESs tested was ERBB2-negative and one was weakly positive (1+//:5%). One of the three MMMTs was ERBB2-negative and two were weakly positive (one 1+//:5% and one 1+//:10%).

Altogether, the majority of uterine sarcomas showed weak to moderate intensity (1+ or 2+ in 8 out of 11 tumors) and focal (,10%) to moderate (10-50%) staining distribution (8 out of 11 tumors) of ERBB2. Two of the 11 tumors were entirely ERBB2-negative. There was one leiomyosarcoma showing strong intensity (3+), but low distribution (30%), and one LGESS showing extensive distribution (80%), but moderate intensity (2+). In Figure 1 a representative section showing sarcoma cells positive for ERBB2 is presented.

Results of the semi-quantitative immunohistochemical score are presented in Table 2. Eight out of 11 tumors had a low immunohistochemical staining score (0-2), and three had a moderate score, while none of the tumors showed strong ERBB2 expression.

**Discussion**

ERBB2-targeted therapy has revolutionized breast cancer treatment in recent years. High-powered, multi-center clinical trials have shown that trastuzumab (Herceptin®, F Hoffmann-La Roche Ltd., Basel, Switzerland), a humanized monoclonal antibody directed against HER-2, improves survival in women with metastatic [13-15], as well as operable breast cancer in the adjuvant setting [16-19]. Furthermore, clinical trials evaluating the efficacy of lapatinib (Tykerb®, GW572016, GlaxoSmithKline), a small-molecule reversibly inhibiting both ERBB1 (EGFR) and HER2, has shown promising results against trastuzumab-refractory metastatic breast cancer [20, 21], and operable breast cancer in the neoadjuvant setting [22].

Given the efficacy of ERBB2-targeted therapy in breast cancers overexpressing ERBB2, application of this type of therapy in uterine sarcomas would be desirable. In view of this possibility, we have immunohistochemically analyzed the expression of ERBB2 in a panel of archival formalin-fixed paraffin-embedded tissue specimens from uterine sarcomas. The majority of tumors tested had a low immunohistochemical staining score (8 out of 11, with two entirely negative), only three had a moderate staining score, while none showed strong ERBB2 expression.

Our results are in line with those of previous studies, most of which showed that ERBB2 was expressed only in a minority of uterine sarcomas by immunohistochemistry [23-29]. In most of these studies ERBB2 expression was primarily analyzed in MMMTs. Cimbaluk et al. [23] found ERBB2 immunopositivity in the epithelial component of two of 30 (6%) MMMTs, while the mesenchymal component of all tumors stained negative. Raspoillini et al. [24] observed HER-2 overexpression in nine of 28 (32.1%) MMMTs, and Livasy et al. [25] in 14 of 55 (25.5%) MMMTs; again the carcinomatous component showed more frequently HER2 overexpression as compared with the sarcomatous component. Sawada et al. [26] found HER-2 immunopositivity in the epithelial component, of nine (56%), and in the mesenchymal component of one (6%) out of 16 cases of MMMTs. Amant et al. [27] reported that all ten adenosarcomas, 21 endometrial stromal sarcomas, and ten leiomyosarcomas tested were HER-2 negative, while HER-2 was positive in the epithelial component of five and the sarcomatous component of one out of 22 MMMTs. Nasu et al. [28] observed ERBB2 expression in one of six (16.7%) leiomyosarcomas, in the carcinomatous area of all six (100%), and in the sarcomatous area of five out of six (83.3%) MMMTs. On the other hand, Swisher et al. [29], found that HER-2 protein was not overexpressed in any adenosarcoma (n = 6) or primary MMMT (n = 20) tested. Interestingly, despite the low proportion of uterine sarcomas exhibiting moderate-to-strong expression of ERBB2, authors did not completely rule out the use of ERBB2-targeted therapy in a subgroup of patients [24, 25].

**Conclusion**

Our data together with findings from previous studies suggest that wide application of ERBB2-targeted therapy in uterine sarcomas seems unlikely. On the other hand, despite the fact that only 20-25% of breast tumors exhibit ERBB2 overexpression, the efficacy of targeted treatment in this subgroup of breast cancer patients is firmly established. Thus, it seems reasonable to suggest that ERBB2-targeted therapy could also be applied in a subgroup of patients with uterine sarcomas overexpressing ERBB2, and this should be tested in future studies.

**Table 1. — ERBB2 (HER2) expression in a panel of uterine sarcomas.**

<table>
<thead>
<tr>
<th>Histological classification</th>
<th>Staining intensity</th>
<th>Tissue staining distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no.</td>
<td>0</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>HGEES §</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>LGESS ‡</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>MMMT†</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

†† IHC-score = immunohistochemical score.

† HGESS: high-grade endometrial stromal sarcomas, ‡ LGESS: low-grade endometrial stromal sarcomas, † MMMT: mixed muellerian mesenchymal tumor.

**Table 2. — Immunohistochemical scores of ERBB2 (HER2) expression in uterine sarcomas.**

<table>
<thead>
<tr>
<th>Histological classification</th>
<th>IHC-score ††</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (0-2 points)</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>4 2 2 -</td>
</tr>
<tr>
<td>HGEES §</td>
<td>2 2 -</td>
</tr>
<tr>
<td>LGESS ‡</td>
<td>2 1 1 -</td>
</tr>
<tr>
<td>MMMT†</td>
<td>3 3 -</td>
</tr>
</tbody>
</table>

§ HGESS: high-grade endometrial stromal sarcomas, ‡ LGESS: low-grade endometrial stromal sarcomas, † MMMT: mixed muellerian mesenchymal tumor.
immunostaining (x 400).

References


Empirical accuracy of fine needle aspiration cytology (FNAC) for preoperative diagnoses of malignant breast lumps in hospitals with restricted health resources

J. Patumanond¹, T. Kayee², U. Sukkasem³

¹Division of Clinical Epidemiology and Medical Statistics, Faculty of Medicine, Chiang Mai University, Chiang Mai
²Department of Surgery, Nakornping Hospital, Chiang Mai
³Department of Surgery, Lamphun Hospital, Lamphun (Thailand)

Summary

Objective: To reevaluate the additional diagnostic value of FNAC from patient profiles in the diagnosis of breast cancer among patients presenting with breast lumps. Methods: A database was reconstructed from routine follow-up data of patients with breast diseases. Predictive ability of the variables were presented with an area under a receiver operating characteristic (ROC) curve using the logistic model. Results: Age and size of breast lumps alone could predict malignancy with a ROC area of 0.85, (95% CI = 0.81-0.89). When FNAC was added into the logistic model in the presence of age and size of breast lumps, the ROC area increased to 0.95 (95% CI = 0.93-0.97) with statistical significance. Given the same FNAC classification codes, the probability of malignancy increased in older patients and in patients with larger breast lumps. Conclusion: In developing countries where health resources are restricted, FNAC seems to be cost efficient. Continued use of the technique should be encouraged.

Key words: Breast mass; Breast cancer; Fine needle aspiration cytology; Diagnostic test; Diagnostic research; Prediction research.

Introduction

Fine needle aspiration cytology (FNAC), a procedure using a narrow gauge needle to collect a sample of a lesion for microscopic examination, was first recognized in 1921 [1]. It was initially introduced to replace open or incisional biopsy, which is an invasive method. In the evaluation of breast lumps, the principles of “triple diagnosis” are recommended. This refers to the application of a combination of three diagnostic tests: clinical breast examination, breast imaging (mammography or echography), and breast pathology (cytopathologic or histopathologic findings). Open biopsy was used in the past for cytopathicologic evaluation, but has been replaced with the FNAC technique. FNAC and similar related procedures such as core needle biopsy are now universally accepted as methods that eliminate the need for an open biopsy or frozen section in cancer diagnosis. More recently FNAC was successfully incorporated into a triple test score for evaluating breast lumps [2] and later into a clinical decision rule to triage women with breast lumps into open biopsy or follow-up [3]. Moreover, FNAC under ultrasound (US) guidance was reported to be highly accurate and minimally invasive [4, 5].

In countries with restricted health resources, mammography and US is not always available in most centers, either due to lack of instruments or skilled personnel. Surgeons are therefore left with only the options of clinical breast examination and cytopathologic diagnosis to justify a more intense medical evaluation for metastatic disease and to institute preoperative adjuvant therapy [6-8]. However, clinical breast examination alone, even in experienced hands, is not a reliable tool to diagnose breast cancer [9]. Therefore, in many developing countries, FNAC still serves as the only cost-effective alternative to open surgical biopsy with, or more often, without imaging study. Its use has been claimed to be efficient and yields a definitive diagnosis of breast lumps [10].

Like any other diagnostic test, FNAC possesses some intrinsic and extrinsic disadvantages. It has limitations that can lead to false-negative and false-positive results [11]. Its extensive use leads to highly inadequate result rates, equivocal results of limited clinical usefulness and an extensive list of pitfalls, together with diminishing use and an increasingly litigious culture broach issues about the safety and appropriate use of this technique [12]. The shortcomings of this technique have become increasingly apparent over the years to the extent that some surgeons are now questioning the continued role of FNAC in breast clinics [13]. Some researchers even suggested that it should be replaced with core needle biopsy, although FNAC may be more cost-effective for palpable lumps, when time and effort are taken into consideration [14]. Discontinued use of FNAC in the evaluation of breast lumps, either with or without imaging studies, is a critical decision as it may be the only efficient option for hospitals with restricted health resources.

The objective of this study was to reevaluate the additional diagnostic value of FNAC in patient profiles for the diagnosis of breast cancer among patients presenting with breast lumps.
Materials and Methods

We used routinely collected clinical information of patients registered with breast diseases at Nakornping Hospital and Lamphun Hospital. Ethical issues were approved by the hospital authorities. The data set was re-explored and the new study was designed as a phase III diagnostic research [15], using a nested case-control comparison in a defined clinical cohort [16]. We selected patients whose codes were assigned according to their clinical complaints notified as “breast lumps”, who presented at Nakornping Hospital, Chiang Mai, or at Lamphun Hospital, Lamphun, Thailand during 2006. These two hospitals are the training hospitals for medical students in conjunction with The Medical School of Chiang Mai University. The patient cohort was conceptualized from those who obtained a FNAC procedure, of which cytotologic diagnostic reports were also available. Manual searching was done to obtain the subsequent final histopathologic diagnosis, from which breast cancer patients were defined as “cases” and those with benign breast diseases as “controls”.

All key information was retrieved from the medical records and included patient age, affected breast side, location and size of breast lumps, FNA cytologic diagnosis and final histopathologic diagnosis.

The cytopathologic diagnosis was given by a group of pathologists of an adjunct university hospital located in the same area, following the Cytologic Category Code System [17, 18]. Code 1 refers to no cell or scanty cells on the smear (which refers to an inadequate or hypocellular smear in our study), code 2 for presence of a substantial number of benign cells on the smear, code 3 for mild atypia (atypical cells) that are inconclusive, code 4 for suspicious cells of malignancy and code 5 for malignancy. Although cytopathologic diagnosis was not blinded from the patient demographic profiles, pathologists did not use this information in their diagnosis.

The final outcome of each lump was obtained from either a frozen section available at the time of surgery, or an operative specimen available postoperatively. The final outcome was defined as either breast cancer or benign disease. The diagnosis based on the frozen section or operative specimen was given by the same group of pathologists, not using any information from the preceding FNAC diagnosis.

Patient age and size of breast lumps were compared between the two contrast groups by t-tests. The percentage distribution of lump side and quadrants were compared by chi-square tests or exact probability tests when appropriate.

FNAC classification code specific likelihood ratios were calculated with a 95% confidence interval (CI) using standard methods.

The diagnostic odds ratio (OR) and 95% CI were calculated to assess the magnitude of association between each predictor variable and the outcome variable (malignant breast lump), using standard calculations for case-control odds ratios.

The predictive contribution of age alone, size of breast lumps alone, age with size of breast lumps, FNAC alone, and FNAC with age and size of breast lump was presented with receiver operating characteristic (ROC) areas obtained after logistic model fittings [19] and were compared using log-likelihood ratio tests.

The predicted logistic probability function of breast cancer by FNAC was graphically displayed, conditionally on the combination of two age groups (over vs ≤ 40 years) and two lump size groups (over vs ≤ 2 cm).

Results

Patient ages ranged from 16 to 80 years old. Left- and right-sided breasts were affected with similar proportion, and both breasts were affected in almost 1%. Lesions palpable at the upper outer quadrant were reported to be more than 50%. The size of breast lumps varied from 0.5 to 15 cm. Patients with a final diagnosis of breast cancer were significantly older than those with benign diagnoses (51.1 ± 11.0 years vs 38.0 ± 11.2 years, p < 0.001). The average size of breast lumps among the former was also significantly larger (3.9 ± 2.6 cm vs 2.3 ± 1.5 cm, p < 0.001). The difference in breast quadrants was also observed, but there were no differences in breast side (Table 1).

Table 1. — General characteristics of the patients and breast lumps.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Postoperative histopathology</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs) [mean (SD)]</td>
<td>Malignant (%)</td>
<td>Benign (%)</td>
</tr>
<tr>
<td>Site of lumps</td>
<td>51.1 (11.0)</td>
<td>38.0 (11.2)</td>
</tr>
<tr>
<td>Right</td>
<td>104 (47.3)</td>
<td>73 (56.2)</td>
</tr>
<tr>
<td>Left</td>
<td>116 (52.7)</td>
<td>57 (43.8)</td>
</tr>
<tr>
<td>Quadrant</td>
<td>100 (51.3)</td>
<td>76 (59.7)</td>
</tr>
<tr>
<td>Upper outer</td>
<td>23 (11.8)</td>
<td>25 (19.7)</td>
</tr>
<tr>
<td>Upper inner</td>
<td>34 (17.4)</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>Lower outer</td>
<td>20 (10.3)</td>
<td>12 (9.5)</td>
</tr>
<tr>
<td>Lower inner</td>
<td>11 (5.6)</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>III stated</td>
<td>7 (3.6)</td>
<td>8 (6.3)</td>
</tr>
<tr>
<td>Size of lump (cm)</td>
<td>3.9 (2.6)</td>
<td>2.3 (1.5)</td>
</tr>
</tbody>
</table>

Numbers are either no. (%) or mean (SD) (not included missing information).

An inadequate or hypocellular smear (c1) was reported in 4.6% among breast cancers compared to 40.8% among benign breast lumps. The likelihood ratio for malignancy for c1 was 0.11 (95% CI, 0.06-0.21). The likelihood ratio increased from 0.24 for c2 to 0.78 for c3, to 9.45 for c4 and to 29.58 for c5 (Table 2).

Table 2. — Classification of fine needle aspiration cytology (FNAC) of malignant and benign breast lumps and the likelihood ratios of positive (LR+).

<table>
<thead>
<tr>
<th>FNAC classification</th>
<th>Postoperative histopathology</th>
<th>LR+ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant no. (%)</td>
<td>Benign no. (%)</td>
<td></td>
</tr>
<tr>
<td>c1 (Inadequate smear/ hypocellular)</td>
<td>10 (4.6)</td>
<td>53 (40.8)</td>
</tr>
<tr>
<td>c2 (Benign cells present)</td>
<td>23 (10.4)</td>
<td>57 (43.8)</td>
</tr>
<tr>
<td>c3 (Mild atypia, inconclusive)</td>
<td>21 (9.6)</td>
<td>16 (12.3)</td>
</tr>
<tr>
<td>c4 (Suspicious of malignancy)</td>
<td>16 (7.3)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>c5 (Malignant cells present)</td>
<td>150 (68.1)</td>
<td>3 (2.3)</td>
</tr>
</tbody>
</table>

Women over 40 years old were 8.05 times more likely to have breast cancer (95% CI, 4.20-15.42). Age alone could predict malignant breast lumps with a ROC area of 0.80 (95% CI, 0.75-0.85). Breast lumps over 2 cm were 5.33 times more likely to be cancerous (95% CI, 3.07-9.28). Size of breast lumps alone could also predict cancer with a ROC area of 0.72 (95% CI 0.67-0.77). Age
Empirical accuracy of fine needle aspiration cytology (FNAC) for preoperative diagnoses of malignant breast lumps in hospitals etc.

Together with size of breast lumps could predict cancer by a ROC area of 0.85 (95% CI 0.81-0.89). FNAC alone predicted cancer by 0.91 (95% CI 0.88-0.94), with the diagnostic OR increasing with an increase in FNAC classification codes. When FNAC was added in the logistic model on top of age and size of breast lumps, the ROC area increased from 0.85 to 0.95 (95% CI, 0.93-0.97). This increase in a ROC area was statistically significant by the log-likelihood ratio test (p < 0.001) (Table 3) (Figure 1).

The predicted probability of malignant breast lumps by FNAC was estimated by a logistic model conditionally on the combination of categorized age groups and sizes of breast lumps. The entire prediction curves shifted from right to left for older age and for larger size of breast lumps. The probability of a malignant breast lump for any given FNAC code was increased with an increase in age and size of breast lump (Figure 2).

### Discussion

Our study showed that the prediction of malignant breast lumps by FNAC alone was, according to the ROC area, as high as 91%. Studies have reported that FNAC is highly reliable in assessing clinically palpable mass lesions [20, 21]. It is difficult to directly compare the diagnostic accuracy of a test across studies due to differences in disease spectrum, disease gradient or the case mix, the cut-off point selected in each individual study and exclusion of inadequate results. For breast cancer diagnostic research, some authors excluded c1 (inadequate or hypocellular smear) and c3 (mild atypia that are inconclusive) from analysis, resulting in an arbitrarily high sensitivity and specificity. Our study used all FNAC classification codes in interpretation.

The probability of malignancy was significantly high for c5 in our study. An unequivocal cytologic diagnosis of malignancy (c5) is a reliable diagnosis [22]. When FNAC reported suspicious for malignancy (c4), it was recommended that further evaluation was needed, as lesions often prove to be malignant [21]. Our study also showed that a c4 lesion was almost nine times more likely to be malignant, while mild atypia (c3) was equivocal. Benign FNAC (c2) was associated significantly with a low probability of malignancy. There was also a report that FNAC was sensitive for the diagnosis of benign breast lumps [23]. For an inadequate or hypocellular smear (c1), few studies mentioned its interpretation or usefulness. Our study showed that the probability of malignancy of c1 was significantly low, even lower than c2. An inadequate or hypocellular specimen was associated with benign disease in some studies [6, 7]. Nevertheless, some authors concluded that the diagnosis of inadequate or hypocellular smears should be further investigated, also of atypia (c3) because the probability of malignancy is still high [24].

When FNAC was added to the patient age and the size

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**Table 3.** Predictive ability of age and lump size, fine needle aspiration cytology (FNAC) alone, and FNAC with age and lump size from the logistic models; the diagnostic odds ratios (dOR) and area under receiver operating characteristics (ROC) curves.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>dOR 95% CI of dOR</th>
<th>p value</th>
<th>ROC Area (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No more than 40</td>
<td>1.00</td>
<td></td>
<td>0.80 (0.75-0.85)</td>
</tr>
<tr>
<td>Over 40</td>
<td>8.05 (4.20-15.42)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Size (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No greater than 2</td>
<td>1.00</td>
<td></td>
<td>0.72 (0.67-0.77)</td>
</tr>
<tr>
<td>Over 2</td>
<td>5.33 (3.07-9.28)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Age &amp; size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FNAC alone</td>
<td>0.85 (0.81-0.89)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>c1</td>
<td>1.00</td>
<td></td>
<td>0.91 (0.88-0.94)</td>
</tr>
<tr>
<td>c2</td>
<td>2.14 (0.93-4.91)</td>
<td>0.073</td>
<td></td>
</tr>
<tr>
<td>c3</td>
<td>6.96 (2.72-17.77)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>c4</td>
<td>84.80 (10.07-173.76</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>c5</td>
<td>265.00 (70.25-999.65</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>FNAC with age &amp; size</td>
<td>0.95 (0.93-0.97)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 1.** — Comparative area under receiver operating characteristic (ROC) curves of age and lump size, fine needle aspiration cytology (FNAC) alone, and FNAC with age and lump size.

**Fig. 2.** — Probability of lumps being malignant in the four combinations of age (older vs ≤ 40 years) and lumps size (larger vs ≤ 2 cm), as predicted by FNAC.
of breast lumps in a logistic model, the prediction increased from 0.91 to 0.95, which was statistically significant (Table 3 and Figure 1). This implies that FNAC has a significant contribution in the discrimination of malignancy from benign breast lesions beyond the already known patient profiles (age and size of breast lumps). Most studies assessed the value of FNAC without considering other potential predicting characteristics [6-10, 20, 21, 23, 25-28]. Only a few studies used patient profiles as combined predictors [2, 3, 29]. Our study is among the latter.

Clinical breast examination alone, even for an experienced clinician, is not a reliable tool for diagnosis of a palpable breast lesion to determine whether it is malignant or not [9]. If the patient age and size of breast lumps were considered in combination with FNAC, the interpretation and the prediction for malignancy could be improved, especially for c3, where the probability of malignancy increased from below 0.25 in patients 40 years or younger and lumps 2 cm or smaller, to above 0.75 in older patients and larger lumps (Figure 2). It should be noted that age seemed to play a more significant role in the changes of predictive probability than size of breast lumps.

We suggest that a FNAC that is positive for cancer (c5) eliminates the need for open biopsy and allows the surgeon to proceed with mastectomy with confidence [30]. Negative cases required repeat FNAC, core needle biopsy or excisional biopsy [25] although with economic restrictions, repeat FNAC may be a more cost-effective option [26].

False-negatives and false-positives (15.1% and 15.4% in our study) can be minimized by applying the triple test strategy [31]. If not applicable, these false-positive and false-negative cases are likely to be picked up at intraoperative frozen section and lead to no over or under treatment [10].

The advantages of FNAC are that it can replace open biopsy in a majority of clinically malignant cases and can help to reassure and relieve patient anxiety [27]. FNAC also plays an important and essential role in the management of patients with breast lesions and also offers a great potential for prediction of patient outcome, disease response to therapy and assessment of risk of developing breast cancer [32]. It may assist clinical decision-making as far as whether patients should progress to surgical management or should have further core biopsy before planning surgery [28]. In health resource restricted hospitals, FNAC should be used as a first-line diagnostic procedure in the evaluation of patients with palpable breast lesions [7, 26].

Before considering whether to abandon the use of FNAC in breast assessment, each individual unit should make a decision based on their own audited results. If proved to be reliable, its advantages in speed, ease and low cost would serve to support its continued use, especially in hospitals with restricted health resources.

Conclusion

Fine needle aspiration cytology performed as a routine investigation of patients with breast lumps has a significant additional predictive contribution to the diagnosis of breast cancer beyond patient age and size of breast lumps. It should be kept as a first-line diagnostic procedure to manage patients presenting with breast lumps in hospitals with restricted health resources, even without the aids of imaging diagnosis. Its continued use is therefore recommended, with the understanding that surgeons interpret FNAC diagnosis conditionally on individual patient age and size of the breast lump.

Acknowledgement

The authors wish to thank the staff of the two hospitals for their contribution, and The Research Network of the Northern Region Hospitals, Thailand, for their support.

References

Empirical accuracy of fine needle aspiration cytology (FNAC) for preoperative diagnoses of malignant breast lumps in hospitals etc.


Address reprint requests to:
J. PATUMANOND, M.D., MPH., DSc.
Division of Clinical Epidemiology and Medical Statistics
Faculty of Medicine, Chiang Mai University
Chiang Mai, 50200 (Thailand)
e-mail: jpatumanond@yahoo.com
Incidence of port-site metastasis after laparoscopic management of borderline ovarian tumors: a series of 22 patients

R. Berretta, M.D.; M. Rolla, M.D.; T.S. Patrelli, M.D.; D. Gramellini, M.D.;
G.M. Fadda, M.D.; G.B. Nardelli, M.D.

Department of Gynecology, Obstetrics and Neonatology, University of Parma, Parma (Italy)

Summary

Purpose: The aim of this work was to evaluate the incidence of port-site metastasis in patients undergoing laparoscopy for borderline ovarian carcinoma (BOT).

Methods: Twenty-two patients who underwent laparoscopy from 2004 to 2008 for BOT were evaluated retrospectively.

Results: In 15 patients an ultraconservative procedure with enucleation of the annexal neoplasia was carried out, while in five (23%) unilateral salpingo-oophorectomy was performed and in two cases (9%) bilateral salpingo-oophorectomy was done. Conclusion: The literature data report few cases of port-site metastasis in BOT patients. Residual cutaneous metastases have been reported to occur within 12 months from the first surgery, generally in association with serous histology. In our analysis, we found 17 out of 22 cases of serous BOT, three mucinous and two endometrioid. In no case was cutaneous metastasis revealed after an average of 30 months of follow-up.

Key words: Borderline ovarian tumors; Laparoscopy; Port-site metastasis.

Introduction

In the group of ovarian tumors, borderline ovarian tumors (BOT) stand out as a separate entity. BOTs are epithelial ovarian tumors characterized by a degree of cellular proliferation and nuclear atypia in the absence of infiltrative destructive growth or obvious stromal invasion. Their two major characteristics are that they occur in patients younger than those with epithelial ovarian cancer and have a better prognosis than the latter. The overall survival rate at five years is approximately 98% for early-stage BOT and varies between 86% and 92% for more advanced stages of the disease.

Histologically, 50% of BOTs are serous, 46% mucinous, and the remaining 4% endometrioid, clear cell or Brenner. Serous BOTs are unilateral in 70% of cases and in up to 37% of patients they may be associated with invasive or non-invasive implantations [1]. Mucinous BOTs are classified as intestinal (85%) or endocervical/Müllerian type (15%), depending on the nature of the epithelial lining.

The standard treatment for BOT is total abdominal hysterectomy, bilateral salpingo-oophorectomy, peritoneal cytology, omentectomy, and multiple peritoneal biopsies, with pelvic and lumbar-aortic lymphadenectomy in selected cases. Although conservative management in BOT patients remains a major concern, it does appear that conservative surgery can be performed safely in young patients with careful follow-up.

Lately, the use of laparoscopy and conservative surgery, which is defined as surgery with complete staging but with preservation of the uterus and at least part of one ovary, has been gaining popularity. Indications for laparoscopic surgery have extended to various cancers, including gynecologic malignancies [2, 3]. Several authors have reported the occurrence of cancerous port-site metastasis and/or intraperitoneal dissemination after laparoscopic surgery [4, 5]. Implantation of ovarian tumors at port sites has also been well documented in borderline malignant or invasive epithelial ovarian neoplasms after laparoscopy [5]. However, the actual incidence of port-site metastasis is unclear due to the lack of long-term follow-up results, and its clinical significance remains controversial.

The purpose of this paper was to evaluate the incidence of port-site metastasis following laparoscopic surgery in BOT patients.

Materials and Methods

The study was conducted retrospectively on 22 patients with a histological diagnosis of Stage I BOT who underwent laparoscopic surgery between 2004 and 2008.

Preoperatively, all patients submitted to a bimanual pelvic examination, a transvaginal color Doppler ultrasound examination, and – if their tumors matched the International Ovarian Tumor Analysis (IOTA) Group classification for high-risk complex neoplasms – to a pelvic-abdominal CT-scan. Standard blood and tumor marker (CA125 and CA19.9) tests were also performed routinely.

All laparoscopic surgical procedures were carried out under general anesthesia. Patients were usually placed in the dor-solithotomy position with the legs in the universal Allen stirrups. After induction of carbon-dioxide pneumoperitoneum with a Veress needle at the level of the umbilicus, a 10 mm trocar incorporating a zero-degree laparoscope was inserted through an umbilical vertical incision. Entrance into the abdominal cavity was made under direct visualization with an optical view device. To facilitate the surgical manoeuvres, in all cases three ancillary access routes were used (with two 5 mm trocars and one 10 mm trocar, respectively), as well as one suprapubic and two lateral. Endoabdominal pressure never exceeded 12 mmHg throughout surgery.

At the beginning of the laparoscopic procedure, an exploration was made of the pelvic-abdominal cavity, the parietocolic areas, and the hemidiaphragms. A peritoneal cytologic test was then performed, which was followed by enucleation of the cystic mass or unilateral salpingo-oophorectomy in accordance
Results

Twenty-two patients were eligible for the study. Mean age at laparoscopic surgery was 35 years (confidence interval: 16-65). The mean CA125 value was 12 (range, 5-34) (Table 1).

In 100% of cases, laparoscopy could be approached without having to resort to laparotomy. The mean duration of surgery was 53 minutes (range, 35-85) and mean blood loss was 100 cc (range, 50-300). The study population was homogeneous for age, parity, CA125 value at diagnosis, size and surgical stage of the tumor. No patient had any intra- or postoperative complications. Mean hospital stay was two days (range, 1-3).

In 15 patients (68%) it was possible to perform an ultraconservative surgical procedure with enucleation of the adnexal neoplasm and a spillage rate of 53% (8/15). Five patients (23%) underwent unilateral salpingo-oophorectomy and only two (9%) had bilateral salpingo-oophorectomy (both were postmenopausal women) (Table 2). In these last two patients, the preoperative endometrial biopsy showed no evidence of neoplasms.

Among the patients undergoing unilateral salpingo-oophorectomy, 60% (3/5) had a serous BOT and 40% (2/5) had a mucinous BOT. Both patients with bilateral salpingo-oophorectomy had a serous BOT.

Among the 15 patients treated by ultraconservative surgery with cyst enucleation, the BOT was serous in 80% (12/15), mucinous in one woman, and endometrioid in the remaining 13% (2/15). In these last cases, the BOT was associated with pelvic/ovarian endometriosis.

Surgical staging in selected cases never showed any evidence of extraovarian disease.

Based on the International Federation of Gynecology and Obstetrics (FIGO) staging system, 100% of cases had a Stage I tumor – namely, Stage IA in eight cases, IB in ten and IC in four. According to the FIGO grading system, 17 of our patients had a G1 tumor, five a G2 and none a G3. All patients were followed up for at least 24 months (range, 24-60). No patient showed any evidence of scar metastasis at the trocar access site.

One of our patients, a 27-year-old woman, had already undergone surgery eight months before the laparoscopic procedure for a left-side serous BOT. This time she had a complex tumor in the right ovary, which after laparoscopic removal also proved to be a serous BOT. The biopsy carried out on the right ovary during the previous surgery had tested negative.

Currently, none of our patients is showing any signs of either abdominopelvic or scar metastasis.

Discussion

BOTs, or low malignant potential tumors (LMPT), account for 10-15% of all ovarian tumors. Since their original description in 1929, our knowledge of their natural history has greatly advanced over the years. As a consequence, management has changed from radical surgery to a more conservative therapy. In the last two decades, laparoscopy has become a good alternative to laparotomy. Laparoscopic surgery has considerably changed the approach to ovarian masses and now laparoscopic management is considered to be safe and adequate even in early invasive ovarian cancer [7, 8].

The estimated incidence of port-site metastasis in patients with laparoscopic management of gynecologic diseases is between 1% and 2% [9]. A number of mechanisms have been discussed in the literature to explain the occurrence of port-site metastasis: the effect of pneumoperitoneum (related to the pressure and/or use of carbon dioxide), exfoliation or spillage of tumor cells along the trocar (caused by gas leakage), inoculation of the trocar site through contact of the laparoscopic instru-

with the recommendations for laparoscopic treatment of benign adnexal masses [6] depending on the case. Tissue was removed from the abdominal cavity using a plastic bag to reduce the possibility of parietal implantation of neoplastic cells. An intraoperative histologic examination of frozen section preparations was performed in all patients. Staging was then carried out through multiple peritoneal/omental biopsies, appendectomy – if not already performed – in patients with mucinous tumors, and biopsy of the contralateral ovary but only in cases of macroscopically suspect ovaries. In no case was peritoneal drainage used; only the 10 mmHg laparoscopic accesses required suture of the abdominal fascia with an Endo Close instrument. All histologic examinations were performed by the same operator specially trained in the management of gynecologic diseases.

All patients were placed on a standard follow-up scheme at the Gynecologic Oncology Center of the University of Parma Obstetrics and Gynecology Clinic. The follow-up consisted of a general examination to be taken every four months including a gynecologic visit, a transvaginal ultrasound examination and biochemical marker tests, as well as an abdominal ultrasound examination and a Pap smear test to be performed once a year.

The average follow-up period was 30 months (range, 60-24).

Table 1. — Patient characteristics.

<table>
<thead>
<tr>
<th>Features</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (16-65 yrs)</td>
<td>35 yrs</td>
</tr>
<tr>
<td>Operative time (35-85 min)</td>
<td>53 min</td>
</tr>
<tr>
<td>Intraoperative blood loss (10-300 cc)</td>
<td>100</td>
</tr>
<tr>
<td>Hospital stay</td>
<td>2</td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
</tr>
<tr>
<td>– serous</td>
<td>16</td>
</tr>
<tr>
<td>– mucinous</td>
<td>1</td>
</tr>
<tr>
<td>– endometrioid</td>
<td>2</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>Ia</td>
<td>8</td>
</tr>
<tr>
<td>Ib</td>
<td>10</td>
</tr>
<tr>
<td>Ic</td>
<td>4</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>17</td>
</tr>
<tr>
<td>G2</td>
<td>5</td>
</tr>
<tr>
<td>G3</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. — Characteristics of surgery.

<table>
<thead>
<tr>
<th>Surgical approach</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laparotomy</td>
<td>0</td>
</tr>
<tr>
<td>Laparoscopy:</td>
<td></td>
</tr>
<tr>
<td>– Cystectomy</td>
<td>15</td>
</tr>
<tr>
<td>– Salpingo-oophorectomy</td>
<td>5</td>
</tr>
<tr>
<td>– Bilateral salpingo-oophorectomy</td>
<td>2</td>
</tr>
</tbody>
</table>
ments with the tumor, contamination of the port sites as resected specimens are extracted through an excessively small incision, and modification of local immune reactions related to the use of a laparoscopic procedure [10].

In the literature there are only a few sporadic reports of port-site metastasis and most of them have been published as case reports of BOT patients. In our review of the literature, we found only ten cases. In these cases, all the patients who had undergone either ultraconservative (cystectomy) or conservative (unilateral salpingooophorectomy) surgery had a serous BOT (Stage I in some, with peritoneal implants in others) (Table 3).

Table 3. — Scientific literature.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Total port site</th>
<th>Histotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morice et al. 2004</td>
<td>3</td>
<td>serous</td>
</tr>
<tr>
<td>Hsiu et al. 1986</td>
<td>2</td>
<td>serous</td>
</tr>
<tr>
<td>Gleeson et al. 1993</td>
<td>3</td>
<td>serous</td>
</tr>
<tr>
<td>Crouet et al. 1991</td>
<td>1</td>
<td>serous</td>
</tr>
<tr>
<td>Shepherd et al. 1994</td>
<td>1</td>
<td>serous</td>
</tr>
</tbody>
</table>

In two cases reported by Hsiu et al. [11] and also in one case reported by Morice et al. [14] port-site metastasis occurred after a simple laparoscopic biopsy, while in the remaining cases it followed salpingooophorectomy or cystectomy. It is worth noting that in the two cases reported by Hsiu et al. [11], in one case reported by Crouet and Heron [12], and in one case reported by Gleeson et al. [9], port-site implantation was associated with peritoneal implants. These peritoneal implants were not detected during the initial laparoscopic procedure in the two cases reported by Hsiu et al. [11]. In two of the cases reported by Morice et al. [14] and in the case reported by Shepherd et al. [13], there was no peritoneal disease at the time of completion surgery.

None of the patients with scar metastasis died as a result of disease progression. The prognosis in patients with a port-site implantation is excellent, as in the case of BOT and non-invasive peritoneal implants, which also have an excellent prognosis [14].

The literature data indicate the appearance of scar metastasis generally within 12 months of the first surgical procedure, especially with serous BOTs. In our case series of 22 patients, 17 were serous BOTs, three mucinous and two endometrioid. At an average 30 months’ follow-up we did not observe any case of scar metastasis. A possible explanation for our results lies in the strategies adopted. In our department, we use laparoscopic surgery only at the initial stages, keep endoabdominal pressures below 12 mmHg, totally remove the cystic mass when possible using an endobag, avoid contact between the trocar and the abdominal viscera, avoid manipulation of abdominal organs with the instruments used to remove the tumor, irrigate instruments and ports before removal, irrigate and suction the abdomen, use a 0.25% to 1% povidone-iodine solution to irritate wounds, and remove trocars before desufflation. In our opinion, systematic removal of port-site implantations should not be performed in patients with no clinical abnormalities.

Although the literature data are scarce, port-site implantation does not appear to occur more frequently after ultraconservative surgery than it does after more radical surgery. Nor is it more frequent in the presence of tumors with peritoneal implants than it is with Stage I tumors because cases of port-site metastasis have been reported in both instances.

Our data confirm that ultraconservative surgery is a safe procedure for preventing both abdominopelvic and scar metastasis.

Thus, laparoscopic surgery in BOT patients should be considered as safe and adequate. Even in patients who develop port-site metastasis, the prognosis after local surgical resection of the port-site implantation remains excellent and is comparable with those of patients without metastasis.

References


Address reprint requests to:
R. BERRÈTTA, M.D.
Department of Obstetrics and Gynaecology
University of Parma
Via A. Gramsci, 14 - 43100 Parma (Italy)
e-mail: rberrett@tin.it
The role of telomerase activity in predicting early recurrence of epithelial ovarian cancer after first-line chemotherapy: a prospective clinical study

B. Özmen, C. İ. Duvan, G. Gümüş, M. Sönmezer, M. Gungor, F. Ortaç

1Center of Reproductive Health and Artificial Reproductive Techniques, University of Ankara School of Medicine
2Department of Obstetrics and Gynecology, University of Ankara School of Medicine
3Department of Obstetrics and Gynecology, University of Fatih School of Medicine
4Department of Medical Biology and Genetics, University of Ankara School of Medicine, Ankara (Turkey)

Summary

Purpose of Investigation: To investigate the value of telomerase activity (TA) in the detection of early recurrence in primary epithelial ovarian cancer (EOC). Method(s): In this study, TA was studied in 30 patients with Stage III EOC and 50 control patients with benign gynecological disease. All enrolled EOC patients had had primary cytoreductive surgery and six cycles of platinum-based first-line chemotherapy previously. Semi-quantitative TA measurements were done by TRAP assay in ascites, taken at second-look surgery, of cancer patients and in peritoneal washings, taken during planned surgery, of the control group. Result(s): Early recurrence was diagnosed in ten EOC patients (33.3%). Mean TA was statistically higher in EOC patients than in patients with benign disease. However, the mean TA was insignificantly lower in early recurrent EOC patients than in disease-free EOC patients. Conclusion(s): The value of TA is limited in the detection of early recurrence in primary EOC.

Key words: Primary epithelial ovarian cancer; First-line chemotherapy; Telomerase activity; Recurrence; TRAP assay.

Introduction

Epithelial ovarian cancer (EOC) constitutes half of the deaths from gynecological malignancies and is mainly characterized by late onset with insidious symptoms and early recurrences. Even though there has been extensive research on the pathophysiology and growth regulation of EOC, there is no current test to predict with high accuracy which patients will develop early recurrences [1, 2]. The frequently used tests such as serum CA 125 level, transvaginal ultrasonography (US) and computerized tomography (CT) commonly fail in the early detection of recurrences, those common after first-line chemotherapy.

Telomerase, a ribonucleoprotein enzyme, is responsible for elongation of telomeric repeats (TTAGGG), those located at the ends of chromosomes and involved in replication [3]. Mainly telomerase prevents chromosomal instability and cell death by maintaining the length of telomeres [3]. Therefore it is suggested to be related with cellular immortalization that allows human cancers to progress indefinitely [4, 5]. In many human primary cancers (85%) increased expression and upregulation of telomerase activity (TA) have been reported whereas down regulation or absence have also been demonstrated in normal somatic cells [5-12]. Increased TA has also been demonstrated to be linked with poor prognosis [6, 7, 13-15]. Thus it emerges as an attractive target for both cancer diagnosis and treatment.

In ovarian tumors TA has been widely investigated among malignant [9-12, 16], borderline [11, 12], and benign lesions [11, 12]. As expected, studies showed that TA is up-regulated or re-activated in nearly 73-88% of ovarian cancer cases [9-12]. Furthermore telomeric repeats were also shown to be shorter in 60% ovarian cancer cases than in benign ovarian cysts [17].

As well the sensitivity and specificity of TA in ascites, pleural or pericardial fluid were suggested to be superior to traditional cytology in detecting malignant conditions [18, 19]. Particularly TA could detect a malignant cell among 100 inflammatory cells; those common causes of misdiagnoses. Therefore a combination of TA and traditional cytology might increase sensitivity and decrease false-negative rates resulting in early detection of recurrences in EOC [19]. In this study we aimed to investigate the role of TA in detecting early recurrences of EOC after first-line chemotherapy.

Material and Methods

Thirty patients with primary EOC who are scheduled for second-look surgery (SLS) and 50 patients with benign gynecological lesions who were planned to undergo surgery were enrolled in this prospective study. Patients with benign disease were taken as a control group to compare semi-quantitative values of TA between cancer and benign ovarian diseases. All EOC patients had previously undergone optimal primary debulking surgery and six cycles of platinum-based chemotherapy. As well all of them proved to be disease-free before SLS by clinical examination, assessment of serum CA 125 (reference range < 35 IU/ml), and imaging methods including abdominopelvic CT and/or US.

The study was approved by the Ethical Committee of the Ankara University (approval no. 15-2002/259), and all of the patients signed informed consents before enrollment. SLS was...
performed to determine the efficacy of chemotherapy and to perform secondary cytoreductive surgery when macroscopic tumor was detected. In EOC patients the SLS procedure was done according to previous descriptions via laparotomy or laparoscopy [20]. TA was studied in ascites in EOC patients and peritoneal washings with saline solution in control subjects and in EOC patients when there was not ascites. A 100 ml sampling fluid, either ascites or peritoneal washing, was taken and divided equally for both cytological examination and assay of TA.

**TRAP assay**

TA was examined in cells obtained from 50 ml of ascites or peritoneal washings. Both samples were taken immediately at the initiation of the surgery. Samples were transferred into 50-ml of tubes and then centrifuged at 900 rpm, + 4ºC for 10 min. Thereafter 50 ml of erythrocyte lysis buffer containing 155 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA was added to the pellet by vortexing. Following the storage of tubes for 5 min at room temperature, samples were centrifuged at 900 rpm, + 4ºC for 10 min. Then pellets were homogenized with 1 ml PBS and transferred to sterile microtubes. Cells were counted with Thoma slide and cell pellets were stored at -80ºC until TRAP assay was performed, and TA was measured using TRAPEze kit (Intergen, catalog # S7700, CA, USA) according to the manufacturer’s instructions.

**Imaging and semi-quantization:**

For visualizing PCR products 1.5-hour electrophoresis was performed at 12.5% polyacrylamide gel with 400 V and painted for 10 min. Thereafter 50 ml of SYBR Green dye to achieve ultraviolet (UV) examination. UV images of all samples were transferred to digital status with the Diana programme (imaging system) for quantization of the telomerase activity. Signals of the region of the gel lane corresponding to the TRAP product ladder bands from all samples including non-heat-treated (x) and heat-treated sample extracts (xo), 1xCHAPS lysis buffer control (primer-dimer/PCR contamination control) (ro), and TSR8 quantization control (r) and signals from the internal standard in non-heat-treated samples (c) and the internal standard in TSR8 quantization control (cr) were measured with the Aida computer programme. Telomerase activity was calculated as the total product generated (TPG) unit with the specific formula given in Figure 1.

Each unit of TPG corresponds to the number of TS primers in examination. After semi-quantization, TA levels were classified as low (0-50 units/TPG), moderate (> 50-100 units/TPG) and high (> 100 units/TPG) TA levels to detect the major differences between control and EOC groups.

**Statistical analysis**

All available data were transferred to SPSS (SPSS for Windows, Chicago, USA), and the Mann-Whitney, Kruskal-Wallis and multiple comparison tests were used for statistical analysis [21]. The criterion for statistical significance was set at p < 0.05.

**Results**

Demographic characteristics including age, gravida, parity, and body mass index (BMI) were similar between the cancer patients and control group (Table 1). As expected the mean age was found to be higher in ovarian cancer patients than in patients with benign gynecological disease. Surgical indications in the control group were also determined (Table 1). In the control group nearly two-thirds of patients underwent endoscopic surgery, whereas only 20% of cancer patients were operated via endoscopy (Table 1). However there was not any difference in mean TA of patients who underwent endoscopic and laparotomic surgery (26.21 ± 27.65 versus 29.49 ± 39.16, respectively) suggesting that no difference is expected in mean TA according to operation type. Mean TA in patients with ovarian cancer was significantly higher than in patients with benign gynecological lesions (Table 1). Particularly almost all of the patients with benign disease had lower TA levels in unit/TPG (0-50 unit/TPG) whereas 76.6% of EOC patients had moderate

<table>
<thead>
<tr>
<th>Table 1. — Demographic characteristics and telomerase activity in patients with benign gynecological lesions and ovarian cancer.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group</strong></td>
</tr>
<tr>
<td>Age (years, mean ± SD)*</td>
</tr>
<tr>
<td>Gravida (mean ± SD)</td>
</tr>
<tr>
<td>Parity (mean ± SD)</td>
</tr>
<tr>
<td>Body Mass Index (mean ± SD)</td>
</tr>
<tr>
<td>Endoscopic surgery (%)*</td>
</tr>
<tr>
<td>Second look telomerase activity†</td>
</tr>
<tr>
<td>(Mean ± SD)*</td>
</tr>
</tbody>
</table>

**Telomerase Activity Levels (%)**

| Lower (0-50 unit/TPG)* | 96 | 10 | 0.002 |
| Moderate (> 50-100 unit/TPG)* | 4 | 76.6 | 0.001 |
| Higher (> 100 unit/TPG) | 0 | 13.3 | – |

| Diagnosis † | |
| Serous carcinoma | NA |
| Mucinous carcinoma | 28 |
| Uterine fibroid/s | 2 |
| Tubal ligation | – |
| Serous cystadenoma | 8 |
| Dermoid cyst | – |
| Endometriosis | 4 |
| Primary infertility | 4 |
| Atypical endometrial hyperplasia | 2 |

†Telomerase activity was given as units/TPG. *Statistics are not applicable. NS; not significant. NA; not applicable. *A p value < 0.05 was considered statistically significant.
The role of telomerase activity in predicting early recurrence of epithelial ovarian cancer after first-line chemotherapy: etc.

There was not any relation between date of SLS or surgery and TA levels in the EOC and control group suggesting that storing samples, after centrifugation and separation of cell pellets with storage at -80ºC until TRAP assay was performed, did not contribute to the results of TA (r = –0.273, p > 0.05).

Ovarian cancer group

All patients had Stage III disease consisting mainly of serous histological subtype (Table 1). Findings of SLS of EOC patients are shown in Table 2; 79% of patients with positive SLS (n = 11) had microscopic disease and the remaining 21% (n = 3) had macroscopic disease. Histological examination confirmed SLS findings for persistent disease in 85% (n = 12) of patients with positive SLS. Conversely cytology was positive only in 43% of patients with positive SLS and had a false-positive rate of 14.28% (Table 2).

The mean TA was not statistically different among patients with positive SLS and with negative SLS (57.6 ± 32.5 unit/TPG (range 8.43-212.14) versus 59.4 ± 69.1 unit/TPG (range 11.57-211.37), respectively), and mainly had moderate values (Figure 3). However the mean TA levels in patients with macroscopic tumor at SLS was high when compared with the mean TA of patients with microscopic disease at SLS (156 ± 82.5 unit/TPG (range 67.42-212.14) versus 65.2 ± 54.7 unit/TPG (range 8.43-113.51), respectively. Nevertheless subject number with macroscopic disease is not enough to form any clear conclusions. There was also not any difference observed in the mean TA of patients with positive cytology (n = 7) and patients with negative cytology (n = 23) at SLS 67 ± 42 unit/TPG (range 53.42-113.51) versus 59 ± 66.3 unit/TPG (range 8.43-212.14).

Recurrent ovarian cancer patients

All patients with macroscopic disease diagnosed by SLS directly underwent secondary cytoreductive surgery rendering them as having residual disease < 0.5 cm. Patients with positive SLS received either six cycles of platinum-based salvage therapy with paclitaxel, or four cycles of topotecan. Conversely the patients with negative SLS (n = 16) received platinum-based consolidation chemotherapy for three cycles. All patients in the EOC group were followed-up at least 12 months by 3-monthly intervals. Mean serum CA 125 levels before and 12 months after primary debulking were 10.7 ± 14.7 IU/ml

Table 2. — Demographic characteristic, second-look findings, and telomerase activity between patients with and without recurrence.

<table>
<thead>
<tr>
<th>Age (mean ± SD)</th>
<th>Recurrence (–)</th>
<th>Recurrence (+)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>46.4 ± 10.4</td>
<td>48.2 ± 8.3</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Gravida (mean ± SD)</td>
<td>2.5 ± 1.98</td>
<td>2.6 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (mean ± SD)</td>
<td>28 ± 3.408</td>
<td>27 ± 4.59</td>
<td>NS</td>
</tr>
<tr>
<td>Second-look surgery (n)</td>
<td>20 ± 10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Second-look cytology</td>
<td>20 ± 10</td>
<td>10</td>
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</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CA 125 at second look (Mean ± SD)*</td>
<td>21.7 ± 5.8</td>
<td>68 ± 54.4</td>
<td>NS</td>
</tr>
<tr>
<td>Second look telomerase activity (Mean ± SD)†</td>
<td>75.46 ± 72.25</td>
<td>4.02 ± 21.71</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 3. — Clinical characteristics and laboratory results of the patients with recurrent epithelial ovarian cancer.

<table>
<thead>
<tr>
<th>Case Age</th>
<th>History</th>
<th>Stage</th>
<th>SLS</th>
<th>Pathology at SLS</th>
<th>CytoLOGY at SLS</th>
<th>Telomerase activity* at recurrence†</th>
<th>CA125 at recurrence</th>
<th>Imaging Studies (CT)</th>
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<td>8.43</td>
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Microscopic disease at SLS (156 ± 82.5 unit/TPG (range 67.42-212.14) versus 65.2 ± 54.7 unit/TPG (range 8.43-113.51), respectively. Nevertheless subject number with macroscopic disease is not enough to form any clear conclusions. There was also not any difference observed in the mean TA of patients with positive cytology (n = 7) and patients with negative cytology (n = 23) at SLS 67 ± 42 unit/TPG (range 53.42-113.51) versus 59 ± 66.3 unit/TPG (range 8.43-212.14).

Recurrent ovarian cancer patients

All patients with macroscopic disease diagnosed by SLS directly underwent secondary cytoreductive surgery rendering them as having residual disease < 0.5 cm. Patients with positive SLS received either six cycles of platinum-based salvage therapy with paclitaxel, or four cycles of topotecan. Conversely the patients with negative SLS (n = 16) received platinum-based consolidation chemotherapy for three cycles. All patients in the EOC group were followed-up at least 12 months by 3-monthly intervals. Mean serum CA 125 levels before and 12 months after primary debulking were 10.7 ± 14.7 IU/ml

Lower | Moderate | High
---|----------|------|
Control | 48 | 2 | 0 | 50 |
EOC | 3 | 23 | 4 | 30 |
| 51 | 25 | 4 | Total 80 |

Table 3. — Clinical characteristics and laboratory results of the patients with recurrent epithelial ovarian cancer.

Case Age | History | Stage | SLS | Pathology at SLS | CytoLOGY at SLS | Telomerase activity* at recurrence† | CA125 at recurrence | Imaging Studies (CT) |
<table>
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Recurrence was diagnosed by clinical examination, imaging studies and measurement of plasma CA 125 level (Table 3). An elevation of > 50% in the plasma CA 125 level, a plateauing CA 125 level, or a suspicious mass previously not depicted on imaging studies were the criteria to diagnose a recurrence [21]. The outcome of the TRAP assay was defined as low (0-50), moderate (> 50-100), and high (> 100) in unit/TPG (total product generated).

Recurrences were diagnosed either by clinical examination, imaging studies and/or by measurement of serum CA 125 level (Table 3). An elevation of > 50% of the serum CA 125 level, a plateauing CA 125 level, or a suspicious mass previously not depicted on imaging studies were the criteria to diagnose recurrence [20]. At the end of the study, ten patients (33%) were diagnosed with recurrence of EOC (Table 3). The mean follow-up was 12.5 ± 2.8 months from the SLS, and the mean interval for detection of a recurrence from the primary surgery was 13.2 ± 2.94 months with the earliest of nine months. Of these patients eight (80%) had positive and two (20%) had negative SLS. Furthermore only four had positive (40%) cytology at SLS demonstrating the low sensitivity and specificity of traditional cytology.

Notably, patients with recurrence had a statistically insignificant lower mean TA compared to those without recurrence (Table 2). Moreover none of the patients in the recurrent EOC group had high TA levels, whereas three had low TA levels and the remaining had moderate TA levels at SLS (Figure 4). On the contrary, among disease-free patients none had low TA levels, whereas four had high and the remaining had moderate TA levels (Figure 4). Unsurprisingly the mean CA 125 serum levels at SLS were insignificantly higher in recurrent EOC patients than disease-free patients (Table 2).

Thus TA alone or along with cytology at SLS did not improve sensitivity in predicting the subgroup of patients with early recurrences. In addition, neither high TA values nor moderate or low TA levels after primary chemotherapy indicated the high risk of early recurrence. Also there was no correlation found between the type of received adjuvant chemotherapy regimen and TA (data not shown).

Discussion

The current diagnostic management of EOC patients, including repeated serum CA 125 levels and further imaging studies, lacks sensitivity for the early detection of recurrences and persistent disease of EOC [20, 22]. In a study, nearly 60% of the patients with normal serum CA 125 levels before SLS had persistent disease confirmed by SLS [20]. Furthermore it has been demonstrated that patients with < 2 cm residual mass rarely have abnormal CA 125 levels [20]. Particularly results obtained by sophisticated imaging techniques such as positron emulsion CT are also limited indicating 10% sensitivity and 42% specificity in detecting minimal volume persistent EOC [22]. Practically 30-50% of the patients with negative SLS present with recurrences mainly within one to three years. Thus, there has been an increasing struggle to discover novel substances that can be used as a screening marker to predict the subgroup of patients more likely to develop early recurrences among these EOC patients. As well, new markers are also precisely needed to determine the efficiency of first-line chemotherapy which is also a high risk for early recurrence, but SLS is not a routine procedure that accurately shows low and no response to chemotherapy.

Many published articles have indicated major roles of TA and human telomerase reverse transcriptase (hTERT) promoter in ovarian cancer pathophysiology and ovarian oncogenesis [23, 24]. It has been shown that TA can detect one malignant cell in a normal population of 100 cells by TRAP assay [25]. Compared to preinvasive lesions, TA has been more highly detected in gynecologic cancers including ovarian cancer [9, 10]. It was claimed that the low diagnostic sensitivity of ascites cytology in EOC, which was reported between 40-60% at primary surgery or at SLS, could be overcome by using traditional cytology with a combination of TA measured in ascites [18, 19]. Furthermore it was also claimed that by combining TA detected by TRAP assay in ascites a false-positive rate of < 7% in EOC patients could be obtained [19]. Nevertheless, measuring solely TA was found to be superior to traditional cytology in detecting disease [26]. The reported low sensitivity of traditional cytology was suggested to be mainly caused by difficulty in discriminating neoplastic cells from atypical inflammatory ones [19]. In addition supposed false-negative results of traditional cytology were also caused by tissue capsulation due to primary chemotherapy [27]. In another study usage of TA assay in detecting micrometastasis in lymph nodes was also suggested to be efficient [28].

Since the fraction of actively dividing cells is altered by primary chemotherapy and by cytoreductive surgery, one can theoretically assume that decreased TA levels should be expected at SLS. However in the current study, in parallel with the literature, TA was found to be higher in malignant tumors than in benign diseases. As well, TA in peritoneal washings at SLS of EOC patients who received adjuvant chemotherapy was also suggested to increase the sensitivity of SLS in detection of residual
The role of telomerase activity in predicting early recurrence of epithelial ovarian cancer after first-line chemotherapy: etc.

The current study investigated the relationship between telomerase activity (TA) and early recurrence of epithelial ovarian cancer (EOC) after first-line chemotherapy. Specifically, the study aimed to determine the clinical value of TA levels in predicting early recurrence risk in patients with EOC.

**Background:**
Early recurrence in EOC is a significant clinical issue. The role of genomic instability, particularly microsatellite instability (MSI-H), in predicting recurrence has been studied. MSI-H tumors are more likely to recur, leading to a lack of telomerase activation.

**Methods:**
The study utilized a sensitive PCR-based method (TRAP assay) to detect TA in samples from patients. The relationship between TA levels and early recurrence risk was analyzed.

**Results:**
- TA levels were lower in recurrent EOC patients compared to disease-free patients.
- There was no significant difference in mean TA levels between SLS (second-line surgery) positive and negative patients.
- TA levels were not related to any clinicopathological factors in the current study.
- The predictive value of TA cannot be affirmed in the detection of early recurrence risk at SLS in EOC patients.

**Conclusions:**
TA levels can be used as a reliable marker for clinical use in differentiating patients with recurrent disease from those with disease-free disease. However, non-quantification of TA might lead to misdiagnoses or false-positive errors in detecting persistent and recurrent disease. The role of telomerase activity in predicting early recurrence is crucial for improving clinical outcomes.

**References:**
- [23]
- [31]
Conclusion

Likewise the recent study [29] detection method of TA is also one of the limitations for the current preliminary report. In addition sample size of the current study was insufficient to make a clear-cut conclusion for the prediction of early recurrences. Since the mean duration of follow-up was approximately one year, we also cannot conclude whether TA detected at SLS has any value in predicting recurrences of EOC. However further studies with a large sample size and long follow-up are required to make a more precise conclusion.

References


[34] B. ÖZMEN, M.D.

Address reprint requests to:
B. ÖZMEN, M.D.
Uyum Sitesi No: 28
Çayyolu 06100, Ankara (Turkey)
e-mail: batuhanozmen@tr.net
Radical abdominal trachelectomy in managing early cervical invasion

K. Jeremić, S. Petković, A. Stefanović, J. Stojnić, M. Maksimović, I. Likić, J. Atanacković

Institute for Gynecology and Obstetrics, University of Belgrade, Clinical Center of Serbia, Belgrade (Serbia)

Introduction
Cervical cancer is one of the most frequent malignancies in the female population. Lately this disease has shown an increased incidence in young women (< 35 years). If future pregnancy is desired there is the task to treat the cancer while possibly preserving fertility [1].

Surgical treatment is the standard method in the treatment of invasive cervical cancer [2]. In certain cases there is a possibility for patients to undergo less radical surgical treatment rather than classic or radical hysterectomy with bilateral salpingo-oophorectomy, and to preserve fertility and attain a better quality of life. This concept is strongly selective, and includes only patients with early stages of cervical cancer. There are several methods of surgical treatment for cervical cancer to preserve fertility [2].

Abdominal radical trachelectomy with pelvic lymphadenectomy
This technique includes preparation of the uterine arteries and sections of both cervical branches, preparation of the ureters, removal of the parametria, cervix, distal vaginectomy, preserving the utero-ovarian ligation, and section of the uterosacral ligaments. It was shown that the uterine arteries have no effect on uterine viability and fertility due to the collateral circulation from the ovarian arteries [3]. Afterwards reanastomosis of the uterine corpus with vaginal mycosis and cerclage with nonesorative rope at the level of the uterine isthmus are performed. During the operation, pelvic lymphadenectomy is performed, and then an extempor biopsy is done to determine the radicality of the operation. Pelvic lymphadenectomy includes a deliberate dissection of the parametrial lymph nodes of the common iliac artery, and external iliac veins. Lateral chains of external iliac lymph nodes are distal to the circumflex iliac veins. Then dissection of the medial chain of the external iliac lymph nodes and obturator and ischiorectal lymph nodes is performed, followed by the paraaortal lymph nodes [4].

Vaginal radical trachelectomy with pelvic lymphadenectomy
This operation is a modification of the Schauta-Stoeckel procedure (vaginal radical hysterectomy). The difference is that in radical vaginal trachelectomy the apical part of the endocervix and uterine corpus are preserved. The technique includes laparoscopic pelvic lymphadenectomy with laparoscopic parametrectomy [5].

The aim of the study was to determine if radical trachelectomy with pelvic lymphadenectomy could be a method of treatment for early-stage cervical cancer to preserve fertility.

Material and Methods
We analyzed 12 patients with early-stage cervical cancer hospitalized at the Institute of Gynecology and Obstetrics, Clinical Center of Serbia in the period from 2002 to 2006. Patients were surgically treated and postoperatively followed during a two-year period. All patients were in the reproductive period.

The diagnostic method for diagnosing cervical cancer was histologic examination by cone or biopsy. Stage and lesion size were preoperative determinants for participation in the study (FIGO Stage Ia1, Ia2 and Ib1). The histologic diagnosis was well differentiated planocellular carcinoma. Adenocarcinoma cases were not included in the study.
Results

In the target group, histopathological examination showed that two of the patients had FIGO Stage Ia1, seven had Ia2, and three had Ib1. The preoperative diagnostic method for cervical cancer was histologic examination - cone in five patients and biopsy in seven patients.

All the patients were in the reproductive period: the average age was 30.55 ± 5.52 (range, 22-40 years). No patient gave a history that suggested problems with fertility.

Histological tumor grade (according to the modified Browder system for planocellular carcinoma) was well differentiated type G1-G2 in all patients.

In all patients, rapid frozen section of the endocervical margins and lymph nodes was performed selectively. In all but one case they were clear. In one patient resectional edges were positive for neoplasia so she was submitted to radical hysterectomy.

Postoperatively we found a positive lymph node in one patient, so radiation therapy was continued. In the other ten patients we did not find any signs of residual cancer in the two-year follow-up period (Table 1).

<table>
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<tr>
<th>Patient</th>
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<td>31</td>
<td>Ia2 cone</td>
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Discussion

There are several methods of fertility preserving surgical treatments for cervical cancer. Besides cone and pelvic lymphadenectomy, chemotherapy could also be performed (taxol, cisplatinum, epiburicin) [2]. Landoni et al. performed a study in 12 patients with invasive cervical carcinoma. After chemotherapy followed by cone biopsy with pelvic lymphadenectomy, in eight patients there was no residual malignancy. However, the safety of this method has not been seriously examined in clinical practice [6].

D’Argent et al. in 1994 described an operation called radical vaginal trachelectomy. Their initial experience has been expanded and repeated by others [6].

Roy and Plante [5] and D’Argent et al. [6] have suggested criteria for performing a radical trachelectomy:

1. a desire to preserve fertility;
2. no clinical evidence of impaired fertility;
3. FIGO Stage Ia2, or Ib;
4. lesion size < 2 cm;
5. absence of adenocarcinoma;
6. absence of capillary space involvement;
7. limited endocervical involvement on colposcopic examination, and
8. no evidence of pelvic lymph node metastasis [6].

All the patients were in child-bearing age. No patient had a history that suggested problems with fertility. Some of the patients were older than 35 years, which is the age of a lower fertility rate. The operation itself can decrease fertility rates because of pelvic adhesions.

Although infertility has been suggested as a contraindication for trachelectomy, Covens et al. described three of five infertile patients who became pregnant after trachelectomy [7].

The diagnostic method for cervical cancer was histologic examination, cone or biopsy, as well as endocervical curettage. We examined the length and width of invasion and involvement of the lymphovascular space to determine if radical trachelectomy should be performed. To evaluate the disease we used clinical examination, exfoliative cytodiagnostics – Papanicolaou smear, pelvic and abdominal ultrasound scans, lung scan and laboratory analyses. FIGO stage and the lesion size are the most important preoperative determinants to perform radical trachelectomy [7].

Surgical treatment is the standard method for treatment of invasive cervical cancer [2]. For Stage Ia1 (stromal invasion less than 3 mm, largest diameter of the lesion less than 7 mm), without lymphovascular invasion, the best choice of treatment is cone, considering that the incidence of lymphatic invasion is less than 1%. Moreover, possible therapy is radical trachelectomy [7], as it was in two of our patients who had a positive resectional edge after therapeutic cone (microinvasive carcinoma).

For Stage Ia2 (stromal invasion 3-5 mm, largest diameter of the lesion less than 7 mm) and Ibl (stromal invasion more than 5 mm, largest diameter of the lesion less than 4 cm), the risk of lymphatic invasion is higher, so not only should the primary tumor be removed, but also the lymph node. In addition to radical trachelectomy, pelvic and paraaortical lymphadenectomy have to be performed. Some authors consider that Stage Ia2 could be equally treated by circular cone with clear resectional edges, as well as tracheectomy or hysterectomy [7]. They consider that in this stage, changes are more lymphatic, and less local. Nonetheless, pelvic lymphadenectomy is still necessary [7, 8].
In our study there were two patients with Stage Ib1 cervical carcinoma, with a lesion diameter less than 2 cm, that underwent radical trachelectomy. Few authors consider that there is a possibility to combine radical cervical lesion excision with pelvic lymphadenectomy for Stage Ib1 [8].

Some authors also consider that a lesion diameter less than 2 cm is important in ulcerative and infiltrative lesions, but exophytic lesions that emanate from the portio of the cervix with narrow bases may be reasonable exception to the criteria size. Recidual disease more often occurs in lesion diameters more than 2 cm [8].

During surgery, the cervix with part of the vagina is removed, as well as the lymph node. Resectional edges and selective lymph nodes are analyzed extemore. If the edges are clean and metastases are not found in the lymph nodes, trachelectomy should be continued. If the edges are positive and/or metastases are found, the planned radical trachelectomy is abandoned and the operation is continued as a radical hysterectomy, as occurred in one of our patients [9, 10].

Pelvic lymphadenectomy that was performed in this series included a deliberate dissection of the parametrial lymph nodes because they are the first order lymph nodes for drainage from the cervix. Their removal is deemed a vital part of radical trachelectomy and pelvic lymphadenectomy for cervical cancer. Afterwards all lymph nodes up to the common iliac, paracervical, and hypogastric (obturator) vessels, common internal and external aortic and presacral and lateral sacral were removed [10].

Some authors do not consider adenocarcinoma as a contraindication for radical trachelectomy. Our study did not include patients with adenocarcinoma, as most other studies. Moreover, histological grade NG III was considered as a contraindication for this method because of the worse prognosis [11].

Abdominal radical trachelectomy is considered as technically less difficult than vaginal, but then there are more often complications like bleeding and infection [12, 13]. One of the complications is cervical stenosis, leading to hematometra and amenorhea. After infection pyometra occurs. Consequently some authors suggest insertion of a Foley catheter through the endocervix before cervical amputation. It remains in place for a few days to avoid pyometra or stenosis and hematometra. If such complication occurs, it is necessary to drain and evacuate the contents [12, 13].

These patients should be controlled once every three months the first year after treatment, then twice a year with cytological analysis the next four years, and then once a year. Once a year patients should undergo pelvic and abdominal ultrasound scan, and lung scan if necessary [14].

The recidual and mortality rate after radical trachelectomy are similar as after radical hysterectomy or radiotherapy [3, 7, 11, 13, 15-18].

Our report adds to the accumulating data on radical trachelectomy and pelvic lymphadenectomy for early-stage cancer of the cervix in women with an intact uterus who wish to preserve fertility. Our experience and short-term results support radical trachelectomy and pelvic lymphadenectomy as reasonable forms of treatment. The average pregnancy rate after surgery is 70% [18]. Term delivery occurs in 50% of these patients; 20% deliver before term and the other 30% have spontaneous miscarriages in the I or II trimester of pregnancy [15, 16, 20]. Fertility issues remain the largest unanswered problem. The literature shows that miscarriages occur more often in early trimesters because of cervical incompetence, or rupture of fetal membranes. All pregnancies after radical trachelectomy are high-risk pregnancies [14].

Currently, our approach to the fertility issue is as follows: pregnancy is avoided until one year of follow-up has been completed. Once a woman becomes pregnant, early involvement with a perinatologist who is familiar with the second trimester loss rate seems very important if insight into causes, possible prophylactic measures, and possible treatment measures are to be gained [13].

According to our results, we concluded that radical trachelectomy with pelvic lymphadenectomy could be an appropriate method for treatment of early-stage cervical cancer preserving a woman’s fertility.

References


Address reprint requests to:

K. JEREMIĆ, M.D.
Institute for Gynecology and Obstetrics
Višegradska 26
11000 Belgrade (Serbia)
e-mail: jeremicj@hotmail.com
Ineffective attempt to preserve fertility with a levonorgestrel-releasing intrauterine device in a young woman with endometrioid endometrial carcinoma: a case report and review of the literature

I. Vandenput¹, K. Van Eygen², Ph. Moerman³, I. Vergote¹, F. Amant¹

¹Leuven Cancer Institute (LKI), Division of Gynecological Oncology, UZ Gasthuisberg, Katholieke Universiteit Leuven
²Department of Medical Oncology, AZ Groeninge, Kortrijk
³Department of Pathology, UZ Gasthuisberg, Katholieke Universiteit Leuven (Belgium)

Introduction

Endometrial cancer is the most common malignancy of the female genital tract in the Western world [1]. Although it is primarily a disease of postmenopausal women, 2-14% of the cases occur in women less than 40 years. Often these women wish to preserve their fertility [2].

The levonorgestrel intrauterine contraceptive device (LNG IUCD) has a T-shaped body and releases daily 20 mg of levonorgestrel, which leads to inactivation of the endometrium [3].

Case Report

A 25-year-old nulligravida presented with intermenstrual bleeding. The pathological analysis of the curettage specimen revealed grade 1 endometrioid endometrial carcinoma (EEC). Imaging studies including transvaginal ultrasound (TVS), computed tomography (CT) and magnetic resonance imaging (MRI) could not detect myometrial invasion or metastatic disease. The immunohistochemical expression of the estrogen and progesterone receptor in the tumor was strongly positive, whereas p53 staining was negative. After extensive counseling, we decided to use a levonorgestrel-releasing intrauterine device to preserve her fertility. Follow-up was organized every three months and consisted of serum CA125 levels, TVS, endometrial biopsy and MRI. The tumor regressed after ten months and the intrauterine device was removed. However, nine months later, recurrent EEC was diagnosed and a hysterectomy performed. Pathological examination confirmed Stage Ia EEC.

Discussion

Total hysterectomy with bilateral salpingo-oophorectomy is the cornerstone treatment for endometrial cancer. In general, EEC in young women has an excellent prognosis. Therefore, clinically early-stage EEC in these women wishing to preserve their fertility poses a dilemma whether or not to perform a definitive hysterectomy. We present a case of a patient with grade 1 EEC, with favorable prognostic factors (ER and PR positive, p53 negative). Despite a temporary regression, the tumor recurred nine months after removal of the device.

Since the patient wanted to preserve her fertility, after extensive counseling we decided to insert a LNG IUCD after curetage. Follow-up was organized every three months and consisted of serum CA125 level, TVS, endometrial biopsy and MRI. After ten months, we performed an endometrial sampling after removal of the IUCD. Pathologically proliferative endometrium was noted. CA125 levels were always normal. We allowed her to get pregnant but she did not conceive in the following nine months. At that stage and without symptoms, an endometrial biopsy revealed recurrent grade 1 EEC. Laparoscopically assisted vaginal hysterectomy with washings (without bilateral salpingo-oophorectomy) was performed. Pathological examination showed EEC without myometrial invasion (Stage Ia).

Summary

Background: The treatment of endometrial cancer in young women who want to preserve their fertility is challenging. Case: A 25-year-old woman (A0P0G0) was diagnosed with grade 1 endometrioid endometrial carcinoma (EEC). Imaging studies including transvaginal ultrasound (TVS), computed tomography and magnetic resonance imaging (MRI) could not detect myometrial invasion or metastatic disease. The immunohistochemical expression of the estrogen and progesterone receptor in the tumor was strongly positive, whereas p53 staining was negative. After extensive counseling, we decided to use a levonorgestrel-releasing intrauterine device to preserve her fertility. Follow-up was organized every three months and consisted of serum CA125 levels, TVS, endometrial biopsy and MRI. The tumor regressed after ten months and the intrauterine device was removed. However, nine months later, recurrent EEC was diagnosed and a hysterectomy performed. Pathological examination confirmed Stage Ia EEC. Conclusion: Despite the presence of favorable prognostic factors of EEC as determined by grade and immunohistochemistry, the levonorgestrel-releasing intrauterine device was unable to preserve fertility.

Key words: Endometrial; Cancer; Fertility; Levonorgestrel; Conservative.
Given the limited experience and potential hazard of uterus sparing treatment, a highly motivated person and detailed counseling concerning the risks are required [4]. Radiologic examinations such as CT, MRI, and TVS are added to improve the accuracy of clinical staging [5]. Ovarian disease is rarely observed in patients with Stage I disease, but can occur in 5% of cases [6]. For this reason, Morice et al. [7] proposed a laparoscopic procedure including adnexal exploration and peritoneal cytology to verify the absence of extrauterine disease.

We consider a thorough assessment of tumor biology a crucial step in the decision making for conservative management. Although endometrial cancer in young women tends to be well-differentiated and estrogen/progesterone dependent, exceptions have been described [8]. Surprisingly, only a few studies have determined the hormone receptor status [8-11]. The absence of hormone receptors is likely to reduce the success rate of hormonal treatment and alternatives (if any) should be investigated.

Information on the p53 status and vascular space infiltration further adds to the risk assessment. Bell et al. [12] associated the presence of vascular space infiltration with the risk of nodal metastasis and cancer recurrence in clinical Stage I endometrial cancer. Kohlerberger et al. [13] demonstrated that 9% of women with early-stage EEC had a p53 mutation/overexpression, which was correlated with a poorer overall survival. Ferrandina et al. [14] reported on a 30-year-old woman diagnosed with grade 1 EEC who was treated with 20 mg of dihydrogestosterone daily on days 15-25 of the menstrual cycle, for a period of three months. She conceived three months after therapy. However, eight months after delivery, recurrence was noted intraperitoneally. The pathology report revealed grade 3 EEC. Immunohistochemistry performed on the primary tumor showed a positive reaction for ER and PR (80% and 90%) and a strong immunoreaction for p53 protein in 70% of the cells. Therefore, if the current case would have been p53 positive, we would not have attempted a conservative approach.

Strategies to preserve fertility include oral progestins, prostegens in combination with aromatase inhibitors, LNG IUCD and operative hysteroscopy. The efficacy of oral progestin therapy of grade 1 EEC, has been investigated in several previous studies. From Table 1 it appears that there is no consensus concerning the duration, dosage and type of progestin therapy. The duration of treatment was between two and 36 months. Tumor regression was noted in 70% of the cases, with a recurrence rate of 43%. The clinical benefit, defined as women who showed tumor regression without recurrence of disease, was however only 27%.

Only one study [15] investigated the response of the combination of medroxyprogesterone acetate (MPA) (160 mg/day) with an aromatase inhibitor (anastrozole). Two cases with grade 1 endometrial cancer and treated with this strategy, reverted to normal endometrium after three and six months. The authors suggest that combining progesterone with an agent that eliminates the adipose production of estrogen might be more effective than the direct effect of progesterone alone. However, this strategy has not yet been proven to be more effective in other hormone sensitive disease such as breast cancer.

The levonorgestrel-releasing intrauterine device has been used as conservative treatment for patients with early endometrial cancer who were at risk for surgery (morbid obesity, hypertension, age). The wish to preserve fertility was the indication in our case (Table 2). Only three out of eight cases (38%) with grade 1 EEC showed complete remission, while 63% had tumor persistence. In one (current case) out of three cases (33%) with response, the tumor recurred. Clinical benefit (tumor regression without recurrence) was thus observed in 2/8 (25%).

---

### Table 1 — Outcomes with oral progestin therapy of Stage 1 EEC (grade 1/2) from retrospective case series as reported in the literature.

<table>
<thead>
<tr>
<th>Author et al.</th>
<th>n</th>
<th>Hormonal agent</th>
<th>Duration of treatment (mths)</th>
<th>Tumor regression n (%)</th>
<th>Tumor persistence n (%)</th>
<th>Tumor recurrence n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim et al. 1997 [18]</td>
<td>7</td>
<td>Megestrol acetate 160 mg/d</td>
<td>3</td>
<td>4/7 (57)</td>
<td>3/7 (43)</td>
<td>2/4 (50)</td>
</tr>
<tr>
<td>Kaku et al. 2001 [19]</td>
<td>12</td>
<td>MPA 200-800 mg/day</td>
<td>2-14</td>
<td>8/12 (67)</td>
<td>4/12 (33)</td>
<td>2/8 (25)</td>
</tr>
<tr>
<td>Imai et al. 2001 [20]</td>
<td>15</td>
<td>MPA 400-600 mg/day</td>
<td>3-16</td>
<td>8/15 (53)</td>
<td>7/15 (47)</td>
<td>3/8 (36)</td>
</tr>
<tr>
<td>Pinto et al. 2001 [31]</td>
<td>1</td>
<td>Megestrol acetate 40mg/d</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gotlieb et al. 2003 [21]</td>
<td>13</td>
<td>Megestrol acetate or OH-prog or MPA</td>
<td>3-37</td>
<td>13/13 (100)</td>
<td>5/13 (38)</td>
<td></td>
</tr>
<tr>
<td>Ota et al. 2005 [32]</td>
<td>12</td>
<td>MPA 600 mg/d</td>
<td>3-12</td>
<td>5/12 (42)</td>
<td>7/12 (58)</td>
<td>2/5 (40)</td>
</tr>
<tr>
<td>Niwa et al. 2005 [9]</td>
<td>9*</td>
<td>MPA 400-600 mg/day</td>
<td>6-10</td>
<td>9/9 (100)</td>
<td>8/9 (89)</td>
<td></td>
</tr>
<tr>
<td>Ferrandina et al. 2005 [14]</td>
<td>1</td>
<td>Dihydrogestosterone 20 mg/day</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chang et al. 2006 [22]</td>
<td>1</td>
<td>MPA 500 mg/twice weekly</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Park et al. 2006 [10]</td>
<td>1</td>
<td>Megestrol acetate 600+320 mg/day</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Shamshirsaz et al. 2007 [23]</td>
<td>1</td>
<td>Megestrol acetate 160 mg/day</td>
<td>36</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yamazawa et al. 2007 [24]</td>
<td>9</td>
<td>MPA 400 mg/day</td>
<td>6</td>
<td>7/9 (78)</td>
<td>2/9 (22)</td>
<td>2/7 (29)</td>
</tr>
<tr>
<td>Ushijima et al. 2007 [25]</td>
<td>22</td>
<td>MPA 600 mg/day</td>
<td>6</td>
<td>12/22 (55)</td>
<td>10/22 (45)</td>
<td>8/14 ** (57)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>2-36</td>
<td></td>
<td>38/124 (31)</td>
<td>38/89 (43)</td>
<td>38/89 (43)</td>
<td>38/89 (43)</td>
</tr>
</tbody>
</table>

mths: months; MPA: medroxyprogesterone acetate; OH-prog: Hydroxy-progesterone; GnRHa: gonadotropin-releasing hormone analogue.

*: only cases with long-term follow-up were included; **: unclear in paper how they come to 14 patients.
References


Table 2.—Outcomes with levonorgestrel-releasing intrauterine device of well differentiated EEC, Stage Ia-b, reported in the literature.

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Duration of treatment (mths)</th>
<th>Tumor regression</th>
<th>Tumor persistence</th>
<th>Tumor recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahamonde et al.</td>
<td>2</td>
<td>3-7</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Giannopoulos (*)</td>
<td>1</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dhar et al.</td>
<td>4</td>
<td>6-36</td>
<td>1/4</td>
<td>3/4</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Current case</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL EEC</td>
<td>8</td>
<td>3/8</td>
<td>5/8</td>
<td>1/3</td>
<td>(38%)</td>
</tr>
</tbody>
</table>

(*) combination of levonorgestrel-releasing intrauterine device with medroxyprogesterone acetate 400 mg/day.

Table 3.—Outcomes of operative hysteroscopy with or without progestins, as treatment of well differentiated EEC, Stage Ia-b, reported in the literature.

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Hormonal agent</th>
<th>Duration of treatment (mths)</th>
<th>Tumor regression</th>
<th>Tumor recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mazzon et al.</td>
<td>1</td>
<td>Megestrol</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sparac et al.</td>
<td>1</td>
<td>Progesterone</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Viñals et al.</td>
<td>1</td>
<td>none</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Hysteroscopic resection has been described in three cases. Two out of three patients received oral progestin therapy following the procedure. Nevertheless, the results look promising in all three cases (Table 3). The concern of transfallopian tube dissemination of malignant cells into the peritoneal cavity has been investigated. Viñals et al. [16] showed a review of the literature and concluded that the frequency of positive peritoneal cytology was between 6.3 and 10% and that this did not effect survival in women with Stage I endometrial cancer.

Conclusion

It appears from these data that oral progestins or LNG IUCD bear little clinical benefit when preservation of fertility is aimed for. The options of combination of hormonal agents or hysteroscopic resection need further attention.


Address reprint requests to:
F. AMANT, M.D.
Leuven Cancer Institute
Division of Gynecologic Oncology
UZ Gasthuisberg
Katholieke Universiteit Leuven
Leuven (Belgium)
e-mail: frederic.amant@uz.kuleuven.ac.be
Adenoid cystic carcinoma of Bartholin’s gland with lung metastasis: a case report

X. Wang, W. Lu, C. Zhu, F. Ye, X. Xie
Women’s Hospital, School of Medicine, Zhejiang University, Hangzhou (China)

Summary

Background: Adenoid cystic carcinoma (ACC) of the Bartholin’s gland is very rare. There is no agreed consensus on the optimal treatment. Case: In the present study we report the case of a 41-year-old Chinese woman who suffered from ACC of the Bartholin’s gland with lung metastases and repeated local recurrence, and discuss the most suitable treatment for late stage ACC of the Bartholin’s gland. Conclusion: Our experience from this patient suggests that initial conservative surgery of a local lesion, even for later stage patients with ACC of the Bartholin’s gland, could be considered.

Key words: Adenoid cystic carcinoma (ACC); Bartholin’s gland.

Introduction

Adenoid cystic carcinoma (ACC) is a malignant epithelial neoplasm that commonly involves the salivary glands, upper respiratory tract, breast, and skin, as well as the uterine cervix [1, 2]. ACC of the Bartholin’s gland is very rare. It constitutes less than 1.0% of all vulvar carcinomas and approximately 10% of all Bartholin’s gland carcinomas [3]. To date only about 50 cases of ACC of the Bartholin’s gland have been reported in the English literature [4]. This tumor is characterized by slow growth, local invasion, and uncommon distant metastasis. There is no agreed consensus on the optimal treatment. Wide local dissection of the vulva or radical vulvectomy is the usual option.

We report the case of a patient who suffered from ACC of the Bartholin’s gland with lung metastases and repeated local recurrence, and discuss the most suitable treatment for late stage ACC of the Bartholin’s gland.

Case Report

A 41-year-old Chinese woman, gravida 2, para 1, was referred to the Women’s Hospital, School of Medicine, Zhejiang University with complaints of introital pain and a gradually increasing vulvar mass of 2-year’s duration in April of 2002. She had no previous illness or surgery and her family history was unremarkable.

Local examination revealed a hard, unmovable mass measuring 1.0 cm × 1.0 cm × 1.5 cm in the left labium major with normal superficial skin. Pelvic examination revealed no positive findings. The bilateral inguinal and femoral lymph nodes were not palpable. Chest computed tomography (CT) showed several lesions with clear margins and soft tissue density measuring 1-2 cm in diameter scattering in the bilateral lung (Figure 1). No signs of mediastinal lymph node metastasis or other abnormalities were found. Pelvic ultrasonography (US) did not show any metastatic disease. Serum tumor markers including CA125, AFP, and CEA were negative.

Biopsy of the vulvar mass was initially taken, and histological examination revealed ACC originating in the Bartholin’s gland. Transthoracic core needle biopsy of the lung lesions was then performed, confirming adenocarcinoma histologically. The patient was diagnosed with Stage IV vulvar carcinoma according to the International Federation of Gynecologists and Obstetricians classification (1988). Wide local resection of the vulva was performed. Postoperative histological examination also showed ACC of the Bartholin’s gland (Figure 2) and tumor involvement of the striated muscle of the left lateral vaginal wall with positive margins of the resected specimen. The patient received three courses of systemic chemotherapy with 5-fluorouracil, cis-diaminedichloroplatinum and adriamycin. The patient’s response to chemotherapy was stable according to the WHO criteria.

During the follow-up period, the patient had no symptoms until she was referred to the hospital with a complaint of vulvar pain in March 2003. Local examination revealed two recurrent lesions, one measuring 1.0 cm × 2.0 cm × 2.0 cm in the left vaginal orifice and the other measuring 2.0 cm × 3.0 cm × 3.0 cm in the left area of the external urethra. A diagnosis of local recurrence of ACC of the Bartholin’s gland was made, and she underwent a wide local excision of the lesions again. Histological examination showed the same diagnosis as before and positive margins. The patient did not receive chemotherapy or radiotherapy postoperatively at this time. During August 2004 to December 2005, she was referred to the hospital twice because of local recurrence. The local masses measured 2.0 cm × 2.0 cm × 3.0 cm in the left lateral/posterior vaginal wall and 0.5 cm × 1.0 cm × 3.0 cm in the left/anterior area of the external urethra, respectively. Chest CT showed that the metastatic lesions grew slowly and the mediastinal lymph nodes metastasized (Figure 1). Only local excisions were performed. Since then, she gave up further treatment and examination, and died in December 2007.

Discussion

ACC of the Bartholin’s gland is an uncommon vulva carcinoma. The mean age of diagnosis is 49 years with a range of 25-80 years. Signs and symptoms are nonspecific but may include local pain, bleeding, dyspareunia, pruritus, drainage from an abscess, and the presence of a...
palpable mass [5]. The tumor is characterized by slow growth, with a marked tendency to perineural and local invasion [6]. Perineural infiltration along the neural sheath is quite characteristic and probably the histopathologic cause of the itching and burning sensation that many patients experience long before the onset of pain or before a palpable mass is evident. The diagnosis of the disease is sometimes difficult. Frequently patients undergo drainage and marsupialization because of misdiagnosis of Bartholin’s gland infection or cyst. The correct diagnosis is not made till the incision does not heal or histological examination is performed. Wheeleck et al. [7] reported an average 3.3 months interval between initial symptoms and the correct diagnosis. For this patient, the correct diagnosis was delayed about two years, during which she was treated for an infection of Bartholin’s gland cyst. Dodson et al. [8] recommend a routine cytological examination at the time of marsupialization for over 40-year old women with a Bartholin’s gland mass.

It is extremely difficult to make a definite recommendation on treatment of ACC of the Bartholin’s gland. Surgery is a common option as initial treatment. The surgical extension is arranged from wide local excision of the lesion to radical vulvectomy. DePasquale et al. [5] suggested that the initial treatment should be surgery and as conservative as possible. If the lesion is small, unilateral, and does not approach the anterior or posterior midline a wide local excision should be performed. If the lesion is extensive, or if it approaches the anterior or posterior midline, a radical vulvectomy should be performed. In accordance with DePasquale et al., Nasu et al. [4] also suggested that the initial surgical management should be as minimal as possible. Wide local excision should be performed for Stage II disease at initial surgery. If the wide local excision margin is positive, radical vulvectomy be should subsequently considered. It has been debated whether inguinal and femoral lymphadenectomy should be included in the initial operation. Dodson et al. [8] treated nine patients by vulvectomy and bilateral inguinal femoral lymphadenectomy. Only one of them presented a metastatic femoral node. The biologic behavior of ACC of the Bartholin’s gland appears to be quite different from adenocarcinoma and squamous cell carcinoma of the Bartholin’s gland. Metastasis to the inguinal femoral nodes is uncommon in ACC. The disease is characterized by late local invasion and distant blood-borne metastasis. It is suggested that routine inguinal femoral lymphadenectomy can be omitted in the surgery for ACC of the Bartholin’s gland unless the patient is clinically suspected to have involvement of the groin nodes.

Adjuvant radiotherapy is recommended when margins of excision are positive or local or perineural spread is involved. However, Rosenberg et al. [1] and Copeland et al. [3] reported that 12 patients postoperatively underwent external beam radiation. Of those, 53% continued to recur consequently.

Chemotherapy is the first choice for metastatic ACC of the parotid and salivary glands. Of those, Adriamycin is regarded to be active in the disease [9]. However, Information about chemotherapy in ACC of the Bartholin’s gland is limited. According to two recent reports, no...
Adenoid cystic carcinoma of Bartholin’s gland with lung metastasis: a case report

Responses were found regardless if nonspecific chemotherapy or combination of methotrexate, actinomycin-D, and cyclophosphamide were used [10, 11]. We gave the patient three courses of systemic chemotherapy with 5-FU, DDP and adriamycin because of Stage IV disease. However the response was very poor suggesting that the sensitivity of ACC of the Bartholin’s gland to chemotherapy might be different from one of the salivary gland.

Most of ACCs of the Bartholin’s gland are early stage at the initial diagnosis. Copeland et al. [3] reported five ACCs of the Bartholin’s gland, two of them were found to be pulmonary metastases. One patient survived 4.5 years with disease metastases without treatment. The other developed pulmonary metastases after 14 months and received chemotherapy. However she died after 63 months of respiratory failure. There is no agreed option of treatment for lung metastases of ACC of the Bartholin’s gland up to date. In our report, the patient was found to have pulmonary metastases at the first diagnosis. She underwent wide local excision of the vulvar tumor and chemotherapy as the initial therapy, but did not receive more chemotherapy or radiotherapy except local excision during recurrence. The progression of metastases of the lung and local vulva were slow and the patient obtained long survival despite no chemotherapy. There seems to be a higher frequency and shorter interval of local recurrence in later stage patients than in early ones. Because the patients with local recurrence suffer from severe pain, local excision is necessary. Our experience from this patient suggests that the initial conservative surgery of a local lesion, even for later stage patients, should be considered.

References


Address reprint requests to:
X. XIE, M.D.
Women’s Hospital
School of Medicine
Zhejiang University
Hangzhou (China)
e-mail: xiex@mail.hz.zj.cn
Small cell neuroendocrine carcinoma of the cervix: report of two cases

F. Puig1, M.D.; C. Rodrigo1, M.D.; G. Muñoz2, M.D.; R. Lanzón1, M.D.

1Department of Gynecology; 2Department of Pathology, Miguel Servet University Hospital, Zaragoza (Spain)

Summary
Small cell neuroendocrine tumor of the cervix is a rare malignancy with aggressive behavior. Metastases and recurrent disease are frequent and multimodal therapy is commonly used. Neoadjuvant or adjuvant chemotherapy should be combined with radiation therapy and surgery, even in early stages. Nevertheless, due to the low prevalence of these tumors, the best treatment has not yet been determined. Two cases of small cell neuroendocrine tumor of the uterine cervix are reported. We describe the clinical course, diagnostic methods and examine treatments applied and survival.

Key words: Neuroendocrine carcinoma; Small cell carcinoma; Uterine cervical neoplasm.

Introduction
Neuroendocrine tumor of the uterine cervix is a very rare neoplasia, accounting for 1-5% of all cervical malignancies [1, 2]. It is extremely aggressive and has an unfavorable outcome, due to the early development of lymph node metastases and vascular invasion [3].

More than 15 varied descriptive terms have been used to describe these neoplasms. To avoid the confusion produced by various different types of terminology for neuroendocrine tumors of the cervix, the College of American Pathologists adopted standardized terminology, including: typical carcinoid tumor, atypical carcinoid tumor, small cell neuroendocrine carcinoma (SCNEC) and large cell neuroendocrine carcinoma (LCNEC) [4].

Among these rare cervical tumors, SCNEC is the most frequent type and histologically has the same features as primary small cell carcinoma of the lung [5]. In this report we present two cases of SCNEC treated with multimodal therapy.

Case Report

Case 1
A 61-year-old woman, gravida 1, para 1, presented with abnormal vaginal bleeding for 15 days. Gynecological examination revealed a 3 x 3 cm tumor involving the cervix. Colposcopy-directed biopsy of the lesion revealed a malignant epithelial tumor with neuroendocrine differentiation. High-risk oncogenic HPV type 16 was detected. Magnetic resonance imaging (MRI) of the pelvis showed a cervical tumor of 3 cm. Complete blood count, blood chemistry and chest X-ray were normal and the neoplasm was clinically defined as Stage IB1. The patient underwent a radical hysterectomy with bilateral pelvic lymphadenectomy.

Pathologic evaluation of the specimen revealed a 36 x 30 x 25 mm solid mass with ill-defined borders. There was diffused lymphovascular space involvement. The vagina, parametria and lymph nodes (total of 26 nodes) were free of tumor (Figure 1). The tumor was reported as a SCNEC of the uterine cervix. Diagnosis was confirmed by immunohistochemical staining (positive immunoreactivity for chromogranin A and neuron-specific enolase). Glandular differentiation was also found.

After surgery a consensus was attained with an oncologist to include the patient in a combined adjuvant therapy program, consisting of adjuvant radiotherapy (50.40 Gy whole-pelvis radiation and 18 Gy vaginal brachytherapy) and chemotherapy (4 intravenous cycles of cisplatin 100 mg/m2). Upon writing the patient was in the 73rd postoperative month and no evidence of disease was detected.

Case 2
A 56-year-old woman, gravida 2, para 2, was admitted complaining of postmenopausal vaginal bleeding for two months. Speculum examination revealed an anterior lip of the uterine cervix replaced by cancerous tissue. Colposcopy-directed biopsy disclosed an invasive malignant epithelial tumor with no distinctive histological type. High-risk oncogenic HPV types 16 and 18 were detected. MRI of the pelvis revealed a cervical neoplasm of 2 x 2 cm and the patient was staged as Stage IB1. Two weeks later she underwent a radical hysterectomy with bilateral pelvic lymphadenectomy.

Histological examination revealed a 28 x 25 x 20 mm tumor with the vagina and parametria free of tumor but two of 20 lymph nodes were metastatic. Tumor cells had abundant cytoplasm, large nuclei, and prominent nucleoli. Rosette-like structures were detected (Figure 2).

Immunohistochemical study showed a positive reaction to neuron-specific enolase but a negative reaction to chromogranin A and S-100 protein. The tumor was reported as a SCNEC of the uterine cervix confirmed by immunohistochemical staining. The patient underwent 46 Gy whole pelvis radiation with concomitant 40 mg/m2 cisplatin weekly for five weeks and vaginal brachytherapy 18 Gy, which were well tolerated. After 24 months she developed brain and vertebral metastases and died of the disease.

Discussion
SCNEC of the uterine cervix is rare, the clinical course is very rapid, the prognosis is unfavorable and it is
reported to have a high incidence of central nervous system metastases, differing from other cervical malignancies [6]. It accounts for 0.3-2% of invasive carcinomas of the uterine cervix [7]. Clinical features usually comprise abnormal vaginal bleeding as the most common symptom [8].

The diagnosis of patients with neuroendocrine tumors has been improved by the introduction of new pathohistologic techniques. To make the diagnosis of a cervix SNEC, pathologists currently immunostain for general neuroendocrine markers that identify the neuroendocrine "nature" of the tumor, as well as cell-specific markers including: neuron-specific enolase, chromogranin A and synaptophysin [5]. Albores-Saavedra et al. [4] indicated that not all of the markers need to be present to make the diagnosis because 60% are negative for chromogranin A and synaptophysin, and 30% for neuron-specific enolase.

Treatment of cervix SNEC remains controversial due to the high rate of recurrence and to the premature development of metastases, even with early-stage disease. The usual treatment for early-stage SNEC is radical surgery. Because of the poor prognosis of these tumors, most patients receive adjuvant radiation therapy and chemotherapy as well [9].

Combination chemotherapy is considered by many authors to be essential for the appropriate management of SNEC of the uterine cervix owing to the early expansion to regional lymph nodes and to distant sites such as the lung, liver, bone, and brain [10]. Most frequently used regimens include: combinations of platinum and etoposide or vincristine, doxorubicin and cyclophosphamide [6, 7]. Due to the limited experience with these neoplasias, most therapeutic methods are derived from the experiences with neuroendocrine tumors of the lung and gastrointestinal tract. The aggressiveness and prognosis of cervix SNEC suggest the need of new chemotherapy regimens. Definitive treatment does not seem to be established.

References


Address reprint requests to:
C. RODRIGO, M.D.
Calle Monasterio Nuestra Señora de los Ángeles, nº 3, portal 4, 2º A.
Zaragoza, 50.012 (Spain)
e-mail: casteval@yahoo.com
Repeat chemosensitivity of epithelial ovarian carcinoma in a BRCA1 mutation carrier to paclitaxel/platinum combination chemotherapy

B. Melichar1, M.D., Ph.D.; P. Fridrichová, M.D.; M. Tomšová, M.D., Ph.D.; E. Malířová, Pharm.D.

Departments of 1Oncology & Radiotherapy, 2Medical Genetics, 3Pathology, and 4Nuclear Medicine, Charles University Medical School & Teaching Hospital, Hradec Králové, and 7Department of Oncology, Palacky University Medical School & Teaching Hospital, Olomouc (Czech Republic)

Summary

We present here a case of a BRCA1 mutation carrier with repeat responsiveness of recurrent EOC to paclitaxel/platinum. The patient had complete response to the combination of paclitaxel/platinum in the first line. Subsequent four recurrences also showed a complete response to this combination. The chronic toxicity, including hypersensitivity and nephrotoxicity could be controlled by modifying the regimen. In conclusion, recurrent EOC in BRCA1 mutation carriers may retain sensitivity to paclitaxel/platinum combination chemotherapy, and this combination could be therapy of first choice in this patient population.

Key words: BRCA1; Epithelial ovarian carcinoma; Paclitaxel; Platinum.

Introduction

Epithelial ovarian carcinoma (EOC) is the leading cause of death from gynecological cancer. At the same time, EOC is a highly chemosensitive tumor. Currently, the standard first-line regimen in advanced EOC is the combination of paclitaxel and platinum (cisplatin or carboplatin), and the response rate to this combination is about 60-70% [1, 2]. Although the complete response rate is also relatively high, the tumor recurs in a vast majority of patients after a median of 16-18 months. The response rate to agents used in the second line of therapy, including topotecan, gemcitabine or liposomal doxorubicin, is substantially lower, and these responses are of limited duration. The response rate to combinations of second-line agents is also relatively low. Recurrent EOC responds to repeat administration of paclitaxel/platinum combination [3, 4]. The probability of response to repeat administration of paclitaxel/platinum increases with the time from the last platinum administration (platinum-free interval), and, based on platinum-free interval, recurrent EOC may be classified as refractory, resistant or sensitive [5].

Although the probability of response to paclitaxel/platinum combination in platinum-sensitive recurrent EOC is relatively high, the optimal management of recurrent EOC is still a matter of dispute, and there is no universally accepted standard of care in these patients [6, 7]. The choice of therapeutic agents in patients with second or subsequent recurrences is even less clear. We present here a case of a BRCA1 mutation carrier with repeat responsiveness of recurrent EOC to paclitaxel/platinum.

Case Report

A 48-year-old woman with a history of breast carcinoma treated with lumpectomy, adjuvant chemotherapy and adjuvant external beam radiation at the age of 44 years had elevation of tumor markers discovered during a routine follow-up in November 2001. Computed tomography (CT) revealed a right ovarian mass, ascites and multiple densities in the omentum. On November 23, 2001 hysterectomy, bilateral salpingo-oophorectomy, omentectomy and sigmoid resection were performed. Peritoneal carcinomatosis was evident during surgery, and a port system for intraperitoneal chemotherapy was implanted. Histology revealed poorly differentiated adenocarcinoma, and the diagnosis of Stage III EOC was made. The patient’s mother had been treated for breast carcinoma at the age of 59 years and for EOC at the age of 63 years, and a maternal aunt had also had breast carcinoma. Subsequent analysis found the patient harboring BRCA1 mutation (exon 20, c.5385dupC).

The patient was treated by four cycles of combination paclitaxel (175 mg/m²) and carboplatin (area under the curve 6) every three weeks starting in December 2001. Subsequently, the same combination was administered intraperitoneally for four cycles. As consolidation therapy, four cycles of intraperitoneal interleukin-2 (3.6 MU days 1-5 and 8-12) and interferon-γ (100 μg days 1, 3, 5, 8, 10 and 12) repeated every 28 days were administered. Subsequently, an intraperitoneal catheter was not patent, and the patient received six more cycles of paclitaxel and carboplatin. A carboplatin allergy manifested, and in the last cycle in February 2003 carboplatin was substituted by cisplatin (80 mg/m²). Second-look surgery was performed which showed no evidence of disease.

In February 2004 the patient complained about abdominal pain. CA125 rose to 1603 U/ml (Figure 1), and CT scan revealed pelvic recurrence. Starting in March 2004, the patient was treated until October 2004 with ten cycles of paclitaxel (175 mg/m²) and cisplatin (80 mg/m²) resulting in complete response.

In July 2005 the patient presented with hematochezia. Three ulcerated lesions (20, 30 and 65 cm from anus) were detected on colonoscopy and were also apparent on control CT scan. The
patient opted for resection of the involved colon. The lesions with involved colon were first resected. The postoperative course was complicated by gangrene of the remaining left colon. Left colectomy with transversostomy was subsequently performed. The course was further complicated by an abdominal abscess that was drained percutaneously. Subsequently, systemic therapy with the same combination was initiated, and between October 2005 and March 2006 the patient was treated with nine cycles, again resulting in a complete response (detected by both CT scan and CA125). In January 2007, the patient presented with a third recurrence. Enlarged para-iliac lymph nodes were evident on control CT scan, and the CA125 concentration rose again. Treatment with the combination of paclitaxel and cisplatin was initiated. The therapy was complicated after the third course by acute renal failure that resolved spontaneously. As a complete response was reached after three cycles in April 2007, no additional therapy was administered.

In November 2007, the patient presented with the fourth recurrence. Recurrence of abdominal lymphadenopathy was evident on control CT scan along with elevation of CA125. Because of earlier nephrotoxicity after administration of cisplatin and carboplatin hypersensitivity, the patient was treated with the combination of paclitaxel (175 mg/m²) and carboplatin (area under the curve 6) every three weeks with carboplatin administered following a desensitization protocol (diluting the drug with the solution of glucose at a ratio of 1:10; three times and administering the drug starting with the highest dilution). After six cycles, a complete response was obtained in March 2008 on control CT scan as well as in CA125 concentrations, and the therapy was subsequently interrupted. The patient is followed regularly, and at the next recurrence the same therapy is planned. At last control in May 2008 the patient was without evidence of disease activity.

Discussion

The present case illustrates repeat sensitivity of recurrent EOC in a BRCA1 mutation carrier to paclitaxel/platinum chemotherapy. The tumor retained sensitivity to this combination even after several recurrences. As expected, the complete response did not lead to eradication of the disease, but repeat response could be obtained with administration of the same chemotherapy regimen even after less than a year. The only limitation in the present case was the toxicity of the repeated chemotherapy. Retrospective analyses indicate that EOC patients carrying the BRCA1 mutation have significantly better prognosis [8-11]. This may be explained by increased chemosensitivity of these tumors that could be linked to the biological role of BRCA1. The BRCA1 protein is one of the molecules responsible for response to DNA damaging agents. BRCA1 protein participates in DNA repair, messenger RNA transcription, cell cycle regulation and protein ubiquitination, and the cells lacking BRCA1 protein are highly sensitive to alkylating agents, platinum derivatives and anthracyclines, although the susceptibility to taxanes could be lower.

The patient in the present report also had breast carcinoma as the first primary. The increased susceptibility of BRCA1-related carcinomas may be observed across the spectrum of tumors. Increased chemosensitivity has been described in BRCA1-related breast carcinoma and EOC [8-10, 12-14]. Experimental data also point out a generally increased chemosensitivity of BRCA1 mutant cells [15].

The present case suggests that the optimal strategy in BRCA1 mutation carriers with recurrent EOC is the re-administration of paclitaxel/platinum that could be repeated. The platinum-free interval in the current case was usually about a year, but the last recurrence manifested after only seven months (probably as a result of shorter treatment duration for the third recurrence). The predictive value of the platinum-free interval may be linked more to the size of the tumor [16]. The present case demonstrates the limitations of cytoreduction by chemotherapy. Despite a complete response the disease always recurred within a year after stopping the therapy. This relatively rapid growth pattern is linked to the biology of the tumor that, however, retained chemosensitivity to the paclitaxel/platinum combination. The course of recurrent EOC in BRCA1 mutation carriers may thus resemble more a chronic disease rather than a rapidly fatal malignant disorder. It is now well established that EOC recurring more than six months after the last administration of platinum-based combination may be sensitive to platinum re-induction chemotherapy [3-7, 17, 18]. A remarkable feature in the current case is that it demonstrates that the disease responds repeatedly to platinum/paclitaxel re-induction chemotherapy. This approach may be in some variation with current practice that favors administration of non-cross resistant agents, e.g., topotecan, gemcitabine or liposomal doxorubicin, in patients with recurrent EOC, especially in cases of shorter platinum-free interval or repeated recurrence. In fact there are little data that could guide us on the use of therapy in patients with repeated recurrence. Some studies have included patients with a second recurrence [17], but little is known about the efficacy of platinum therapy in patients with the third or fourth recurrence as the number of these patients included in prospective trials has been extremely limited [18]. In one large trial of paclitaxel/platinum combination administered in recurrent EOC (mostly second line), only 8% of patients
received paclitaxel in the next line [18]. While the paclitaxel/platinum combination is considered the standard of care in platinum-sensitive EOC in the first recurrence, most physicians would probably opt for combinations including other agents during subsequent relapses.

In conclusion, recurrent EOC in BRCA1 mutation carriers may retain sensitivity to paclitaxel/platinum combination chemotherapy, and this combination could be therapy of first choice in this patient population.

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Address reprint requests to:
B. MELICHAR, M.D., Ph.D.
Department of Oncology
Palacky University Medical School
& Teaching Hospital
IP Pavlova 6, 775 20 Olomouc (Czech Republic)
e-mail: bohuslav.melichar@fnol.cz
Bone metastasis from endometrioid ovarian carcinoma: a case study and literature review

N. Baize, A. Mahamat, E. Benizri, M.C. Saint-Paul, N. Mounier
Onco-Haematology Department, Archet Hospital, Nice (France)

Summary

Introduction: Bone metastases from epithelial ovarian carcinoma are rare, usually discovered post-mortem. The survival of these patients is poor. Furthermore, only two cases of endometrioid ovarian carcinoma with metastasis to the skeletal structures have been described in the literature. Case report: We present the case of a 58-year-old woman with a lytic metastasis in the left iliac ramus from endometrioid ovarian carcinoma that occurred seven years after the initial diagnosis. Discussion: A review of the literature since 1966 on bone metastasis of ovarian cancer is also presented. In patients suffering from a neoplasm that rarely metastasises to bone, histological proof should be obtained to diagnose uncommon sites of disease relapse.

Key words: Endometrioid carcinoma; Malignant ovarian tumour; Bone metastases.

Introduction

Epithelial ovarian cancer usually remains confined to the pelvis and abdomen [1, 2]. Distant metastases may occur anywhere but the liver is the most commonly involved site [1-3]. Bone metastases are anecdotal, especially in epithelial ovarian cancer [2, 4]. The majority of these bone metastases were revealed in autopsy findings [3-9]. Bone metastases discovered antemortem are exceptional [1, 2, 4, 10, 11]. Endometrioid ovarian cancer represents 12% of all ovarian carcinomas [6].

We reviewed the literature since 1966 and only two cases premortem were described in patients with endometrioid ovarian tumours [12, 13]. We report a third case with an exceptional late recurrence of bone metastasis occurring seven years after the initial diagnosis.

Case Report

In June 2000, a 58-year-old woman presented with abdominal pain and a palpable lower abdominal mass. Abdominal computed tomography (CT) showed a right-sided ovarian cancer. Tumor markers were normal (CA 125: 23 U/ml, normal < 35U/ml). Subsequently, the patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy, extended omentectomy and lymphadenectomy. Histological examination revealed well differentiated endometrioid carcinoma of the right ovary and right fallopian tube. The uterus was myomatous and associated with endometriosis. There were small implantations at the peritoneal surface. The disease was classified as Stage III A according to the FIGO 1986 staging system. Postoperatively, in September 2000, the patient presented with a rectovaginal fistula. CT scan showed involvement of the rectum and vagina. The surgical procedure was optimised and no visible residual disease remained. Six cycles of chemotherapy of paclitaxel (175 mg/m²) and carboplatin (area under the curve 5) were administered every three weeks. Five years later in October 2005 a blood test showed an elevation of serum CA125 to 51 U/ml. The tumour had recurred in the pelvis with involvement of the right ureter. A right uretero-nephrectomy was performed without residual disease. The patient received six cycles of the same regimen by paclitaxel and carboplatin and normalisation of CA125 was observed. Two years later in September 2007 she had a pain in the left pelvis. CT scan showed an isolated 50 mm osteolytic tumour of the left iliac ramus (Figure 1). Tumour markers were raised (CA125: 42 U/ml). A bone scan did not reveal other sites of metastases. The lesion was histologically confirmed as metastatic endometrioid ovarian carcinoma by fine-needle aspiration immunostaining with CA125 antibodies (Figure 2). The patient was treated with palliative radiotherapy (20 Gy/5 fractions) and bisphosphonates were given every month. Acceptable pain relief was achieved after treatment. In October 2007, a gynaecological examination performed secondary to bleeding showed a vaginal recurrence. A third line of chemotherapy with caelyx was given. Since then, the patient’s disease has remained stable with normalisation of CA125 (14 U/ml).

Discussion

A review of the literature shows that bone metastases from epithelial ovarian cancer has been well documented in many autopsy series [3-9] and in some clinical studies [1, 2, 10, 11]. Of these data, the incidence of bone metastases was 0.7 to 15% [1-11]. The incidence in autopsy studies is higher than that reported in clinical studies. In autopsy studies, the incidence of bone metastasis was up to 15% because about 50% of the sites of metastases were asymptomatic and unknown during the lifetime [2].

In clinical studies the incidence of bone metastases was 0.7 to 3.3%. Thus, since 1966, a total of 109 cases with osseous metastases among 1,638 patients presenting with epithelial ovarian carcinoma have been diagnosed, of which only 11 bone metastases were discovered antemortem (Table 1). This reflects the rarity of bone involvement in this malignancy and the rarity of our observation.

When bone metastases in ovarian carcinoma are discovered antemortem, they were usually associated with...
complaints of bone pain [1, 10]. These lesions are osteolytic [4, 10, 11], rarely osteoblastic [10]. The most common site observed was the vertebrae [1, 4, 10] followed by the ribs [4], femur [4, 11], skull [4], clavicle [1] and pelvic bones [4]. Many feel that the route of extension of bone metastases seems to be by haematogenous spread [4, 11].

Variation in histological type of carcinoma [3, 5, 8] and degree of histological malignancy [3, 5] did not significantly affect the pattern of bone metastases. Conversely, Abdul-Karim et al. showed that bone metastases occurred in high-grade carcinomas, but not in low-grade cases [4]. Few previous reports have specified the histological types of skeletal metastases [4, 9, 11]. In an autopsy series, Abdul-Karim et al. described three cases with papillary serous adenocarcinomas, two mixed adenosquamous carcinomas and one clear cell carcinoma [4]. Julian et al described three patients with bone metastases, two with papillary serous carcinoma and one with mucinous carcinoma [9]. Brufman et al. noted one mesonephric carcinoma [11].

Bone metastases from ovarian endometrioid cancer are exceptional. Two clinical cases have been described in the literature [12, 13]. The first case reported by Turan et al. was osteolytic metastasis in a phalanx discovered on a past medical history of pain and swolleness [12]. This patient had advanced disease with pulmonary metastases and subcutaneous lumps on the thorax. Histological analysis showed low-differentiated ovarian endometrioid bone metastasis. She died one month after the diagnosis. Sansom et al. reported a case of bone metastasis of endometrioid ovarian cancer in the left acetabulum. This patient was Staged IC and developed a bone lesion one month after surgery without evidence of intraabdominal disease or other distant metastases. She developed a local recurrence eight months later, with a pathological fracture in the left pubic ramus. She died 30 months after diagnosis of the bone lesion [13]. In our patient, the interval from primary diagnosis to bone tumour detection occurred late after the initial diagnosis of a bone lesion in contrast to Turam et al. and Sansom et al. [12, 13]. The bone lesion was osteolytic with a rapid bone resorption like Turam et al. [12] and was localised in the left iliac ramus like Sansom et al. [13].

Osseous metastases are rarely present at the time of diagnosis [1, 2, 4]. Median time to development of bone metastases is 21.8 months [1], ranging from 3-49 months [1, 4, 11]. In the present report, our patient developed isolated bone metastasis seven years after the diagnosis of an ovarian tumour. The presence of bone metastases in a patient with epithelial ovarian carcinoma is a grim prognostic sign indicating widespread disease. There were no differences in survival rate between the patients with serous and endometrioid carcinoma [5]. Survival time from radiographic detection of bone metastases was only four months, range 1-7 months [1]. Our patient’s disease became more aggressive after the diagnosis of isolated bone metastases because she presented vaginal recurrence just one month later.

Several studies suggest that the introduction of paclitaxel in the last decade has prolonged survival and con-
tributed to an increased frequency of uncommon metastatic sites [2, 6]. These studies showed that the median interval time between diagnosis of ovarian cancer and documentation of distant disease was much longer than 20 years ago (44 months and 15 months, respectively) [1, 2]. In 1987, Dauplat et al. reported that distant metastases may occur anywhere but for Cormio et al. the earliest metastases occurred in the liver, brain and skin while the later ones occurred in the bones and pleura [2]. Guth et al. suggested a changing pattern of disease spread in patients with ovarian cancer receiving cisplatin-based therapy. They compared patients who had received chemotherapy according to previous standards with patients who received current chemotherapy regimens. They showed that in this group the area significantly increased the incidence of liver metastases and less involvement of the lung and pleural cavity [6].

The treatment of ovarian cancer patients with bone metastases is not clear. Our patient had treatment with radiotherapy and biphosphonates. Radiotherapy is an established treatment for metastatic bone pain. Biphosphonates are effective for reducing skeletal complications such as bone pain and pathological fractures. Hirata et al. showed a direct inhibitory effect of biphosphonates on various ovarian cancer cell lines [14].

The recent study suggests that bone scans should be recommended for patients with skeletal symptoms, advanced clinical stage or a high-grade neoplasm [10]. Our observation supports the rarity of bone metastases and shows that bone metastases can occur late in the course of disease. We suggest that the long interval time between diagnosis of ovarian cancer and bone metastasis in our patient probably reflects the multimodality treatment.

References
Limbic encephalitis associated with immature teratoma

H. de Lins e Horta¹, ², M.D.; A. Fonseca de Castro¹, ³, M.D.; R. Porto Fonseca¹, ², ³, M.D.; A. Soares Fernandes Jr.¹, ², ³, M.D.; V. Soares Lima¹, ², ³, M.D.; L. Carvalho Neuenschwander¹, ², ³, M.D.

¹Oncomed, ²Department of Oncology, Felício Rocho Hospital, ³Department of Oncology Luxemburgo Hospital, ⁴Department of Oncology, Governador Israel Pinheiro Hospital (IPSEMG), ⁵Department of Oncology, São Francisco de Assis Hospital, Belo Horizonte (Brazil)

Summary

Paraneoplastic neurological syndrome (PNS) includes rare manifestations of different forms of cancer, in which the specific syndrome of limbic encephalitis can be found. This report describes a case of a previously healthy young lady who developed severe limbic encephalitis associated with an immature teratoma. After surgical treatment, the patient showed rapid progressive neurological improvement with complete regression of the symptoms during follow-up. Although rare, correct recognition and management of PNS is of great importance especially considering the fact that PNS can carry the risk of permanent disability or even death, even when associated with tumors in which high cure rates are expected.

Key words: Germ cell; Teratoma; Limbic encephalitis; Ovarian cancer; Paraneoplastic; Neurological syndromes.

Introduction

Paraneoplastic neurological syndrome (PNS) is characterised by signs or symptoms related to tissue damage at sites which are distant from the primary tumor or its metastasis. PNS is not related to metabolic, infectious, ischemic, or nutritional complications nor side-effects from the oncologic therapy [1]. Although some cases have been associated with benign tumours of indolent behaviour, in which high recovery rates are expected, such syndromes may have severe or even fatal outcomes. A severe case of limbic encephalitis associated with an ovarian teratoma is presented together with a brief review of the literature.

Case Report

A previously healthy 32-year-old female presented with a two-month history of abnormal behaviour and anxiety followed by depression, mental confusion, amnesia, and aphasia. The patient was initially treated by a psychiatrist with risperdal. Upon progressive worsening in the psychiatric and neurological states, the patient began to have a convulsive crisis and fever states, the patient began to have a convulsive crisis and fever and was subsequently transferred to a tertiary hospital. Empirical treatment with acyclovir was begun due to the suspicion of herpetic encephalitis with no improvement. A magnetic resonance imaging (MRI) brain scan revealed signs of rare abnormality spots in the subcortical white matter and in periventricular frontoparietal convexities. The patient deteriorated into a critical condition on the second postoperative day. A few days later, the patient was discharged from the hospital with discrete short-term memory impairment. Afterwards, she was submitted to adjuvant chemotherapy with the BEP (cisplatin, etoposide, and bleomycin) regimen and then underwent follow-up. Twenty-eight months after surgery the patient remains with no neurological deficits, negative tumour markers as well as no evidence of activity of the disease.

Discussion

Paraneoplastic neurological syndrome (PNS) is a rare manifestation of different forms of cancer, in which the specific syndrome of limbic encephalitis can be found. It is believed that the majority of PNS are immune-mediated, and their pathogeneses are related to the tumour’s ectopic expression of an antigen, which is normally expressed exclusively in the nervous system. This antigen is recognised by the immune system as foreign, leading to an abnormal immune response that can attack the nervous system (either by producing antibodies or through cellular response: B- and T-lymphocytes) [1-3].

Normally, neurological abnormalities precede the identification of the tumour. While in some cases the tumour presents indolent behaviour, neurological paraneoplastic-associated disorders may present rapid, severe, disabling, and, in some cases, lethal outcomes. Today many antibodies are recognised as being able to bind specific neurological and tumour targets, representing powerful tools for diagnosis. However, PNS can occur without the presence of an onconeural antibody, or the antibody may be present in the absence of the neurological syndrome; hence, the presence of an antibody is not the only condition that defines a paraneoplastic neurological syndrome [4-6].

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Considering their pathogeneses, the treatment of these syndromes is based on two pillars: withdrawing the antigen source through the treatment of the tumour and/or the suppression of the immune response through immunotherapy (e.g., corticosteroids, plasma exchange, intravenous immune globulin, cyclophosphamide, cyclosporine, and tacrolimus). Improvement of neurological symptoms can vary according to the nature of the paraneoplastic syndrome. Usually, in cases in which damage occurs in sites where the nervous system is able to regenerate (e.g., myelin layer, synapses), improvement is expected with treatment. When neurons are destroyed, the treatment can prevent the progression of neurological damage, but regression of the symptoms is rarely observed.

Limbic encephalitis is a classic PNS characterised by a progressive acute or sub-acute change in behaviour, mood, cognitive dysfunctions, hallucinations, and partially-complex convulsive crises. The selective loss of recent memory is a common alteration [7, 8]. Hypothalamic dysfunction leading to hyperthermia, somnolence, and endocrine abnormalities may also occur. Ventilation difficulties have been reported (central hypoventilation) [9]. Fluid exams show inflammatory alterations in up to 80% of the cases and alterations in nuclear MRI or CT can be found in 65-80% of the cases. Electroencephalographic findings include focal or generalised slowing, and occasionally epileptic activity, mostly in the temporal areas [1].

Symptoms of limbic encephalitis can also be found in other diseases, such as viral encephalitis (especially herpes simplex), systemic lupus erythematosus, Wernicke-Korsakoff encephalopathy, toxic effects of doxifluridine, and non-paraneoplastic limbic encephalopathy related to voltage-gated potassium channels [10, 11].

The most frequent tumours associated with this syndrome are lung cancer (especially small cell lung cancer (SCLC)), testicular cancer, thymoma, breast cancer, Hodgkin’s lymphoma, and immature teratoma. Antineuronal autoantibodies can also be found in up to 60% of the cases, including anti-Hu (normally related to SCLC),

Figure 1. — (A) Computed tomography of the pelvis showing a calcified ovarian mass. (B) Dry tumour section observed: (B.1) Well differentiated area with scaly tissue and skin annexes (H&E 100 X); (B.2) Poorly differentiated area with neural tissue (H&E 400X); (B.3) Mesenchymal areas with bone-cartilage differentiation (H&E 100 X).
anti-Ma2 (associated to testicular cancer), anti-Ma1, anti-CV2/CRMP5 (thymoma and SCLC), and anti-VGKC (thymoma and non-paraneoplastic limbic encephalitis), which is the most common [1].

The majority of patients with paraneoplastic limbic encephalitis do not respond well to treatment, although in some cases improvement of symptoms can occur (usually associated with primary tumour treatment and, rarely, with spontaneous resolution).

**Conclusion**

Ovarian germinative tumours are relatively rare, constituting 2% to 3% of all ovarian cancers. These tumours typically affect children and young women and present high cure rates. Immature teratomas are responsible for 10% to 20% of ovarian tumours found in women under 20 years of age, representing the second most common germinative cell tumour [12]. Patients with Stage I and histological grade 1 present a 5-year survival rate of 90% with surgical treatment. Although there are still controversies, adjuvant chemotherapy with BEP is recommended after surgery for patients with histological grades of 2 to 3 (Stage I) or Stages II to IV. A long-term survival rate of 75% to 80% is expected even in cases of diseases at advanced stages with incomplete resection [12, 13].

The present case study shows a previously healthy young patient, carrier of a tumour with a high rate of cure, and yet her paraneoplastic neurological conditions were considered severe, almost fatal. After appropriate treatment, regression of the symptoms could be observed, and the patient is now living a normal life with no symptoms at all. Although rare, it is important both for the oncologist as well as for the gynaecologist to clearly recognise PNS symptoms. Because of the risk of permanent neurological disability, a specific oncologic treatment or immunosuppressive therapy (even in the absence of a diagnosis of cancer) should not be delayed [14].

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Address reprint requests to:
H. DE LINS E HORTA, M.D.
Oncomed: 3106 Bernardo Guimaraes Street
30140-083, Belo Horizonte, MG (Brazil)
e-mail: henriquellih-onco@yahoo.com.br
Leiomyosarcoma of the broad ligament:
a case report and review of the literature

A. Kolusari¹, G. Ugurluer², M. Kosem³, M. Kurdoglu¹, R. Yildizhan¹, E. Adali¹

¹Department of Obstetrics and Gynaecology, ²Department of Radiation Oncology, ³Department of Pathology,
Yuzuncu Yil University, Faculty of Medicine, Van (Turkey)

Summary

Leiomyosarcoma of the broad ligament is a rare tumour, since only 15 cases have been reported thus far in the English literature. We describe the case of a 35-year-old patient with primary leiomyosarcoma of the broad ligament. The histologic diagnosis and management of this rapidly progressive and highly malignant tumour are also discussed. The tumor had high mitotic activity and more than ten mitotic figures were found for ten high-power fields. The treatment consisted of total abdominal hysterectomy, bilateral salpingo-oophorectomy and pelvic lymph node dissection. The patient received pelvic radiotherapy and chemotherapy considering the high grade of malignancy. No evidence of metastasis has been noted after a follow-up of 12 months.

Key words: Cervical cancer; Microsatellite instability; Clinicopathological parameters.

Introduction

Primary malignant tumors of the broad ligament are rare. The most common solid tumor of the broad ligament is a leiomyoma. According to the criteria for diagnosis of broad ligament tumours by Gardner et al. [1] the disease should be “completely separated from and in no way connected with either the uterus or the ovary”. Only 15 cases of primary leiomyosarcoma originating in the broad ligament have been reported in the English literature (Table 1) [2-15]. We describe here a new case of primary leiomyosarcoma of the broad ligament. The histologic diagnosis and management of this rapidly progressive and highly malignant tumor are also discussed.

Case Report

A 35-year-old, gravida 6 para 5, with one spontaneous abortion, was referred to our department by another institution after recurrent episodes of pelvic pain and bleeding with a diagnosis of pelvic-abdominal mass for further management. The patient was hospitalized with the same presumptive diagnosis. The pain had a dull character. There had been no alterations in bowel and/or bladder habits. Body weight had been stable. No other systemic disease was found. No surgical history was noted. On physical examination, the abdomen was distended with a large pelvic mass reaching the umbilicus on the right side. CT scan confirmed a 103 x 110 x 114 mm solid mass filling the pelvis with a heterogeneous density and irregular contours.

The gynecological examination was normal except for the finding of a mass arising from the pelvis. Laboratory findings were within normal values except Hgb 8.95, LDH 557 U/l, CA 125 59.9 U/Ml, CA 15-3 86.9 U/ml. Preoperative exams did not show any distant metastases. At laparotomy, there was a large solid, fibrotic, lobulated mass, about 15 cm, in the left adnexal area. It was surrounded by omentum. The uterus, the tubes and the ovaries were normal and all seemed completely independent of the mass. There was small amount of serous ascitic fluid. After peritoneal washings for cytology, the patient underwent excision of the mass (frozen section evaluation was performed and reported as malignant), total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, appendectomy and pelvic and paraaortic lymph node dissection. The patient received pelvic radiotherapy and chemotherapy postoperatively.

The chemotherapy protocol consisted of ifosfamide, mesna and adriamycin. The patient is being followed as an outpatient and remains free of disease even after 12 months.

Discussion

Primary leiomyosarcoma of the broad ligament of the uterus is a rare neoplasm. Definition is not obvious, but Gardner et al. [1] have developed the criteria that are still commonly followed for the diagnosis of this neoplasm. These authors established the definition of tumours of the broad ligament, requiring that they “occur in or on the broad ligament, but completely separated from and in no way connected with either the uterus or the ovary.” Using these criteria, the presented case is the 16th reported case of leiomyosarcoma of the broad ligament (Table 1) in the English literature. Our patient is also of interest, since her age of presentation was 35, with the mean being 55.4. Two patients in the literature were unusually young, 31 and 36 years [8, 9].
According to Gardner et al.’s criteria, the most common solid tumour of the broad ligament is leiomyoma. Leiomyosarcoma is rare and not only is the lack of any extension or connection with the uterus and other annexes mandatory, but also the microscopic pattern of the disease appears to play a crucial role in defining overall prognosis and postsurgical therapy. It occurs mainly in the postmenopausal years with nonspecific clinical manifestations and carries a poor prognosis. No cases have been diagnosed correctly before surgery. The preoperative and intraoperative differential diagnosis includes any tumour that takes its origin from or nearby the broad ligament. The differential diagnosis of microscopic findings should be carefully made between

Table 1. — Leiomyosarcoma of the broad ligament. Review of the available literature.

<table>
<thead>
<tr>
<th>Case</th>
<th>Author (year)</th>
<th>Age</th>
<th>Mitosis</th>
<th>Surgery</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>Lowell and Karsh (1968) (2)</td>
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<td>0-4</td>
<td>TAH, BSO</td>
<td>None</td>
<td>12 months OS</td>
</tr>
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<td>2</td>
<td>Ullmann and Roumell (1973) (3)</td>
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<td>15</td>
<td>TAH, BSO</td>
<td>RT, CT</td>
<td>7 months, DOD</td>
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<tr>
<td>3</td>
<td>Weed and Podger (1976) (4)</td>
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<td>12</td>
<td>TAH, BSO</td>
<td>CT</td>
<td>19 months, DOD</td>
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<tr>
<td>4</td>
<td>DiDomenico et al. (1982) (5)</td>
<td>48</td>
<td>10.5</td>
<td>TAH, BSO</td>
<td>None</td>
<td>NR</td>
</tr>
<tr>
<td>5</td>
<td>Raj-Kumar (1982) (6)</td>
<td>70</td>
<td>&lt; 10</td>
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<td>None</td>
<td>NR</td>
</tr>
<tr>
<td>6</td>
<td>Herbold et al. (1983) (7)</td>
<td>73</td>
<td>21</td>
<td>TAH, BSO</td>
<td>None</td>
<td>1 months, DOD</td>
</tr>
<tr>
<td>7</td>
<td>Shimm, McDonough (1987) (8)</td>
<td>31</td>
<td>8</td>
<td>Excision</td>
<td>RT, CT</td>
<td>&gt; 30 months OS</td>
</tr>
<tr>
<td>8</td>
<td>Lee et al. (1991) (9)</td>
<td>36</td>
<td>&gt; 10</td>
<td>TAH, BSO</td>
<td>CT, RT</td>
<td>&gt; 33 months</td>
</tr>
<tr>
<td>9</td>
<td>Lee et al. (1991) (9)</td>
<td>65</td>
<td>&gt; 10</td>
<td>STA H, BSO</td>
<td>CT</td>
<td>30 months, DOD</td>
</tr>
<tr>
<td>10</td>
<td>Cheng et al. (1995) (10)</td>
<td>59</td>
<td>&gt; 10</td>
<td>TAH, BSO</td>
<td>None</td>
<td>&gt; 12 months OS</td>
</tr>
<tr>
<td>11</td>
<td>Pekin et al. (2000) (11)</td>
<td>56</td>
<td>8</td>
<td>TAH, BSO</td>
<td>RT</td>
<td>&gt; 30 months OS</td>
</tr>
<tr>
<td>12</td>
<td>Shah et al. (2003) (12)</td>
<td>87</td>
<td>30-40</td>
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<td>None</td>
<td>2 months, DOD</td>
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<tr>
<td>13</td>
<td>Agarwal et al. (2003) (13)</td>
<td>55</td>
<td>&gt; 10</td>
<td>TAH, BSO</td>
<td>CT</td>
<td>&gt; 12 months OS</td>
</tr>
<tr>
<td>14</td>
<td>Murialdo et al. (2005) (14)</td>
<td>53</td>
<td>&lt; 10</td>
<td>TAH, BSO</td>
<td>None</td>
<td>&gt; 13 months OS</td>
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<tr>
<td>15</td>
<td>Ben Amara et al. (2007) (15)</td>
<td>49</td>
<td>NA</td>
<td>TAH, BSO</td>
<td>NA</td>
<td>5 months OS</td>
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<tr>
<td>16</td>
<td>This article</td>
<td>35</td>
<td>&gt; 20</td>
<td>TAH, BSO</td>
<td>RT, CT</td>
<td>&gt; 12 months OS</td>
</tr>
</tbody>
</table>

TAH, total abdominal hysterectomy; STA H, subtotal abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy; RT, radiotherapy; CT, chemotherapy; NR, not reported; NA, not available; OS, overall survival; DOD, died of disease.
leiomyosarcoma and leiomyoma, especially atypical leiomyoma and cellular leiomyoma. Mitotic figures (equal to or more than five vs less), cellularity of the tumour (hypercellular vs normocellular) and nuclear atypia (yes vs not) are considered as the most important criteria to differentiate leiomyosarcoma from leiomyoma [14]. These criteria were commonly used in previous recent reports, considering the mitotic figures (per ten high-power fields). Only one study [12] uses the criteria modified by Hendrickson and Kempson [16] for the diagnosis of uterine smooth muscle neoplasms and they have extrapolated their criteria to this unusual tumour in the absence of established parameters specific to smooth muscle tumours originating in the broad ligament (degree of cytological atypia none to mild or moderate to marked, presence or absence of coagulative tumor cell necrosis, mitotic index if moderate/severe atypia is present without necrosis).

As very few cases are reported, the exact nature and biological activity of leiomyosarcoma of broad ligament is still poorly understood and the staging and management are currently based on criteria used for uterine leiomyosarcoma [17]. As the Table 1 shows, the cornerstone remains total abdominal hysterectomy with bilateral salpingo-oophorectomy, but the adjuvant treatment is quite variable, ranging from no therapy to chemo- and/or radiotherapy. Adjuvant chemotherapy and/or radiotherapy may be used in selected cases. The treatment following the surgical procedures would be driven by the histologic grade of the neoplasm, which has shown a prognostic value. The case described in the present study showed a high histologic grade and was therefore considered as having a poor prognosis and received adjuvant therapy postoperatively.

References


Address reprint requests to:
A. KOLUSARI, M.D.
Yuzuncu Yil Universitesi
Arastirma Hastanesi
Kadin Hastaliklari ve Dogum Anabilim Dali
Van (Turkey)

e-mail: dralikolusari@yahoo.com
Fallopian tube carcinoma metastatic to the pericardium and breast

S. Buyukkurt¹, M.A. Vardar¹, H. Zeren², B. Guzel¹, I. Tuncer²

¹Department of Obstetrics & Gynecology, ²Department of Pathology, University of Cukurova School of Medicine, Adana (Turkey)

Summary

Introduction: Fallopian tube carcinoma is a rare gynecological tumor and simultaneous pericardial and breast metastasis of this cancer is an extremely exceptional event. Case: A 46-year-old woman with FIGO Stage IIIc, grade 3 adenocarcinoma of the fallopian tube was left at the end of the operation. The pathological examination of the specimen revealed grade 3 adenocarcinoma of the fallopian tube received cyclophosphamide and carboplatin subsequent to surgery. The disease had been completely silent for 41 months and then it relapsed with pericardial and breast metastasis consecutively. She expired one year after the relapse. Conclusion: Although clinical and biological behavior and response to the treatment of fallopian tube carcinoma is quite similar to epithelial ovarian carcinoma, breast and pericardium are unusual sites of metastasis for each malignancy. As survival is prolonged with new chemotherapeutics these atypical cancer metastases will be observed more frequently.

Key words: Fallopian tube carcinoma; Adenocarcinoma; Breast metastasis; Pericardial metastasis.

Introduction

Primary fallopian tube carcinoma accounts for a very small portion of female cancers. The clinical course of the disease is quite similar to epithelial ovarian cancer. Although fallopian tube carcinoma usually spreads intraperitoneally, distant metastases to the common target of the metastatic disease such as the brain, lung and bone have been reported previously [1-3]. Pericardium and breast are not common sites for metastasis. In this paper we report a case of fallopian tube carcinoma metastasizing simultaneously to the breast and pericardium which are rarely involved by metastatic disease.

Case Report

A 46-year-old, gravida 3, para 2, abortus 1 woman presented with weight loss, abdominal distention and fatigue in May 2003. These complaints had started two months before and her personal and familial medical history were unremarkable. A 3 x 4 x 4 cm solid, heterogeneous mass was discovered in the left adnexal area with diffuse ascites using sonography and computed axial tomography (CAT). Tumoral implantation on the peritoneum and omentum was also found. Cytological examination of the ascites fluid revealed malignant epithelial carcinoma. As the serum level of CA 125 was 345 U/ml, the initial diagnosis was ovarian carcinoma. Exploratory laparotomy was performed and a mass filling the left tube was seen accompanying the peritoneal carcinomatosis and omental caking. The opposite tube, both ovaries and uterus appeared normal. Total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, pelvic and paraaortic lymphadenectomy were performed. Any tumoral implant greater than 1 cm in diameter was left at the end of the operation. The pathological examination of the specimen revealed grade 3 adenocarcinoma of the uterine tube without lymph node involvement (Figure 1).

At first six cycles of paclitaxel and carboplatin as first-line chemotherapy were employed but the patient developed an allergic reaction to the former agent on the first day of the treatment and the chemotherapy regimen was changed to six cycles of cyclophosphamide with carboplatin. This chemotherapy regimen ended in October 2003. In October 2006 her CA 125 level was found to be 183 U/ml following three years where the disease had been radiologically and biochemically silent. Computed tomography (CT) of the thorax and abdomen was only remarkable for peritoneal carcinomatosis. Malignant epithelial cells were found in the peritoneal fluid. Five cycles of second-line chemotherapy were started with docetaxel and carboplatin. She received five cycles of third-line chemotherapy with liposomal doxorubicin, because her CA 125 level was still high (113 U/ml). The CA 125 level was persistently elevated (162 U/ml) and the patient’s respiratory complaints started when the chemotherapy regimen finished in June 2007. The chest radiogram was remarkable only for a minimally enlarged cardiac silhouette. Echocardiography demonstrated pericardial effusion and the ejection fraction was 55%. A catheter was placed in the pericardium and 220 ml of serosanguinous fluid was drained. The patient’s complaints improved considerably and the catheter was removed on the third day. The cytological examination of the pericardial fluid revealed malignant epithelial carcinoma (Figure 2). Simultaneously a 3 x 3 cm solid mass was palpated at the upper outer quadrant of the left breast. The breast examination was not significant for peau d’orange, nipple discharge or retraction. Excisional biopsy of the mass revealed adenocarcinoma with disseminated intra-lymphatic tumoral thrombus (Figure 3). The immunohistochemical evaluation of the sample was negative for estrogen receptor, progesterone receptor, c-erb 2 and gross cystic disease fluid protein (GCDFP)-15.

The patient refused additional therapy and expired in October 2007, 53 months after the initial diagnosis.

Discussion

Carcinoma of the fallopian tube accounts less than 0.5% of all female cancers [1]. The classical symptoms

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of tubal carcinoma are pelvic pain, pelvic mass and serosanguinous vaginal discharge which are known as the Latzko triad [3]. The biological and clinical behavior of fallopian tube carcinoma closely resemble that of ovarian cancer. While dissemination of the tumor usually occurs in the peritoneal cavity, lymphatic and hematogenous spread of the tumor may rarely be seen [1-3].

Breast involvement is a rare entity in oncology. The incidence of secondary breast cancer in autopsy series varies from 0.4% to 6.6% while nearly 1% is observed clinically. The range is dependent on whether hematological malignancies are included or not [4]. The most common source of metastasis to the breast is the opposite breast. Leukemia, lymphoma, malignant melanoma and lung carcinoma are the most common cancers metastasizing to the breast. Ovarian cancer is the commonest gynecological cancer that spreads to the breast [5]. The clinical findings of breast metastasis are not similar to that of primary malignant breast cancer. While metastatic lesions in the breast tend to be superficial, they cause neither skin nor nipple retraction, nor nipple discharge. The mass is usually firm, mobile and painless. The diagnosis requires a strong suspicion and knowledge of the history of primary malignant carcinoma [4, 5]. Metastatic lesions to the breast do not show the mammographic features of primary malignancy, such as microcalcification and spiculation. Metastasis to the breast may be through the lymphatic or hematogenous route and each has different mammographic features. Lymphatic metastasis shows subcutaneous trabeculation and irregularity. Glandular stroma is denser as well. Lymphatic metastases to the breast and inflammatory breast carcinoma have similar mammographic appearances. Hematogenous metastasis to the breast may exhibit a solitary, well demarcated lesion which seems to be benign fibroadenoma. On the other hand, multiple or diffuse involvement may also be seen in hematological metastasis [6]. Fine needle aspiration cytology, incisional or excisional biopsy are the appropriate ways of the histological diagnosis. Radical excision of the breast mass is redundant unless it becomes an ulcerated or excessively huge mass [5].

The incidence of neoplastic cardiac invasion is 10% to 20% in autopsy series and 85% of them have pericardial involvement [7]. Nearly one-third of pericardial involvement is symptomatic [8]. Symptoms of pericardial involvement are related to the amount of fluid and the rapidness of fluid accumulation. A huge amount of fluid collected slowly in the pericardium may be asymptomatic whereas a small amount of pericardial effusion may produce cardiac tamponade when it is stored quickly.
Clinical findings are usually nonspecific and the diagnosis can be made easily via echocardiography [9]. Pericardiocentesis is an easy procedure to relieve the symptoms and collect a fluid sample for pathological analysis. Pericardial effusion can be managed via catheter drainage, systemic chemotherapy, intrapericardial chemotherapeutics, radioisotopes or sclerosing agent instillation and external radiotherapy. Stage and histopathology of the disease should be kept in mind when selecting a treatment modality stated above. Catheter drainage should be used initially because it is an easy procedure and effective in most cases. When malignant pericarditis relapses, instillation therapies should be considered. On the other hand, systemic chemotherapy or external radiotherapy may be favored for cancers which are extremely sensitive to these therapies, like lymphoma or leukemia [7, 9].

Breast or pericardial invasion of gynecological malignancies usually signifies widely disseminated disease. Extra abdominal metastasis of fallopian tube carcinoma has rarely been reported. In this report, we have presented a case of tubal carcinoma which was asymptomatic for 41 months and then pericardial and breast metastasis developed respectively. A review of the previous reports showed that this is the first case of pericardial metastasis and the second case of breast metastasis of the primary fallopian tube. As new chemotherapeutical drugs gain success in the treatment of epithelial ovarian cancer as well in that of tubal cancer, we believe that these atypical sites of metastasis will be involved more frequently. Precise diagnosis of the metastatic disease is very important for accurate management. The aim of the treatment should only be to relieve the symptoms and avoid radical excision of the breast tumor or extensive pericardiectomy.

References

Address reprint requests to:
S. BUYUKKURT, M.D.
Department of Obstetrics & Gynecology
University of Cukurova School of Medicine
01330, Adana (Turkey)
e-mail: selimbuyukkurt@gmail.com
Virilizing ovarian hilus (Leydig) cell tumor with concurrent contralateral hilus cell hyperplasia: a rare diagnosis

M. Zafrakas¹, I.D. Venizelos², T.D. Theodoridis¹, L. Zepiridis¹, T. Agorastos¹, J.N. Bontis¹

¹1st Department of Obstetrics & Gynecology, Aristotle University of Thessaloniki, Papaogeorgiou General Hospital
²Department of Pathology, Hippokrateio General Hospital, Thessaloniki (Greece)

Summary

Ovarian hilus or Leydig cell tumor and ovarian hilus cell hyperplasia are rare clinical entities, causing virilization in both pre- and postmenopausal women. Differentiation between these two conditions is not always straightforward; the former is usually unilateral appearing as a single, grossly visible, circumscribed mass of hilus cells, while the latter is usually bilateral, appearing as diffuse microscopic aggregates of hilus cells. We report herein an extremely rare case of ovarian hilus or Leydig cell tumor, presenting concurrently with contralateral ovarian hilus cell hyperplasia in a postmenopausal woman with virilization. To the best of our knowledge, only four such cases have been previously reported in the literature. Ovarian hilus cell tumors and hilus hyperplasia almost always have benign biological behavior, thus making bilateral salpingo-oophorectomy an appropriate and sufficient therapeutic approach.

Key words: Ovarian Leydig cell tumor; Ovarian hilus cell tumor; Ovarian hilus cell hyperplasia; Virilization; Ovarian tumor.

Introduction

Virilization in postmenopausal women is a rare clinical condition. The underlying cause in such cases is hyperandrogenemia due to androgen over-production in either the adrenal glands or the ovaries. Ovarian Leydig or hilus cell tumor is a rare cause of postmenopausal virilization [1]. An extremely rare case of ovarian Leydig or hilus cell tumor presenting concurrently with contralateral ovarian hilus cell hyperplasia in a postmenopausal woman with virilization is reported.

Case Report

A 55-year-old white female, G2, P2, was referred to our Department, due to partial scalp alopecia and facial hirsutism. On admission, physical examination revealed increased hair growth on the patient’s breasts, back and extremities, while mild clitoris enlargement was noted. There was no palpable ovarian tumor on pelvic examination, and no abnormal findings on transvaginal ultrasound scan. Measurement of circulating hormones showed increased levels of testosterone, while androstenedione and dehydroepiandrosterone (DHEAS) were normal. In contrast, cortisol metabolites were within the normal range, and Cushing’s syndrome was ruled out by an overnight dexamethasone suppression test. Magnetic resonance imaging (MRI) of the head showed a normal pituitary gland. Computed tomography (CT) of the upper and lower abdomen did not show any pathological signs; particularly both adrenal glands appeared normal. Subsequently, the patient underwent an exploratory laparotomy with bilateral salpingo-oophorectomy due to the presumptive diagnosis of virilization of ovarian origin. On macroscopic examination, a well circumscribed, 2-cm, rubbery, yellowish-orange tumor was found in the left ovary; there were no abnormal macroscopic findings in the contralateral gonad. Microscopically, the well-circumscribed tumor of the left ovary consisted of polyhedral cells almost identical to normal hilus cells, infiltrating the adjacent ovarian stroma and vascular spaces (Figure 1). Interestingly, microscopic examination of the right ovary also showed foci of Leydig cells infiltrating the adjacent ovarian stroma and vascular spaces, a growth pattern consistent with hilus cell hyperplasia (Figure 2). No components of Sertoli cell tumor were observed. Immunohistochemical analysis showed that Leydig cells in both ovaries were positive for α-inhibin (Figure 3) and vimentin, findings consistent with morphological diagnosis, and negative for pancytokeratin (AE1/AE3), epithelial membrane antigen (EMA), CD68, desmin and smooth muscle actin (SMA), findings excluding epithelial or muscular origin [3]. Testosterone...
levels were measured one month postoperatively and were found to be normal. No additional therapy was given to the patient postoperatively, and two years later she was still doing well, with no signs of tumor or virilization recurrence.

Discussion

Ovarian hilus or Leydig cell tumor is a rare clinical entity, causing virilization in both pre- and postmenopausal women. These tumors consist of hilus cells which are normally found in the hilus of most normal ovaries and are morphologically identical to testicular Leydig cells [1, 3]. In this report an extremely rare case of a unilateral ovarian hilus cell tumor with concurrent hilus cell hyperplasia in the contralateral ovary is presented. To the best of our knowledge, only four such cases have been previously reported in the literature [1, 4]. It should be noted, that bilateral hilus cell tumors are also rare, with only four reported cases [3, 4-7].

Differentiation between hilus cell tumor and hilus cell hyperplasia on histologic examination can sometimes be difficult; a single, grossly visible, circumscribed mass of hilus cells is usually characterized as an adenoma or a tumor, while diffuse microscopic collections or aggregates of hilus cells are usually called hyperplasia [3,8]. Both pathologic entities may cause androgen over-production and virilization. Ovarian hilus cell tumors are usually unilateral, while hyperplasia usually affects both ovaries [3], and is sometimes found lining the wall of ovarian cysts [8,9].

Ovarian Leydig cell tumors should not be confused with ovarian Sertoli-Leydig tumors, which may also cause clinical virilization in 70-85% of patients [10, 11]. Normally, there are no Sertoli cells in the adult ovary, while Leydig-like cells are usually found in the hilus – in at least 83% – of normal female gonads [1, 3, 10]. It has been suggested that in Sertoli-Leydig cell tumors, cells of the sex cord differentiate into Sertoli cells, while Leydig cells originate from normal cells of the hilus [10-12].

The ovarian origin of virilization can be suspected by the presence of elevated levels of circulating androgens, with normal levels of cortisol metabolites and a negative dexamethasone suppression test. Suppression of androgen levels after administration of GnRH analogues and exogenous estrogens has been described as part of the diagnostic work-up of these tumors [13, 14], but histological examination is the only way to establish a definitive diagnosis. Co-existence of hilus cell tumors with a normal pregnancy [1, 15, 16], other ovarian tumors [17] as well as pathologic conditions of the endometrium, including polyps, hyperplasia, and carcinoma [1, 18, 19], has been previously reported.

The therapeutic management of ovarian hilus cell tumors and hilus cell hyperplasia is primarily operative, consisting of bilateral salpingo-oophorectomy, either laparoscopically [20, 21] or with a classical open procedure. Since these tumors almost always have benign biological behavior [1], hysterectomy is not usually necessary. After oophorectomy androgen levels usually rapidly return to the normal range, and virilization subsides progressively to a varying extent thereafter [8].

References


Address reprint requests to:
M. ZAFRAKAS, M.D.
1st Department of Obstetrics & Gynecology
Aristotle University of Thessaloniki
Papageorgiou General Hospital
Periferiaki Odo Thessalonikis, N. Efkaris
56403 Thessaloniki (Greece)
e-mail: mzafrakas@gmail.com
Hepatoid carcinoma of the ovary.
A case report and review of the literature

A. Zizi-Sermpetzoglou, N. Petrakopoulou, M.E. Nikolaidou, N. Tepelenis, V. Savvaidou, Th. Vasilakaki

Department of Pathology, Tzaneion General Hospital of Pireaus, Piraeus (Greece)

Summary
Hepatoid carcinoma (HCO) is a rare ovarian tumor and is thought to be a different subtype from hepatoid-type yolk sac tumor based on its pathologic features. In contrast to hepatoid yolk sac tumor in which the patients are usually young, patients with HCO are elderly with a peak incidence during the sixth decade of life. None of the patients with HCO have had gonadal dysgenesis or recognizable germ cell components within the tumors. We describe a case of a 42-year-old woman who presented to our hospital complaining of abdominal pain. Physical examination and CT scan revealed a large tumor in the left adnexa. She underwent total hysterectomy and bilateral salpingo-oophorectomy with omentectomy. A left ovarian mass measuring 11 cm in diameter was found. Histological diagnosis was hepatoid carcinoma of the left ovary. Immunohistochemical findings suggest that hepatoid carcinoma of the ovary is probably a most likely variant of a common epithelial carcinoma by a process of neometaplasia or transdifferentiation.

Key words: Hepatoid carcinoma; Ovary; Immunohistochemical markers.

Introduction
Hepatoid carcinomas are a rare group of extrahepatic tumors and can be found in the stomach, pancreas, kidney and urinary bladder. These tumors show liver differentiation and produce α-fetoprotein (AFP) [1, 5]. In 1987, Ishikura and Scully described five cases of ovarian carcinoma with hepatoid features [4, 5]. Hepatoid carcinoma of the ovary (HCO) is rare and only a few cases have been reported in the literature. The age of patients ranges between 42 and 78 years. In contrast to the hepatoid type of yolk sac tumor, HCO does not occur in association with any germ cell elements and seems to represent a somatic-derived variant of adenocarcinoma [5].

We report an additional case of hepatoid carcinoma of the ovary in a 42-year-old woman. The histological and immunohistochemical findings are briefly discussed through a literature review.

Case Report
A 42-year-old premenopausal woman, gravida 2, para 0, presented with abdominal pain, persisting for about three months. There was no familial history of gynecological disease. On pelvic examination a left ovarian tumor, approximately 11 cm in diameter was palpable, while the size of the uterus and right ovary were normal.

Biochemical tests including renal and liver functions were within normal range, except for a minimal elevation of serum CA 125 (70 u/ml, normal 0-35 u/ml). Serum α-fetoprotein (AFP) concentration was not measured preoperatively. Computed tomography (CT) scan revealed a solid tumor of low density in the pelvic cavity. No abnormalities were seen in the liver, pancreas, or kidneys on magnetic resonance imaging (MRI) or CT. The patient underwent a total hysterectomy, bilateral salpingo-oophorectomy with omentectomy. The left ovary was enlarged to 11 x 7 x 7 cm and the right ovary was 4 x 3 x 2 cm. Uterine tubes were normal and no other intraabdominal abnormalities were noted.

The cut surface showed a solid tumor, soft and yellow with foci of necrosis and cystic areas containing blood. Microscopically the tumor was composed of sheets of cells with moderate to abundant amounts of eosinophilic cytoplasm and round to ovoid nuclei. Most of the cells were uniform in size and shape but occasionally giant cells, some of them multinucleated, were also observed (Figures 1/2). Green-yellow pigment was found within the canaliculi stained by the Fouchet method for bile. PAS-positive, diastase-resistant hyaline globules could be seen and glycogen was demonstrated within the cytoplasm of the tumor cells. Immunohistochemically the tumor cells were positive for AFP, hepatocyte paraffin 1 antibody (Hep Par 1), polyclonal CEA, albumin, A1AT (α1-antitrypsin) and EMA. Polyclonal CEA antibody showed a membranous pattern of staining (Figure 3). The cells were negative for cytokeratins AE1 and AE3, CK7, CK18, CK19, CK20, CD10, CD30, TTF1, hCG, vimentin and inhibin (Table 1).

Discussion
The term “hepatoid” has been used in the literature to describe a heterogenous variety of tumors in several organs [1, 4, 6, 9, 11], which can be subdivided into three wide categories.

The first is a variant of a germ cell tumor, arising either in the ovary or mediastinum and shows an origin of germ cells (germinal differentiation) with some hepatoid areas. These lesions stain intensely for AFP (especially in the hepatoid areas), show scarce positivity for A1AT (α1-antitrypsin) and EMA. Polyclonal CEA antibody showed a membranous pattern of staining (Figure 3). The cells were negative for cytokeratins AE1 and AE3, CK7, CK18, CK19, CK20, CD10, CD30, TTF1, hCG, vimentin and inhibin (Table 1).
carcinoma” which is a rare group of extrahepatic tumors found in the stomach, pancreas, cervix, endometrium, renal pelvis and urinary bladder [10, 11]. It is characterized histologically by an admixture of areas of tubulopapillary adenocarcinoma and foci of hepatoid differentiation. These tumors show a variable pattern of staining for AFP (frequently patchy). They stain consistently for A1AT and in a few cases some of the tumor cells are positive for pCEA.

The third category (non-hepatoid AFP-producing carcinoma) includes tumors that produce AFP without any morphological features of hepatocellular differentiation.

In 1987 Ishikura and Scully first recognized the entity “hepatoid adenocarcinoma of the ovary” [4]. They described five cases of ovarian carcinoma with hepatoid features, three of them primary and two probably primary. The age of the patients ranged between 42 and 78 years. Clinically patients usually present with symptoms and signs related to the presence of an adnexal mass. The main symptom is abdominal enlargement which may be associated with pain, malaise and weight loss [5]. Macroscopically the tumors are solid or solid and cystic and measure up to 20 cm in the greatest diameter. Histologically HCO resembles hepatocellular carcinoma and is composed of solid sheets or aggregates of uniform cells with moderate or abundant eosinophilic cytoplasm, distinct cell borders and centrally located nuclei with prominent nucleoli. Mitoses, some even atypical, are frequent. PAS-positive diastase-resistant hyaline globules may be seen and glycogen can be demonstrated within the cytoplasm of the tumor cells [14, 16, 17]. Immunohistochemical studies indicate that a significant number of tumor cells are positive for AFP, albumin, and A1AT. There is also focal positivity for pCEA (usually membranous). A new antibody, hepatocyte paraffin 1, is a monoclonal antibody which reacts with normal as well as with neoplastic hepatocellular carcinoma. Table 1. — Immunohistochemical results of our case (hepatoid ovarian carcinoma).

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Source</th>
<th>Type/PM</th>
<th>Dilution</th>
<th>HCO</th>
</tr>
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<tbody>
<tr>
<td>AFP</td>
<td>Dako</td>
<td>M</td>
<td>Ready</td>
<td>+</td>
</tr>
<tr>
<td>Hep Par 1</td>
<td>Dako</td>
<td>M</td>
<td>1:100</td>
<td>+</td>
</tr>
<tr>
<td>pCEA</td>
<td>Dako</td>
<td>P</td>
<td>1:200</td>
<td>+*</td>
</tr>
<tr>
<td>Albumin</td>
<td>Dako</td>
<td>M</td>
<td>1:80</td>
<td>+</td>
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<td>A1AT</td>
<td>Biogenex</td>
<td>M</td>
<td>1:100</td>
<td>+</td>
</tr>
<tr>
<td>EMA</td>
<td>Novocastra</td>
<td>M</td>
<td>1:200</td>
<td>+</td>
</tr>
<tr>
<td>Cytokeratin AE1 and AE3</td>
<td>Novocastra</td>
<td>M</td>
<td>1:50</td>
<td>–</td>
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<tr>
<td>CK7, CK18, CK19, CK20</td>
<td>Novocastra</td>
<td>M</td>
<td>1:50</td>
<td>–</td>
</tr>
<tr>
<td>CD10</td>
<td>Novocastra</td>
<td>M</td>
<td>1:50</td>
<td>–</td>
</tr>
<tr>
<td>CD30</td>
<td>Novocastra</td>
<td>M</td>
<td>1:20</td>
<td>–</td>
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<td>TTF1</td>
<td>Novocastra</td>
<td>M</td>
<td>1:50</td>
<td>–</td>
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<td>hCG</td>
<td>Novocastra</td>
<td>M</td>
<td>1:30</td>
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<td>M</td>
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<td>Inhibin</td>
<td>Zymed</td>
<td>M</td>
<td>1:40</td>
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</tr>
</tbody>
</table>

* pCEA (polyclonal carcinoembryonic antigen) showed a membranous pattern of staining.

P polyclonal antibody, AFP alpha-feto protein, Hep Par 1, Hepatocyte paraffin 1, A1AT alpha 1 antitrypsin, EMA epithelial membrane antigen, hCG beta human chorionic gonadotropin.
The differential diagnosis of HCO is very difficult to make on three years after surgery and pelvic irradiation. The differential diagnosis usually includes disseminated disease and death of disease within a few years of diagnosis. Our patient remains free of disease, and the findings suggest that HCO is probably derived from carcinoma, the cytokeratin profile of these cells resembles that of the hepatocyte phenotype. The HCO cells share a hepatocyte phenotype. The immunohistochemical studies demonstrated that HCO cells are not stained by AFP except in one case of endometrioid carcinoma for which there occurs mainly in younger patients. The immunohistochemically a Hyst stains negative for Hep Par1 and focally positive (cytoplasmic staining) for pCEA. In contrast to this HCO, as already mentioned, stains positive for Hep Par1 as well as for pCEA with a diffuse membranous stain (Table 2). HCO must also be distinguished from other ovarian tumors, such as several undifferentiated carcinomas, steroid cell (lipid cell) tumors, endometrioid carcinomas, and clear cell carcinomas[2, 5, 7, 14, 15]. All these neoplasms may have features resembling hepatocellular carcinoma but they are not stained by AFP except in one case of endometrioid carcinoma for which there were positions of transformation to a yolk sac tumor.

Finally, the histogenesis of hepatoid adenocarcinoma remains a matter of debate. Many authors believe that a multipotent cell origin or a differentiation of tumor cells in an endodermal direction is responsible for the hepatoid characteristics of this rare tumor. Scully et al. regard HCO as a variant of a common epithelial carcinoma[4].

Later, Tochigi et al. reported three cases of HCO admixed with a common surface epithelial carcinoma[13]. Using immunohistochemical studies they demonstrated that HCO cells share a hepatocyte phenotype. The cytokeratin profile of these cells resembles that of the associated common epithelial adenocarcinoma. These findings suggest that HCO is probably derived from carcinoma of surface epithelial origin by a process of neometa/plasia or transdifferentiation.

In conclusion, hepatoid carcinoma of the ovary is a highly malignant neoplasm. Most patients present with disseminated disease and die of disease within a few years of diagnosis. Our patient remains free of disease three years after surgery and pelvic irradiation. The differential diagnosis of HCO is very difficult to be made on morphological grounds alone. Immunohistochemistry and in situ hybridization techniques are of great help in the distinction of HCO and other ovarian tumors with hepatoid features.

Table 2. — Comparison of hepatoid yolk sac tumors with hepatoid carcinomas of the ovary.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HYST*</th>
<th>HCO*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of patients</td>
<td>7-54 yrs (average 22)</td>
<td>42-78 yrs (average 63)</td>
</tr>
<tr>
<td>Associated ovarian disease</td>
<td>Gonadal dysgenesis, dysgerminoma, other pattern of YST</td>
<td>No</td>
</tr>
<tr>
<td>Cellular uniformity</td>
<td>Present</td>
<td>Usually absent</td>
</tr>
<tr>
<td>Giant, bizarre cells</td>
<td>Usually absent</td>
<td>Present</td>
</tr>
<tr>
<td>Hyaline globules</td>
<td>Many</td>
<td>Many</td>
</tr>
<tr>
<td>Glandular structures</td>
<td>Occasionally (3/14)</td>
<td>Rare</td>
</tr>
<tr>
<td>Hep Par 1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AFP</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pCEA</td>
<td>+ (focal cytoplasmic)</td>
<td>+ (diffuse membranous)</td>
</tr>
</tbody>
</table>


References

Address reprint requests to: A. ZIZI-SERMPEZTOGLOU, M.D. P.O. Box 3143 - Alikos 19400 Ag. Marina, Koropi Athens (Greece) e-mail: adserbet@yahoo.gr
Giant uterine leiomyomas causing bilateral hydronephrosis coexisting with endometrial cancer in polyp: a case study

A. Semczuk¹, P. Skorupski¹, P. Olcha¹, D. Skomra², T. Rechberger¹, M. Gogacz¹

¹² Department of Gynecology, ¹Deptmen of Pathology, Lublin Medical University, Lublin (Poland)

Summary

Multiple uterine leiomyomas are present in a large population of women and may cause several uncommon clinical symptoms, including disseminated vein thrombosis and hydronephrosis. We report a case of giant uterine leiomyomas causing bilateral hydronephrosis coexisting with endometrial cancer (EC) deriving from a uterine polyp. A 50-year-old woman was admitted due to bilateral hydronephrosis caused by monstrous abdominal tumor to the II¹ Department of Gynecology, Lublin Medical University, Lublin, Poland. A bilateral double-J catheter was inserted. Pelvic examination revealed a huge, rough tumor, originating from the uterus. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed, and a giant uterus weighing 15.2 kg and measuring 35 x 29 x 18 cm was removed. Histopathological examination revealed multiple uterine leiomyomas with calcification and partial necrosis, and well-differentiated (G1), endometrioid-type EC (Stage IA) concomitant with atypical endometrial hyperplasia, deriving from a uterine polyp. The postoperative recovery was without complications, and the patient was discharged on postoperative day 10. In conclusion, giant uterine leiomyoma may incidentally compress the urinary tract organs, causing hydronephrosis.

Key words: Uterine leiomyoma; Hydronephrosis; Endometrial carcinoma; Endometrial polyp.

Introduction

Uterine leiomyomas are the most common benign tumors of the uterus, present in up to 30% of women over 30 years of age [1]. They are asymptomatic in general, however, clinical presentation and additional symptoms are associated with their size and localization. Mitotic activity of the leiomyoma is usually low, less than five mitotic figures per 10-high-power fields which differentiate them from uterine leiomyosarcoma [2]. Even small submucosal leiomyomas may cause abnormal uterine bleeding and compromise the vascular supply. In selected cases, giant leiomyomas are detected during pelvic examination and may be connected with many signs and symptoms, including abdominal pain, a sensation of pressure and abnormal uterine bleeding [3].

Giant uterine leiomyomas may be associated with mechanical obstruction of the venous system as well as compression of the urinary tract organs. For example, thrombosis caused by compression of the pelvic veins by uterine leiomyomas has been independently reported by Dekel and co-investigators [4], and by Khilanani and Dandolu [5]. Hawes et al. [6] described complete compression of the distal inferior vena cava and both ureters (with associated hydronephrosis) by giant uterine fibroids measuring 25 cm at the maximum diameter. There are only a few case reports describing giant leiomyomas originating from the urinary bladder [7] or from the ovary [8], causing hydronephrosis. Obstructive symptoms of the urinary tract are usually associated with the localization of the leiomyomas as well as their monstrous size.

In the current study, we report a case of giant uterine leiomyomas causing bilateral hydronephrosis coexisting with well-differentiated (G1), endometrioid-type endometrial cancer (EC) deriving from a uterine polyp. It is worth pointing out that the diagnosis of cancer was made incidentally after careful histopathological examination of the postsurgical material.

Case Report

A 50-year-old women (gravida 1, para 2), presenting with a giant abdominal tumor, was admitted the to II¹ Department of Gynecology, Lublin Medical University, Lublin, Poland, for surgical intervention. At first, the patient was hospitalized at the Department of Urology of the University, due to bilateral hydronephrosis caused by a monstrous abdominal tumor, where bilateral double-J catheters were inserted. Pelvic examination revealed a huge, rough tumor, likely originating from the uterine corpus (Figure 1A). Physical examination of the heart and lungs revealed no abnormalities. Transabdominal ultrasound (US) scans showed an approximately 30-cm tumor, circumferential and heterogeneous in appearance, with focal calcification. The mass showed complete compression of both ureters. An abdominal computed tomography (CT) scan revealed that the tumor originated from the uterine corpus; there were no abnormalities in the ovaries and in the oviducts, and there was no fluid in the abdominal cavity. Before surgery, subcutaneous treatment of enoxaparin (40 mg/d) was initiated and 2 U of packed red blood cells were prepared for intraoperative transfusion. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed in which a monstrous uterus, weighing 15.2 kg and measuring 35 x 29 x 18 cm, was removed (Figure 1 B-C). Histopathological examination revealed giant uterine leiomyomas with calcification and partial necrosis, and a well differentiated (G1), endometrioid-type EC deriving from a uterine polyp (Figure 2A). This endometrial...

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Figure 1. — Preoperative view of the patient showing a giant abdominal tumor (A). Hysterectomy in process (B). Monstrous uterus was surgically removed (C).

Figure 2. — Early-stage endometrioid-type EC developed in an endometrial polyp (A). An area of complex atypical hyperplasia was also detected (B).
polyp was found postoperatively during careful evaluation of the uterine cavity. Atypical endometrial hyperplasia coexisted with uterine cancer which did not infiltrate the myometrial wall (Figure 2B). Tumor was Stage IA according to the FIGO classification [9]. The postoperative course was without any complications, and the patient was discharged on postoperative day 10. After one week, the patient returned to the Urology Outpatient Department where the double-J catheters were removed.

**Discussion**

Multiple uterine leiomyomas are present in a large population of women and may cause several rare clinical symptoms, including disseminated vein thrombosis, hydroureter, hydronephrosis, and intestinal gangrene [6]. Among the various, dangerous complications during pregnancy ascribed to massive uterine leiomyomas are spontaneous abortions, PROM (premature rupture of the membranes), cervical dystocia, inversion of the uterus, and postpartum hemorrhage [1].

The present report has described an unusual case of monstrous uterine leiomyomas compressing both ureters, causing bilateral hydronephrosis. The patient was first admitted to the Urology Department at the Lublin Medical University, Lublin, Poland, where double-J catheters were inserted. The diagnostic imaging (USG, CT) performed showed the origin of the abdominal masses from an enlarged uterus. Detailed histopathological assessment of the postsurgical material revealed multiple uterine leiomyomas with calcification and partial necrosis. In the literature, Nakata et al. [10] described a rare case of bladder leiomyoma with marked bilateral hydronephrosis caused by chronic urinary retention. Giant uterine leiomyoma causing acute abdomen and bilateral hydronephrosis has been reported by Khaafaf and co-investigators [8]. Large uterine fibroids causing compression of both ureters concomitantly with mechanical obstruction of the inferior vena cava and subsequent thrombosis, have been previously noted [6]. Therefore, it is worth pointing out that monstrous uterine fibroids may rarely compress the urinary tract organs, causing hydronephrosis.

In general, about 5% of uterine polyps contained EC, whereas 12-34% of endometrial malignancies coexisted with uterine polyps [11-13]. It has been suggested that endometrial polyps may represent a marker of increased cancer risk because they reflect the ability of the endometrium to develop proliferative lesions [11]. In the current study, well-differentiated, endometrioid-type histology (similar to the data presented herein). They finally suggested to implicate surgical procedures in most postmenopausal women affected by complex atypical hyperplasia/uterine carcinoma in a polyp even if these changes were confined to the polyp in the initial diagnostic procedures.

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**References**

Renal cell carcinoma in pregnancy: a case report

J. Stojnić, K. Jeremic, S. Petković, G. Lazović, A. Stefanović

Institute for Gynecology and Obstetrics, University of Belgrade, Clinical Center of Serbia, Belgrade (Serbia and Montenegro)

Summary

Renal cell carcinoma is seen most frequently after childbearing years, but occasionally is diagnosed in pregnancy. The pregnancy demands special considerations in terms of the diagnostic evaluation and management. A case of a patient with renal cell carcinoma diagnosed in the first trimester of pregnancy, which suddenly enlarged at the end of the second trimester, is presented. She underwent radical nephrectomy after delivery. Since the mother’s welfare is the primary concern, surgical management should not be delayed.

Key words: Renal cell carcinoma; Pregnancy; Prognosis.

Introduction

More than 70 cases of renal cell carcinoma in pregnancy have been reported in the literature [1]. Renal cell carcinoma is rare in pregnancy, yet cases of nephrectomy have been performed by an open technique or by simultaneous cesarean section and radical nephrectomy. Successful laparoscopic nephrectomy has even been reported during the first trimester of pregnancy with normal delivery of a healthy baby at term [2].

Ultrasound (US) is the safest method to diagnose a renal mass in pregnancy and has a relatively acceptable sensitivity (82%) [3, 4]. Since most renal tumors at this stage are large, additional imaging is warranted for more accurate staging. Magnetic resonance imaging (MRI) is advisable because it is the least harmful in pregnancy.

Still, it remains unclear about the timing and route of the nephrectomy in pregnancy. Loughlin [5] recommended that surgery be performed in the first or third trimester and be delayed after 28 weeks of gestation if the renal mass is found in the second trimester. Other investigators recommend immediate nephrectomy irrelevant of the pregnancy stage because the mother’s welfare is the primary concern [6-8].

Case Report

A 22-year-old woman was referred to our Institute for a left ovarian cyst at eight gestational weeks of her first pregnancy in April, 2003. Her general condition was good, without any history of earlier gynecological or urologic problems. A routine US scan revealed a 40 x 45 x 56 mm anechogenic cyst of the left ovary, most likely a corpus luteum cyst. However an abdominal US scan and MRI were performed at 13 gestational weeks which confirmed the earlier renal findings.

A detailed abdominal US scan and MRI were performed at 13 gestational weeks which confirmed the earlier renal findings. The appearance was highly suggestive of a renal neoplasm. Neither of these studies suggested involvement of other organs, including the left renal vein, inferior vena cava, or liver. A chest X-ray showed no evidence of pulmonary metastases. A full blood count, urea, creatinine, electrolytes and urinalyses were normal. Urine cytology was negative. A microbiological urine culture was negative and no further evaluations were performed at the time.

After extensive counseling and consultations with the obstetric and urological staff, it was recommended that she should undergo a left radical nephrectomy to be performed at 15 weeks of gestation. The patient and her partner wanted to continue the pregnancy and requested the surgery be conducted after the term delivery.

The patient and fetus were under monthly evaluation by a team composed of gynecologists and urologists. The evaluations included abdominal and renal US scans, blood tests, urea, creatinine, electrolytes, urine analysis, cytology and microbiological culture. All tests were within normal range except for mild anemia. A 24-hour urea and creatinine urine clearance were also performed. Obstetric US was regularly performed and identified a live fetus, morphologically and functionally developed as could be expected for the gestational age. At 23 weeks of gestation percutaneous umbilical blood sampling (cordocentesis) was performed and revealed a female fetus (46XX). At gestational week 27 an abdominal US scan revealed the renal hyperechogenic lobular tumor had doubled in size, and now the greatest diameter was 93 mm with a central cavity (probably necrosis) without signs of other organ involvement. The patient developed left flank pain and asymptomatic bacteriuria. The microbiological urine culture showed Enterococcus species and 2 g of ceftriaxone was applied daily intramuscularly for ten days.

The tumor had a slow progression according to abdominal US findings from 27 to 36 gestational weeks.

After an amniocentesis performed at 36 weeks for prediction of fetal lung maturity showed a lecithin/sphingomyelin (L/S) ratio less than 1.0 and number of polygonal cells 0.4 x 10⁴ dexametasone (4 mg), was directly applied to the fetus in the region of the left gluteus to induce lung maturity under US surveillance. Three days later, an elective cesarean section was performed through a 6 cm-long Pfannenstiel incision. A viable female infant was delivered (48 cm, 2750 g, Apgar 6/8). The postoperative course for the mother was complicated with septic fever (up to 38.5°C) due to an urinary infection with E. Coli and hematometra. Ceftriaxone, imipenem and metronidazole were used. Lactation was discontinued. Dilatation and vacuum aspiration of uterine contents were performed 12 days later.

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later. The patient had one blood transfusion and blood analyses showed a hemoglobin level of 107 g/l, 14.2 x 10⁹/l leucocyte level and the erythrocyte sedimentation rate was 74 in the first hour, with c-reactive protein 200.

Three weeks after the cesarean a left radical nephrectomy with ipsilateral adrenalectomy was performed. A 1300 g specimen including the rest of the left kidney and tumor was removed. Pathological examination revealed renal cell carcinoma (clear cell nuclear grade 2) pT2pN(2/10)M0. The patient received six courses of chemotherapy and was reoperated the same year for local recurrence of the tumor. After regular follow-up without signs of metastatic disease for almost a year, she was admitted to the surgery ward for intensive abdominal and intractable back pain, and multiple metastases were diagnosed in the liver, lumbar spine, intestine and paraaortic lymph nodes. The patient was transferred to the oncology unit for pain relief and other symptomatic therapy.

Discussion

Although renal cell carcinoma accounts for 3% of all adult malignancies, it is rare in women of childbearing age. To date there are reports of about 70 cases having been diagnosed during pregnancy; it is the most common renal neoplasm reported in pregnancy, accounting for half of all primary tumors [6, 7]. Sex and age matching data do not exist to allow comparison of the relative proportion occurring outside of pregnancy, although in general there is no evidence of an increased incidence of malignant neoplasm in pregnancy [8].

In most cases the biological behavior of malignancy is not influenced by pregnancy and the prognosis of each stage is similar to that in non pregnant women [9].

Although most cases are incidentally diagnosed in pregnancy, the classic triad of abdominal mass, pain, and hematuria appeared in 26% of cases [3]. Most patients presented a palpable mass (88%), probably because of the more frequent physical examinations during pregnancy. Hypertension is seen in 18% of patients and may mimic preeclampsia [10]. In addition, urinary tract symptoms are experienced by many pregnant women and are often due to non-neoplastic causes, such as calculi or urinary tract infection. This means that renal cell carcinoma may not be considered as a potential cause for such symptoms, thus leading to delay in diagnosis and treatment.

In the current case the renal tumor was suspected during routine obstetric US examination in the first trimester.

During pregnancy, diagnosis of extra pelvic abdominal pathology is difficult by US and the enlarged uterus may cause technical difficulties in tissue characterization.

Diagnostic evaluation of a pregnant patient with a possible renal carcinoma requires special consideration of non-invasive techniques and as little radiation exposure as possible to mother and fetus. The evaluation should involve complete physical examination including palpation, complete blood count, renal and liver function tests, urine culture and urine analysis (with cytology) [7, 10].

Abdominal US along with MRI can adequately identify, differentiate, and stage solid renal masses in most cases, since they avoid radiation exposure to the fetus, these are the investigations of choice [11].

There are several issues to consider when treating a pregnant woman with a renal mass suspicious for malignancy. First, the clinician’s primary responsibility is to the mother, though the management must take into account her wishes regarding the welfare of the fetus.

Such cases should be managed in a multidisciplinary setting involving obstetricians, urologists, neonatologists, radiologists, oncologists and histopathologists. The standard surgical treatment of most stages of renal cell carcinoma is a radical nephrectomy, involving the removal of the entire kidney and perinephritic fat within Gerota’s fascia.

The timing of surgery is guided by the biological behavior of such tumors and neonatal survival rates for different gestations. The doubling time of renal cell carcinoma is estimated at 300 days [12]. The management of solid masses in pregnancy was reviewed by Loughlin [5] and he recommended that surgery should not be delayed in the first and third trimester. However, if a mass is diagnosed in the second trimester then it is reasonable to wait until fetal viability before proceeding to surgery. Surgery may be postponed until after delivery if the renal mass is discovered near term [5].

Cases of nephrectomy in pregnancy have always been performed by an open technique (via both transperitoneal and extraperitoneal approaches) and also the first case of successful laparoscopic nephrectomy in the first trimester was reported in 2004 [2].

In the current case, the patient and her partner wanted to keep the pregnancy but wanted the surgery to be conducted after term delivery. Nephrectomy was performed three weeks after the term delivery instead of in the first trimester. Three years later the child is healthy and the mother is faced with metastases to the liver, spine and intestines after two surgeries and six chemotherapy courses.

It appears that similar to the management of acute surgical problems in pregnancy, timely management need not be deferred.

References


Address reprint requests to:
J. STOJINIĆ, M.D.
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